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## Prognostic significance of lymphocyte recovery in children with standard and high-risk precursor B acute lymphoblastic leukemia<sup>☆</sup>

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### ABSTRACT

**Background:** The primary aim of this study was to determine if absolute lymphocyte count (ALC) at end of induction is an independent prognostic marker of event free survival (EFS) in children with precursor B-ALL.

**Material & Methods:** This is a retrospective analysis of children (aged 1–18 years) diagnosed with standard and high-risk precursor B ALL. At day 29 of induction ALC ( $ALC_{29}$ )  $\geq 1.5 \times 10^9/L$  was considered high and  $<1.5 \times 10^9/L$  was considered low.

**Results:** Total 260 children with (183 standard risk and 77 high risk) Precursor B ALL were included. The mean age of the study cohort was  $5.2 \pm 4.0$  years. Fourteen patients had an event (12 relapse, 4 deaths). Mean follow-up duration was 41 months  $\pm 20$  months (median 41 months).

Overall survival (OS) of the entire study cohort was 98.5% (standard risk 99.4%, high risk 96.1%,  $p = 0.045$ ). EFS of the entire study cohort was 94.6% (standard risk 96.1%, high risk 90.9%,  $p = 0.072$ ).

ALC at any of the studied time points did not correlate with relapse. MRD at day 29 was the strongest predictor of both death ( $p = 0.00$ ) and relapse (0.034).  $ALC_{29}$  did not differentiate EFS among MRD negative patients.

However, once patients relapsed, the OS was 100% for those who had  $ALC > 1.5 \times 10^9/L$  at day 29 of initial induction chemotherapy vs 40% for those with  $ALC < 1.5 \times 10^9/L$  ( $p = 0.01$ ).

**Conclusion:** We conclude that  $ALC_{29}$  cannot be used to risk stratify children with pre B ALL.

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## 1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common leukemia in children. Survival outcomes have greatly improved with combination chemotherapy and the intensification of treatment [1]. Recent improvements in ALL management have largely been due to optimizing treatment according to various risk stratification schema. Minimal residual disease (MRD) at the end of induction treatment has been a valuable addition in this regard [2]. Still 50% of relapses occur in MRD negative cases [3]. Identifying additional

prognostic markers may be useful in further improving the treatment of childhood ALL. However, 80% of childhood ALL occurs in developing countries where there is limited access to MRD studies [4]. The availability of a less expensive and technically less involved prognostic marker would allow better risk stratification for these children and potentially improve the outcomes of more children with ALL globally. One such proposed marker is absolute lymphocyte count (ALC).

Early lymphocyte count recovery has been shown to be an important prognostic variable in stem cell transplantation [5,6] high-risk Ewing sarcoma [7], osteosarcoma [8], acute myeloid leukemia [9] and in aplastic anemia patients treated with immunosuppressive therapy [10]. Recently, it was shown that lymphocyte recovery is a significant prognostic marker for pediatric ALL and is independent of MRD in predicting the outcomes of children with ALL [11]. When both MRD and ALC recovery were combined the prognostic importance was further enhanced [12]. This has not been confirmed by another group [13]. Another study showed

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significance at lower ALC cut-off of  $0.35 \times 10^9/l$  and another one showed significance of lymphocyte recovery at a cut-off of  $1 \times 10^9/l$  [14]. St Jude's study used a cut-off of  $0.5 \times 10^9/l$  and showed lack of significance on multivariate analysis [15]. Recently, another study demonstrated usefulness of ALC recovery and outcome in children [16].

The primary aim of this validation study was to determine if ALC at end of induction and early in maintenance therapy is an independent prognostic marker of relapse free survival, event free survival or overall survival in children with precursor B-ALL.

## 2. Patient & methods

This is a retrospective analysis of children (aged 1–18 years) diagnosed with precursor B ALL over 6 years between 01 Jan 2004 to 31 Dec 2009 at The Hospital for Sick Children, Toronto. Children with T-ALL, very high risk ALL at diagnosis and infant ALL were excluded to maintain homogeneity in patient population. The study was approved by the IRB. Absolute lymphocyte count (ALC) was measured at days 8, 15 and 29 of induction therapy; start of maintenance therapy; and at 6 months into maintenance therapy. ALC was performed as an automated and standardized test with routine CBC measurements. At day 29 of induction  $ALC \geq 1.5 \times 10^9/l$  was considered high and  $<1.5 \times 10^9/l$  was considered low. ALC was also analysed with cut-offs of  $ALC \geq 1.0 \times 10^9/l$  vs  $<1.0 \times 10^9/l$  as high and low and  $ALC \geq 0.350 \times 10^9/l$  vs  $<0.350 \times 10^9/l$  as high and low. Due to larger number of children in both arms when cut-off of ALC  $1.5 \times 10^9/l$  was used, data are therefore presented in greater detail for this group.

Standard risk patients were treated with either of POG 9904, POG 9905 and AALL0331, and high-risk patients treated with either of POG 9906 and AALL0232. MRD analysis was done using flow cytometry. 0.1% was the MRD cutoff used in the AALL0331 and AALL0232 studies. Data was analysed using SPSS v. 11.5. Survival outcomes were assessed by using Kaplan- Meier curve and log-rank test.

## 3. Results

There were 326 children diagnosed with ALL over the 6 year period. Sixty six children were excluded, including 33 children with T-ALL phenotype, 1 with biphenotypic ALL, 8 with L3/burkitt phenotype ALL, 8 with infant ALL and 16 with very high risk ALL. In all, 260 children treated for Precursor B ALL were included. Table 1 shows the demographic profile of the study cohort. The mean age of the study cohort was  $5.2 \pm 4.0$  years and median presenting WBC count was  $9.1 \times 10^9/l$  ( $1-841 \times 10^9/l$ ). Six patients had end of induction MRD between 0.1 and 1% and 2 had  $>1\%$ . Fourteen patients had an event (12 relapse, 4 deaths). Mean follow-up was 41 months  $\pm 20$  months (median 41 months). Table 2 describes the association of ALC as continuous variable at different time points with overall survival (OS) and event free survival (EFS). It shows that ALC at days 8, day 15 and day 29 of induction was associated with superior overall and event free survival. ALC at start of maintenance therapy had no significance but at 6 months into maintenance was again associated with superior OS and EFS.

### 3.1. ALC350

There were only 9 children (6 high risk and 3 standard risk children) with  $ALC < 0.35 \times 10^9/l$  at day 29 of Induction. OS was 77 months for patients who had  $ALC \geq 0.350 \times 10^9/l$  vs 55 months for children with  $ALC < 0.350 \times 10^9/l$  ( $p = 0.001$ ). Similarly, EFS was 77 months vs 55 months ( $p = 0.013$ ) for high and low ALC children.

**Table 1**  
Demographic profile of patients.

Variable	Number of patients	
Gender	Male	146
	Female	114
<b>Risk Group</b>	<b>Protocol</b>	<b>(n = 260)</b>
Standard risk	POG 9904	30
Average risk	POG 9905	12
Standard risk	AALL0331	141
High risk	POG 9906	9
High risk	AALL0232	65
High risk	SickKids Standard of Care	3
<b>CNS involvement (n = 258)</b>	CNS 1	219
	CNS 2	28
	CNS 3	11
<b>Response (n = 243)</b>	Rapid Early Responder	215
	Slow Early Responder	28
<b>ALC</b>	D 29 $ALC \geq 1.5$	187
	D 29 $ALC < 1.5$	72
<b>MRD (n = 139)</b>	Negative	131
	Positive	8
<b>Events</b>	Relapse	12
	Death	4

ALC-absolute lymphocyte count; MRD-minimal residual disease; CNS-central nervous system; POG-Pediatric Oncology Group.

### 3.2. ALC1000

There were only 39 children (22 high risk and 17 standard risk children) with  $ALC < 1.0 \times 10^9/l$  at day 29 of Induction. OS at 5 years was 78 months for patients who had  $ALC \geq 1.0 \times 10^9/l$  vs 64 months  $ALC < 1.0 \times 10^9/l$  ( $p = 0.018$ ). Similarly, EFS was 78 months vs 55 months ( $p = 0.096$ ) for high and low ALC children.

Table 3 shows the survival outcomes in different subgroups by ALC on day 29 of Induction. OS of the entire study cohort was 98.5%. Actuarial survival at 4 years was 100% for children with higher ALC at D 29 vs 94% for those with  $ALC < 1.5 \times 10^9/l$  ( $p = 0.001$ ). Fig. 1A–D describes overall and event free survival in different risk categories. Also, there was no significant difference in standard or high-risk group in relation to ALC at day 29. However, once patients relapsed, the OS was 100% for those who had  $ALC > 1.5 \times 10^9/l$  at day 29 of initial induction chemotherapy vs 40% for those with  $ALC < 1.5 \times 10^9/l$  ( $p = 0.01$ ).

Fig. 1a and b demonstrate the OS and EFS of the cohort by NCI risk stratification. There was no correlation with ALC at start of maintenance with any of the study variables. Higher ALC at 6 months into maintenance correlated with MRD at D29 ( $p = 0.046$ ), OS ( $p = 0.011$ ) and EFS ( $p = 0.012$ ). ALC at any of the studied time points did not correlate with relapse (Fig. 2C). MRD at day 29 was the strongest predictor of both death ( $p = 0.00$ ) and relapse (0.034).

Table 4 shows the death in this study group. All the children were male.

## 4. Discussion

There is conflicting data regarding usefulness of ALC as prognostic marker as well as its numeric value and timing of testing.

A pediatric study has tried to correlate ALC with MRD and found that ALC recovery at day 22 of induction chemotherapy is significantly correlated with MRD level at day 22 of therapy and can be a good prognostic factor for childhood B-cell precursor ALL [17]. Furthermore, lymphocyte count at initial diagnosis is correlated with MRD level at day 22 in childhood BCP-ALL. Another study showed that ALC is an important predictor of outcome in pediatric ALL treated with chemotherapy alone [11]. Zweidler-McKay presented data to suggest that ALC was independent of MRD in

**Table 2**  
Mean ALC as continuous variable at different time points.

ALC ( $\times 10^9/L$ )	Day 1	Day 8	Day 15	Day 29 (1.5)	350	Maintenance 1	Maintenance6
Mean $\pm$ SD	3.9 $\pm$ 2.8	1.1 $\pm$ 0.8	1.3 $\pm$ 1	2.8 $\pm$ 2.1		1.2 $\pm$ 1.1	0.9 $\pm$ 0.9
Median	3.6	0.8	1.2	2.2		0.6	0.5
OS (p)	ns	0.002	0.001	0.084*		ns	0.011
EFS (p)	ns	0.002	0.001	0.083*	0.001	ns	0.012
Relapse (p)	ns	ns	ns	ns		ns	ns
Death (p)	ns	0.031	0.033	0.034#		ns	ns

Maintenance 1-Start of maintenance; Maintenance6-6 months into maintenance; ns-not significant; OS-overall survival; EFS-Event free survival; ALC-absolute lymphocyte count; \*p value significant when divided into ALC high vs low; #p = 0.001 for high vs low ALC.

**Table 3**  
Survival of different risk stratification by ALC at Day 29.

	Total	ALC>1.5	ALC <1.5	P
<b>Overall Survival (%)</b>	<b>98.5</b>	<b>100</b>	<b>94.4</b>	<b>0.001</b>
<b>Standard risk (%)</b>	99.4	100	97.1	0.040
<b>High risk (%)</b>	96.1	100	91.9	0.057
<b>p</b>	0.045	-	0.316	-
<b>Event free survival (%)</b>	<b>94.6</b>	<b>95.7</b>	<b>91.7</b>	<b>0.145</b>
<b>Standard risk (%)</b>	96.1	96.6	94.3	0.533
<b>High risk (%)</b>	90.9	92.5	89.1	0.463
<b>P</b>	0.072	0.308	0.366	-
<b>MRD Negative</b>	<b>94.6</b>	<b>95.7</b>	<b>91.7</b>	<b>0.144</b>

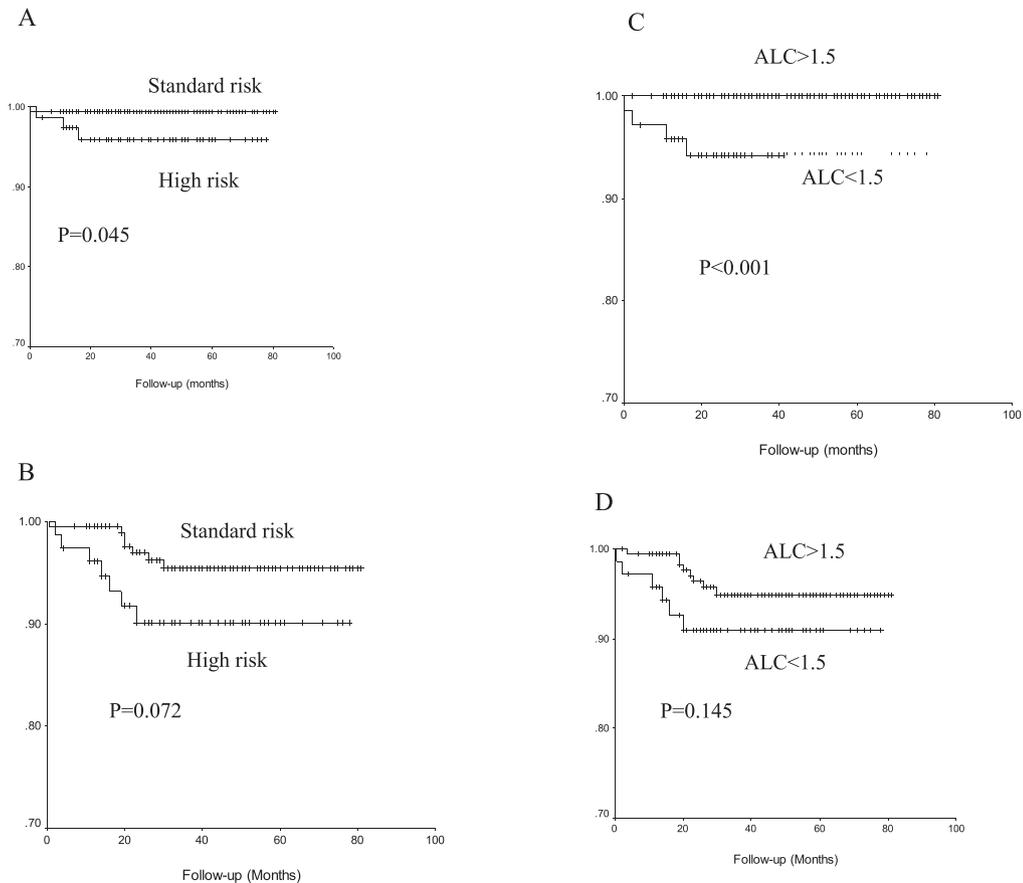
ALC-absolute lymphocyte count; MRD-minimal residual disease.

predicting outcomes of children with ALL [12].

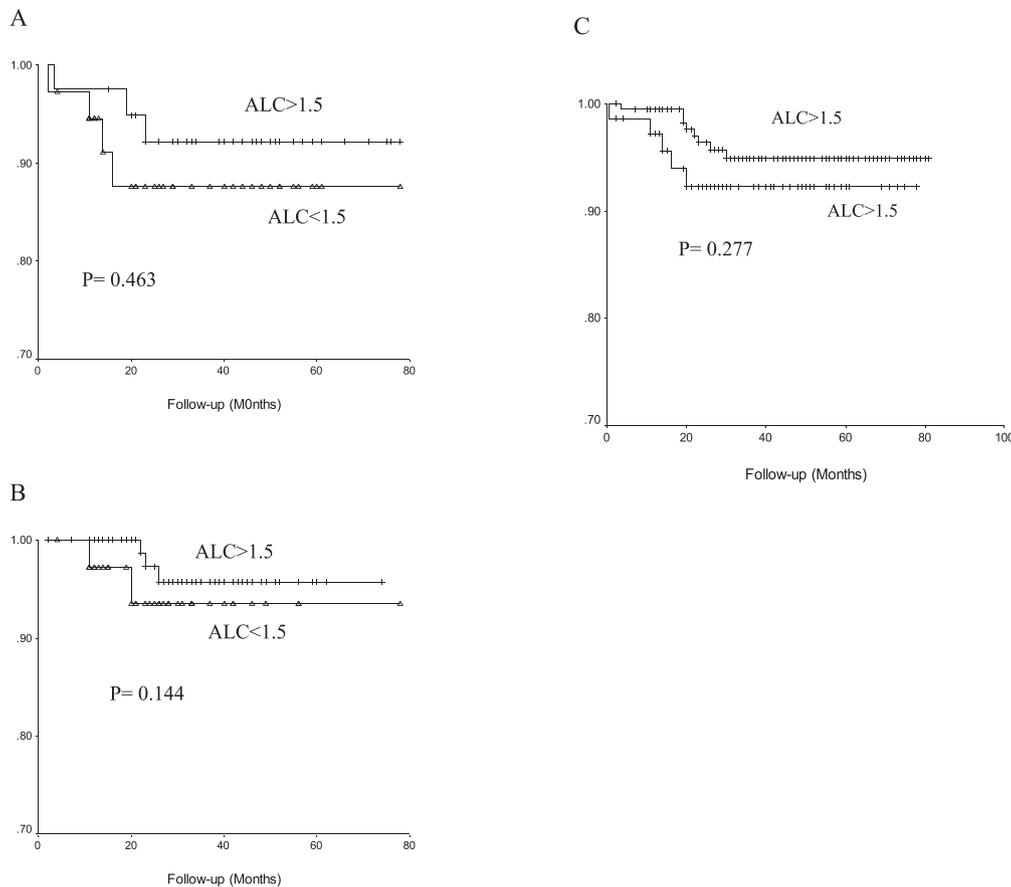
We sought to validate these results to potentially provide an additional early and simple prognostic variable. We chose to assess ALC at the end of induction, start of maintenance and 6 months into

maintenance as reasonable time points to intensify the treatment in higher risk disease if it predicted relapse. We focused more on day 29 of induction therapy as this is generally the time where therapeutic decisions are made with regards to up gradation of risk stratification if required as is true with minimal residual disease at day 29 of induction.

We found an improved OS in our study cohort for higher ALC at early and later time points. However, ALC was not able to predict relapse and the clinical relevance of this finding is unclear. For those patients who subsequently relapsed, the OS was 100% for those who had high ALC at day 29 vs 40% for those with low ALC ( $p = 0.01$ ). There was no difference in relapse free survival in standard or high-risk group either group with any correlation with ALC at any time points. The higher number of high risk children showing lower ALC ( $ALC < 0.35 \times 10^9/l$  and  $ALC < 1 \times 10^9/l$ ) may largely be due to more intensive induction regime used in these children. Continuous superiority of ALC at all 3 cut-offs, however,



**Fig. 1.** A. Overall Survival according to risk stratification. B. Event free survival according to risk stratification. C. OS according to ALC at D29. D. EFS as per ALC on D29.



**Fig. 2.** A. Effect of ALC on high risk ALL. B. EFS by ALC at D29 in patients who were MRD negative. C. Relapse free survival as per ALC on D29.

**Table 4**  
Deaths in study population.

Patient	Age/ Sex	Initial WBC (x10 <sup>9</sup> / L)	CNS	Cytogenetics	Risk group	ALC at D29 (x10 <sup>9</sup> / L)	Relapse Cause	Survival (Months)
1	15/M	17.6	3	Hyperdiploid	High	0.31	No Candida sepsis with HLH	2
2	15/M	3.9	0	t (1; 14)	High	1.0	Yes Induction failure followed by BMT, died 9 months after BMT	16
3	5/M	1.4	0	t (12; 21)	Standard	0.6	No Candida lusitanae and albicans sepsis	1
4	1/M	72.1	3	Non-specific	High	0.12	Yes Disease	11

ALC-absolute lymphocyte count; CNS-central nervous system; BMT-Bone marrow transplantation; HLH-Hemophagocytic lymphohistiocytosis.

does document importance of lymphocyte recovery in overall outcome.

The 2 early deaths in induction due to fungal sepsis and third induction failure and post BMT death could have happened in either group but happened in low ALC group. All four deaths occurred among patients with low ALC at Day 29 (had low ALC at all studied time points).

Absolute lymphocyte count at Day 29 did not differentiate EFS among MRD negative patients in our study. Rubnitz et al. documented that ALC ≥500 cells/μL was significantly more prevalent among patients with B-lineage ALL, in those with favourable presenting features, and in those who achieved negative MRD status on day 43 of treatment. In multivariable analyses, ALC was not a significant predictor of outcome after controlling for age, leukocyte count, lineage, risk group, and MRD status at the end of induction. However, among MRD-negative patients, those with low ALC had a 5-year OS rate of 84.2% versus 97.3% for patients with higher ALC (P = 0.036) [16].

Our study is limited by a very small number of events in these patients. Also, MRD was positive in only 8/139 patients tested. Efficacy of salvage therapy after relapse has significantly improved in recent times. This may have contributed to lack of difference in overall and event free survival. Also, our median follow-up duration is 40 months which is relatively short for ALL studies. This duration should still be able to pick very early and early relapses.

The ability of ALC to predict better survival in relapsed patients is difficult to understand. Lymphocytes have been shown to be an important component of immune surveillance and lymphocyte recovery plays a major part in tumor control [18].

It is possible that lack of prognostic significance of ALC is likely due to differing induction regimen intensities [13,16]. We have earlier demonstrated that early lymphocyte recovery did not influence outcomes in T Cell ALL [19]. Even though different studies have varied on time point to assess ALC and its numeric cut-offs, it does appear that early lymphocyte recovery may be a useful marker of favourable prognosis.

## 5. Conclusions

Absolute lymphocyte count recovery at end of induction and at 6 months maintenance was an important prognostic variable statistically but was not significant clinically in terms of treatment intervention. ALC recovery cannot be used to risk stratify children with pre B ALL as it did not predict relapse.

## Declaration of interest

Authors do not have any financial interests or other conflicts of interests to disclose. This study was approved by an Institutional Review Board.

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