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Prognostic Impact of Absolute Lymphocyte Counts at the End of Remission Induction in Childhood Acute Lymphoblastic Leukemia

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Abstract

Background—Absolute lymphocyte counts (ALC) during treatment have been associated with outcome in children and adults with hematologic malignancies. However, the impact of ALC relative to that of other prognostic factors on the outcome of children with acute lymphoblastic leukemia (ALL) treated in recent trials is unknown.

Methods—Outcomes of 399 patients 18 years of age with newly diagnosed ALL who were enrolled in the Total Therapy XV study at St. Jude Children's Research Hospital were analyzed according to ALC at the end of remission induction therapy.

Results—ALC 500 cell/ μ L was significantly more prevalent among patients with B-lineage ALL, favorable presenting features and in those who achieved minimal residual disease (MRD) negativity on day 43 of treatment. Both overall survival (OS) and event-free survival (EFS) were superior among patients with higher ALC, but only the association with OS was statistically significant in a univariate analysis. In multivariable analyses, ALC was not a significant predictor of outcome after controlling for age, leukocyte count, lineage, risk group, and MRD at the end of induction ($p > 0.1$ for all comparisons). However, among MRD-negative patients, those with low ALC had a 5-year OS of $84.2\% \pm 8.9\%$ versus 97.3 ± 1.0 for patients with higher ALC ($P = .036$).

Conclusion—ALC at the end of induction is related to favorable presenting features and good initial treatment response but does not independently predict outcome in the context of contemporary, MRD-guided, therapy.

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Keywords

acute lymphoblastic leukemia; lymphocytes; pediatrics; prognosis

INTRODUCTION

In patients with acute leukemia undergoing hematopoietic cell stem transplantation (HSCT), absolute lymphocyte counts (ALC) post-transplant serve as an indicator of the speed and quality of engraftment and hematopoietic cell reconstitution and may also reflect the potential for graft-versus-leukemia activity. Thus, several studies have shown that higher ALC post-transplant predict a superior outcome in patients with either acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL).^{1–9} A rapid regeneration of normal hematopoiesis after intensive chemotherapy should also be a favorable prognostic indicator, as this may reflect a deeper leukemia cytoreduction with a consequently higher functional capacity of the hematopoietic microenvironment. Conceivably, immune cells might also exert an anti-leukemic effect and suppress relapse. However, whether ALC have a prognostic impact in patients receiving chemotherapy alone is less clear. To this end, Behl et al.⁴ reported that ALC $> 500/\mu\text{L}$ after induction chemotherapy was associated with superior overall survival (OS) and event-free survival (EFS) in 103 patients with AML. Investigators of the Children's Oncology Group (COG) examined the effect of ALC in childhood leukemia.^{5,6} In an analysis of 171 patients ≤ 21 years old, De Angulo et al.⁵ found that ALC during induction therapy was a significant predictor of survival in both ALL and AML.⁵ In a subsequent study of 171 pediatric ALL cases, Rabin et al.⁶ observed that ALC at day 29 of induction therapy was independently associated with relapse-free survival and OS.⁶

The level of minimal residual disease (MRD) during and after remission induction chemotherapy is the strongest prognostic factor in childhood ALL.^{10–13} MRD is an independent predictor of outcome and can define risk subgroups among clinically or genetically defined ALL subtypes. In the St Jude Total XV study for children and adolescents with newly diagnosed ALL, we incorporated MRD measurements in risk-classification algorithms and used them to guide therapy, a strategy that resulted in 5-year EFS and OS rates of 85.6% and 93.5%, respectively.¹⁴ The study of Rabin et al.⁶ indicated that ALC after remission induction therapy predicted outcome even after adjusting for MRD status, suggesting that these were independent prognostic factors. To determine whether ALC held its prognostic significance in the context of MRD-directed therapy, we examined the impact of ALC on outcome in our Total XV cohort.

PATIENTS AND METHODS

Patients

From June 2000 to October 2007, 411 patients ≤ 18 years of age with newly diagnosed ALL were enrolled in the Total Therapy XV study at St. Jude Children's Research Hospital.¹⁴ Treatment regimens, risk classification, and MRD methods have been previously described.¹⁴ ALC values were obtained at the time of remission evaluation, after six weeks of remission induction therapy. The study was approved by the institutional review board, and written informed consent or assent, as appropriate, was obtained for all patients.

Statistical Methods

ALC values were analyzed separately as a categorical variable (< 500 cells/ μL vs. ≥ 500 cells/ μL) and as a continuous variable. Comparisons of ALC, treated as a categorical variable, between groups were performed by using the chi-square test. ALC treated as a continuous variable between groups was compared using the Wilcoxon rank-sum test when

two groups were compared, and by the Kruskal-Wallis test when more than two groups were compared simultaneously. The duration of EFS was measured from the date of enrollment to the date of the first treatment failure of any kind (relapse, death, lineage switch, or second malignancy) or to the date of the most recent follow-up. Failure to induce remission was considered an event at time zero. The duration of OS was calculated from the date of enrollment to the date of death due to any cause or the date of the most recent follow-up. Data for patients who were alive at the most recent contact date were censored at the time of that contact. The Kaplan-Meier method¹⁵ was used to estimate the probability of OS and EFS, standard errors were determined by the method of Peto and Pike,¹⁶ and comparisons between groups were made using the log-rank test. The Cox proportional hazards regression model¹⁷ was used to assess each potential prognostic factor in univariate and multivariable analyses, and hazard ratios were estimated with 95% confidence intervals.

All analyses were performed using SAS software (SAS Institute, Cary, NC), Windows version 9.2. All reported *P* values are 2-sided. The criterion for significance in all analyses was a probability of 0.05 or less (*P* .05), and no *P*-value adjustments were made for multiple comparisons.

RESULTS

ALC at the end of remission induction therapy and relation with clinical features of ALL

ALC at the end of remission induction ranged from 120 to 6942 cells/ μ l (median, 1024 cells/ μ l). Because ALC was associated with OS when cutpoints of 500 cells/ μ l and 600 cells/ μ l were used but was marginally associated with EFS only when a cutpoint of 500 cells/ μ l was chosen, we used 500 cells/ μ l for subsequent analyses. As shown in Table 1, patients with ALC $\geq$ 500 cells/ μ l at the end of induction were more likely than patients with lower counts to have B-lineage ALL (*P* < .001), have low-risk leukemia as defined by age and leukocyte count (*P* = .001) and be MRD negative (< .01% leukemic cells among bone marrow mononucleated cells) at the end of induction (*P* = .005). When ALC was analyzed as a continuous variable (data not shown), higher counts were associated with age 1 to 9 years (*P* < 0.001), B-lineage ALL (*P* < .001), and low-risk features (*P* < .001). Thus, higher ALC was characteristic of patients with favorable prognostic features and excellent initial treatment response.

Low ALC at the end of induction therapy may reflect a delayed recovery of bone marrow function. Thus, we compared the leukocyte, platelet, and absolute neutrophil counts between patients with ALC values above or below 500 cells/ μ l. Indeed, the median leukocyte count in patients with ALC < 500 cells/ μ l was 1800/ μ l (range, 400/ μ l to 3900/ μ l) as compared to 2900/ μ l (range, 500/ μ l to 33300/ μ l) for patients with higher ALC (*P* = .003); platelet counts were 135×10^3 / μ l (range, 17×10^3 / μ l to 344×10^3 / μ l) vs. 277×10^3 / μ l (range, 23×10^3 / μ l to 1220×10^3 / μ l) (*P* < .001).

Relation between ALC and treatment outcome

In a univariate analysis, ALC $\geq$ 500 cells/ μ l was significantly associated with a superior OS: the 5-year OS estimate was $95.0\% \pm 1.2\%$ versus $82.4\% \pm 6.8\%$ for patients with ALC values lower than 500 cells/ μ l (*P* = .023) (Fig. 1a); ALC was also significantly related to OS when analyzed as a continuous variable (*P* = .029). Among other factors included in the analysis, age (*P* < .001), MRD status (*P* < .001), leukemic cell lineage (*P* = .029), and initial risk group (*P* = .001) were predictive of OS (Table 2). Age (*P* = .005), MRD status (*P* < .001), leukemic cell lineage (*P* = .002), initial risk group (*P* < .001), and leukocyte count at diagnosis (*P* = .040 when analyzed as a continuous variable) were also significantly associated with EFS (Table 2). However, ALC was not significantly associated with EFS:

the 5-year EFS estimates for patients with ALC values greater or lower than 500 cells/ μ l were $88.0\% \pm 1.8\%$ vs. $76.5\% \pm 7.6\%$, respectively ($P = .053$; Fig. 1b); likewise, ALC lacked prognostic significance if analyzed as a continuous variable ($P = .092$).

In a multivariable Cox proportional hazards regression model that included age, leukocyte count at diagnosis, leukemic cell lineage, initial risk group, and MRD at the end of induction, ALC, whether analyzed as a categorical ($P = .362$, Table 3) or continuous ($P = .234$, data not shown) variable, was not associated with OS. We also performed a multivariable analysis that included ANC in the Cox model. However, the inclusion of ANC in the model did not change the results—ALC was not significantly associated with OS. Similarly, ALC was not significantly associated with EFS when analyzed as a categorical ($P = .930$, Table 3) or continuous ($P = .506$, data not shown) variable. In the multivariable analyses, MRD remained significantly associated with OS and EFS ($P < .001$); age was associated with OS ($P = .005$) and initial risk group with EFS ($P = .003$). To assess whether ALC may provide useful prognostic information in settings in which MRD measurement is not possible, the multivariable analyses were repeated without MRD in the model. In these analyses, ALC was not significantly associated with OS ($P = .176$) or EFS ($P = .534$).

Although the multivariable analyses showed no evidence that ALC was independently associated with outcome, we nevertheless performed subgroup analyses to determine whether ALC values might be prognostically useful for a subset of patients (Table 4). After adjusting for age, ALC was not significantly associated with OS or EFS in MRD-positive, low-risk, or standard/high-risk patients, but was associated with OS among MRD-negative patients (Fig. 2; Table 4). The 5-year estimates of OS were $97.3\% \pm 1.0\%$ for MRD-negative patients who had ALC values ≥ 500 cells/ μ l and $84.2\% \pm 8.9\%$ for patients with lower ALC ($P = .036$, Fig. 2). Three hematologic relapses occurred in the low ALC, MRD-negative group, including two patients with T-ALL and one patient with B-precursor ALL, all of whom had MRD levels greater than 1% at day 15 of remission induction therapy.

DISCUSSION

We assessed the prognostic impact of ALC at the end of remission induction in the context of contemporary MRD-directed therapy. We found that a higher ALC (≥ 500 cell/ μ L) was significantly more prevalent among patients with B-lineage than T-lineage ALL, in those with favorable presenting features, and in those who had a good response to remission induction therapy and achieved MRD negativity on day 43. Patients with higher ALC at the end of induction also had higher leukocyte and platelet counts at the same time point. Both OS and EFS were superior among patients with higher ALC, but only the association with OS was statistically significant in a univariate analysis. ALC was not significantly associated with EFS or OS after adjusting for other risk factors, including age, leukocyte count, lineage, risk group, and MRD.

A recent COG study that showed that end-of-induction ALC is a significant predictor of outcome in children with ALL even after accounting for MRD.⁶ Several factors may explain the differences between the COG report and our findings. First, remission induction therapy on the COG trials consisted of 3 or 4 drugs given over a 4-week period, whereas the Total Therapy XV trial included 8 drugs in a 6-week induction period. Thus, ALC and MRD were evaluated at day 29 in the COG study and at approximately day 43 after more intensive therapy in our study. This difference in timing and intensity of therapy was reflected in higher ALC values in the COG study (median, 2280 cells/ μ l; range, 192–9890 cells/ μ l) than in our study (median, 1024 cells/ μ l; range, 120–6942 cells/ μ l). The most important difference, however, was the outcome for MRD-positive patients in the two studies. In the COG report, relapse-free survival and OS rates for patients with low ALC and positive

MRD were 33% and 41% respectively, compared with EFS and OS rates of 69% and 77% for the corresponding group in our study. In the COG study, ALC identified MRD-positive patients with dismal or very good OS rates (41% vs. 92% for those with low or high ALC, respectively), whereas ALC was not associated with survival among MRD-positive patients in our study (77% vs. 84%).

Our results suggest that the modification of therapy based on MRD, pharmacogenetics, and pharmacodynamics, along with careful supportive care, can abrogate the prognostic effect of both traditional and novel factors. Although age was associated with OS and risk group was predictive of EFS, MRD was the only factor that was independently associated with both OS and EFS. By contrast, multivariable analysis showed that leukemic cell lineage, leukocyte count, and ALC were not independently associated with OS or EFS. This suggests that any negative effect potentially associated with low ALC, particularly among MRD-positive patients, was overcome by intensification of chemotherapy. Of note, MRD-negative patients with ALC values <500 cells/ μ l in our study had a significantly worse outcome than those with higher counts within this group, a finding that requires further investigation.

It has been proposed that the favorable impact of higher ALC after chemotherapy or HSCT for hematologic malignancies reflects enhanced immunologic effects of autologous or allogeneic lymphocytes.^{2,6,8,9} Anti-leukemic effects have been ascribed to T-cell subsets^{18,19} and NK cells.^{2,9} It is clear that among patients undergoing HSCT, allogeneic NK cells play a key role, as donor NK cells may exert potent antileukemic effects when the patient's leukemia cells lack the class I epitope for the donor's inhibitory killer immunoglobulin-like receptors.^{20–22} In addition, the normalization of NK cell receptor expression may be prognostically important in patients receiving chemotherapy. A study of adult AML patients revealed that the acquisition of an NK cell–triggering receptor (NCR)–dull phenotype was reversible in patients who achieved remission.²³ An association between the NCR–dull phenotype and poor survival in these patients suggests that recovery of normal NCRs may allow the recovery of normal NK cell function, which, in turn, contributes to sustained remission. While this and other studies suggest an important role for immune surveillance after chemotherapy,^{2,5,6} the results of the present study indicate that intensive chemotherapy may compensate for the lack of such immune effects.

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Figure 1. Overall survival (a) and event-free survival (b) according to ALC at the end of remission induction therapy.

Figure 2. Overall survival (a, b) and event-free survival (c, d) according to ALC at the end of remission induction therapy and minimal residual disease (MRD) status (neg, negative; pos, positive).

Table 1

Absolute lymphocyte count distribution

Variable	Total (n=399)	ALC < 500 cells/ μ L (n=34)	ALC $\geq$ 500 cells/ μ L (n=365)	P-value *
Age at Diagnosis (%)				
1 to 9 years	293	21 (7.2)	272 (92.8)	.107
10 years	106	13 (12.3)	93 (87.7)	
Leukocyte count (%)				
< $50 \times 10^3/\mu$ L	293	25 (8.5)	268 (91.5)	.989
$50 \times 10^3/\mu$ L	106	9 (8.5)	97 (91.5)	
MRD (%)				
Negative (< 0.01%)	318	19 (6.0)	299 (94.0)	.005
Positive ($\geq$ 0.01%)	77	13 (16.9)	64 (83.1)	
Missing	4	2 (50.0)	2 (50.0)	
Lineage (%)				
B	336	19 (5.7)	317 (94.3)	< .001
T	63	15 (23.8)	48 (76.2)	
Initial risk group (%)				
Low	223	10 (4.5)	213 (95.5)	.001
Standard/High	176	24 (13.6)	152 (86.4)	

ALC, absolute lymphocyte count; MRD, minimal residual disease

* Pearson's chi-square test

Table 2

Univariate Analysis of Event-Free and Overall Survival

Variable	n	Event-free survival		Overall survival	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
ALC (continuous)	399	1.00 (1.00, 1.00)	.092	1.00 (1.00, 1.00)	.029
ALC (< 500 cells/ μ L vs. $\geq$ 500 cells/ μ L)	34 vs. 365	2.10 (0.99, 4.46)	.053	2.82 (1.15, 6.91)	.023
Age at diagnosis (1–9 vs. $\geq$ 10 years)	295 vs. 107	0.46 (0.26, 0.79)	.005	0.22 (0.11, 0.46)	<.001
Leukocyte count at diagnosis (continuous)	402	1.00 (1.00, 1.00)	.040	1.00 (1.00, 1.00)	.826
Leukocyte count at diagnosis (< 50 vs. $\geq$ 50 $\times 10^3/\mu$ L)	295 vs. 107	0.68 (0.38, 1.19)	.175	0.71 (0.33, 1.52)	.375
Leukocyte count at end of induction (continuous)	397	0.96 (0.83, 1.12)	.605	1.04 (0.94, 1.15)	.447
ANC at end of induction (continuous)	396	1.00 (1.00, 1.00)	.519	1.00 (1.00, 1.00)	.024
Platelets at end of induction (continuous)	398	1.00 (1.00, 1.00)	.354	1.00 (1.00, 1.00)	.270
MRD (Negative vs. Positive)	321 vs. 77	0.223 (0.13, 0.39)	<.001	0.17 (0.08, 0.35)	<.001
Lineage (B vs. T)	339 vs. 63	0.39 (0.22, 0.70)	.002	0.42 (0.19, 0.92)	.029
Initial risk group (low vs. standard/high)	225 vs. 177	0.25 (0.14, 0.46)	<.001	0.27 (0.12, 0.61)	.001

ALC, absolute lymphocyte count; ANC, absolute neutrophil count; MRD, minimal residual disease

Table 3

Multivariable Cox Proportional Regression Model (ALC as a categorical variable)

Variable	Event-free survival		Overall survival	
	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
ALC (< 500 cells/ μ l vs. 500 cells/ μ l)	1.04 (.44, 2.42)	.930	1.62 (.57, 4.56)	.362
Age at diagnosis (1 to 9 vs. 10 years)	.90 (.49, 1.66)	.736	.30 (.13, .70)	.005
Leukocyte count at diagnosis (< 50 vs. $50 \times 10^3/\mu$ L)	1.39 (.71, 2.70)	.334	1.00 (.41, 2.50)	.996
Lineage (B vs. T)	.80 (.39, 1.65)	.548	.90 (.33, 2.45)	.836
Initial risk group (low vs. standard/high)	.30 (.14, .66)	.003	.70 (.24, 2.01)	.505
MRD (Negative vs. Positive)	.30 (.17, .53)	< .001	.22 (.10, .46)	< .001

ALC, absolute lymphocyte count; MRD, minimal residual disease

Table 4

Outcome according to ALC, risk group, and MRD

Variable	N	5-yr EFS	P-value	5-yr OS	P-value
ALC			.053		.023
< 500 cells/ μ l	34	76.5 $\pm$ 7.6		82.4 $\pm$ 6.8	
500 cells/ μ l	365	88.0 $\pm$ 1.8		95.0 $\pm$ 1.2	
ALC in initial low-risk patients			.580		.319
ALC < 500 cells/ μ l	10	90.0 $\pm$ 10.1		90.0 $\pm$ 10.1	
ALC 500 cells/ μ l	213	94.2 $\pm$ 1.7		98.6 $\pm$ 0.8	
ALC in initial S/H-risk patients			.336		.182
ALC < 500 cells/ μ l	24	70.8 $\pm$ 9.3		79.2 $\pm$ 8.3	
ALC 500 cells/ μ l	152	79.2 $\pm$ 3.5		90.0 $\pm$ 2.5	
ALC in MRD-negative patients			.065		.036
ALC < 500 cells/ μ l	19	78.9 $\pm$ 10.0		84.2 $\pm$ 8.9	
ALC 500 cells/ μ l	299	92.2 $\pm$ 1.7		97.3 $\pm$ 1.0	
ALC in MRD-positive patients			.875		.887
ALC < 500 cells/ μ l	13	69.2 $\pm$ 12.1		76.9 $\pm$ 11.1	
ALC 500 cells/ μ l	64	68.0 $\pm$ 6.2		84.4 $\pm$ 4.7	

ALC, absolute lymphocyte count; S/H, standard/high; MRD, minimal residual disease; EFS, event-free survival; OS, overall survival