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Clinical features and outcome of pediatric acute lymphoblastic leukemia with low peripheral blood blast cell count at diagnosis

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Abstract

Peripheral blood (PB) blast cell count on day 8 of prednisone therapy has been considered one of the strongest predictors of outcome in children with acute lymphoblastic leukemia (ALL). However, little is known about the clinical features and prognostic impact of PB blast cell count at diagnosis in these patients. The aim of this study was to evaluate the relationship between initial PB blast cell count and clinical prognosis of pediatric ALL.

The study comprised 367 patients with ALL, aged 0 to 14 years, enrolled and treated using the Chinese Children's Leukemia Group-ALL 2008 protocol between 2011 and 2015. The majority (91.6%) of patients were B-cell precursor ALL (BCP ALL), and 8.4% were T-cell ALL (T-ALL).

Patients with BCP ALL in the low PB blast cell count group ($<1 \times 10^9$/L) had significantly superior survival rates to those in the high count group ($\geq 30 \times 10^9$/L). In T-ALL, the low count group showed significantly inferior survival rates compared to both the intermediate count group ($1-29.9 \times 10^9$/L) and high count group. Multivariate analysis revealed that the initial white blood cell count and minimal residual disease at the end of induction therapy were independently predictive of BCP ALL outcome, while risk stratification was shown to be an independent prognostic factor for T-ALL outcome.

These results indicated that low blast cell count in PB at diagnosis was associated with different clinical outcomes in patients with BCP ALL and T-ALL, although it was not an independent outcome predictor by multivariate analysis.

Abbreviations: 6-MP = 6-mercaptopurine, ALL = acute lymphoblastic leukemia, BCP ALL = B-cell precursor acute lymphoblastic leukemia, BFM = Berlin–Frankfurt–Münster, CAM = cyclophosphamide, cytarabine and 6-mercaptopurine, CCLG = Chinese Childhood Leukemia Group, CNS = central nervous system, CR = complete remission, EFS = event-free survival, FAB = French-American-British, HR = high-risk, IR = intermediate-risk, MRD = minimal residual disease, MTX = methotrexate, OS = overall survival, PB = peripheral blood, SR = standard-risk, T-ALL = T-cell acute lymphoblastic leukemia, WBC = white blood cell.

Keywords: acute lymphoblastic leukemia, children, peripheral blood blast cell, prognostic factor

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1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy. Based on multiagent chemotherapy regimens and risk-stratified antileukemic therapy, cure rates for children with ALL have improved from 10% to 90% over the past 5 decades.[1,2] B-cell precursor ALL (BCP ALL) accounts for nearly 90% of childhood ALL while T-cell ALL (T-ALL) comprises the remaining cases.[3] Previous studies have demonstrated that patients with T-ALL have an unfavorable prognosis compared to those with BCP ALL.[4–6] Therefore, commonly utilized prognostic factors for BCP ALL may not necessarily be suitable for T-ALL due to differences in their biological and clinical features.[7–9]

Clinical, biological, genetic and response-based variables, such as age, gender, white blood cell (WBC) count, immunophenotypic, cytogenetic, and molecular characteristics are known to predict relapse in childhood ALL. Early response to treatment measured by minimal residual disease (MRD) is currently the single most powerful prognostic factor in childhood ALL.[10–13] Even with identification of novel biomarkers, initial WBC count is still considered one of the strongest independent predictors of induction failure and risk of relapse in pediatric ALL.[14] As shown in previous studies, both age, and initial WBC count were

independent prognostic factors in patients with BCP ALL, although they were significantly less predictive in T-ALL.[3,15] Additionally, T-ALL patients with either an initially low or high WBC count were found to have a significantly lower survival rate than those with an intermediate count.[16]

According to the Berlin–Frankfurt–Münster (BFM) group, prednisone response characterized by the peripheral blood (PB) blast cell count is considered one of the strongest prognostic factors for treatment outcome in children with ALL. A blast cell count of $< 1000/\mu L$ in PB on day 8 of treatment after a 7-day treatment course of prednisone plus one intrathecal dose of methotrexate (MXT) shows significantly improved long-term survival.[17–19] However, the relationship between PB blast cell count at diagnosis and clinical prognosis of children with ALL remains uncertain. The objective of this study was to explore whether low blast cell count in PB at diagnosis was associated with an improved clinical outcome in childhood BCP ALL and/or T-ALL.

2. Materials and methods

2.1. Patients

From May 2011 to May 2015, hospitalized children diagnosed with BCP ALL or T-ALL based on Chinese Children's Leukemia Group-ALL 2008 (CCLG-ALL 2008) protocol at West China Second University Hospital in Southwest China were retrospectively included in this study. The diagnosis of ALL was based on evaluation of bone marrow smears according to morphologic and cytochemical criteria of French-American-British (FAB),[20] and immunophenotypic criteria. The recorded clinical variables included age, gender, fever or infection, pallor, bleeding tendency, splenomegaly, hepatomegaly, lymphadenopathy, infiltration of the central nervous system, WBC count and PB blast cell count at diagnosis, FAB morphology, risk stratification, cytogenetic abnormalities, prednisone response, and MRD on day 33. This study protocol was approved by the Ethical Review Board of Investigation in Human Beings of West China Second University Hospital (no.155/2020). Informed consents were obtained orally from the patients or guardians of each patient.

2.2. Risk stratification and treatment

According to CCLG-ALL 2008 protocol, children with ALL were categorized into standard risk (SR), intermediate risk (IR), and high risk (HR) based on age, WBC count at diagnosis, immunophenotype, cytogenetic features, early response treatment, and MRD level at the end of induction therapy. All patients were treated with the CCLG-ALL 2008 protocol as described previously.[21,22] Following 7 days of treatment with prednisone, the treatment regimen was divided into 5 phases:

1) remission induction [vincristine, daunorubicin, L-asparaginase, and dexamethasone];
2) early intensification [1 course of cyclophosphamide, cytarabine and 6-mercaptopurine (6-MP) (CAM) for SR ALL, and 2 courses of CAM for IR/HR ALL];
3) consolidation [high-dose MTX + 6-MP for SR ALL ($2.0 \, g/m^2$ MTX) and IR ALL ($5.0 \, g/m^2$ MTX), and 2 course of BFM High Risk block-1', BFM High Risk block-2', and BFM High Risk block-3' for HR ALL];
4) delay intensification ([vincristine, daunorubicin, L-asparaginase, and dexamethasone + CAM for SR/HR ALL, 1 course of

6-MP + MTX between 2 courses of delay intensification for IR ALL); and
5) maintenance treatments [6-MP + MTX/vincristine + dexamethasone for SR/IR ALL, and 6-MP + MTX/cyclophosphamide + cytarabine/vincristine + dexamethasone for HR ALL].

2.3. WBC and differential count

Venous PB samples were collected with vacutainer tubes containing K2EDTA (Becton Dickinson, Franklin Lakes, NJ). Complete blood count was measured using an automated hematology analyzer Sysmex XE-2100 (Sysmex, Kobe, Japan). Manual WBC differential count of 200 cells was performed by 2 trained technicians who had more than 10 years of experience in manual slide review. If blast cells were not found after manual counting by the technicians, the slides were reviewed by a third technician or a hematopathologist. If not enough cells were present on 1 slide, 2 or more slides were microscopically reviewed to assure that a minimum of 100 cells were counted.

2.4. Flow cytometric analyses

Bone marrow samples collected from all patients were processed for immunophenotyping at the time of diagnosis, and assessed for MRD at the end of induction chemotherapy (day 33). The staining procedure, protocol for immunophenotyping, and MRD detection by flow cytometry have been described in detail previously.[23–25] Flow cytometry data were recorded using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) and analyzed on BD Cell-Quest™ Pro software (Becton Dickinson, San Jose, CA) within 24 hours after sample collection. The following monoclonal antibodies were used: CD2 PE (clone S5.2), CD3 FITC (clone SK7), CD5 FITC (clone L17F12), CD7 FITC (clone M-T701), CD10 FITC (clone HI10a), CD13 PE (clone L138), CD19 PE (clone 4G7), CD20 PE (clone L27), CD22 PE (clone SJ10.1H11), CD33 FITC (clone P67.6), CD34 FITC (clone 8G12), CD45 PerCP (clone 2D1), CD117 PE (clone 104D2), CD79a PE (clone HM47), and HLA-DR PE (clone L243). All the monoclonal antibodies were obtained from BD Bioscience except CD22 which was obtained from Beckman Coulter. Cells were gated based on CD45 and side scatter parameters. The sample was considered positive for an antigen if it was expressed on 20% or more of leukemic cells.

2.5. Fusion transcript analysis

Bone marrow mononuclear cells were enriched by density gradient centrifugation with Ficoll solution. Total RNA was extracted using Trizol (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. BCR-ABL, TEL-AML1, E2A-PBX1, and MLL-AF4 fusion genes were assessed by reverse transcriptase-polymerase chain reaction.

2.6. Definition

Complete remission (CR) was defined as less than 5% bone marrow blasts and no evidence of extramedullary leukemia. Relapse was defined as recurrence of ≥20% blasts in bone marrow or local leukemia infiltration after CR. Central nervous system (CNS) disease at diagnosis was defined as the presence of neurological symptoms and signs or an elevated number of mononuclear cells ($\geq 5 \times 10^6/L$) in cerebrospinal fluid and leukemic blasts identified on a cytocentrifuge slide.

2.7. Statistical analysis

Statistical analysis was conducted using SPSS 13.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL). Categorical variables were compared with Chi-squared test or Fisher exact test when necessary. A linear regression model was performed to investigate the correlation between PB blast cell count and WBC count. Event-free survival (EFS) was defined as the time from diagnosis to the first adverse event (failure to induce remission, relapse, or death from any cause) or to the last follow-up date. Overall survival (OS) was measured from the date of diagnosis to the date of death or last follow-up. A censored observation was defined as the time from initial diagnosis to the date of last hospital visit, when the patient was subsequently lost to follow-up. EFS and OS were calculated using the method of Kaplan–Meier. Survival curves were compared using the log-rank test. Prognostic factors were assessed using a Cox proportional hazards regression model in univariate and multivariate analyses. The significant variables from the univariate analyses were simultaneously entered into the multivariate Cox proportional hazards model. Furthermore, variables that resulted in a P value $>.05$ but were thought to be clinically relevant were retained for multivariate analysis. Hazard ratio and 95% confidence intervals (95% CI) were reported. $P < .05$ was considered statistically significant.

3. Results

3.1. Patients

A total of 367 pediatric patients aged 0 to 14 years were enrolled in the present study according to CCLG-2008 protocol. Baseline characteristics of children with ALL were summarized in Table 1. CR was achieved in 341 cases (93.0%) after 1 course of remission induction therapy. Thirteen patients (3.5%) died during induction therapy, and 13 patients (3.5%) had resistant disease. The probabilities of EFS (pEFS) and OS (pOS) at 5 years were $75.6 \pm 2.9\%$ and $83.2 \pm 2.2\%$, respectively. Low PB blast cell count $(<1 \times 10^9/L)$ at diagnosis was present in 160 of 336 patients (47.6%) with BCP ALL, and 5 of 31 patients (16.1%) with T-ALL. No PB blasts were found in 75 of the 165 patients (45.5%) in the low PB blast cell count group.

3.2. Correlation between WBC and PB blast cell count

Linear regression analysis revealed a significant correlation between PB blast cell count and WBC count at diagnosis for both BCP and T-ALL patients ($r=.987$, $P=.000$; and $r=.987$, $P=.000$; respectively). In both BCP and T-ALL subgroups, PB blast cell count strongly correlated with WBC count in the high PB blast cell count group $(\geq 30 \times 10^9/L)$ ($r=.996$, $P=.000$; and $r=.983$, $P=.000$; respectively), but not in the low PB blast cell count group (r=.150, $P=.058$; and $r=.387$, $P=.519$; respectively).

3.3. Relation between PB blast cell count and clinical features

PB blast cell count at diagnosis was not significantly related to age, gender, fever/infection, pallor, CNS disease, FAB classification, MLL-AF4, or E2A-PBX1 (Table 2). Of the low PB blast cell count group, 160 (97.0%) were BCP ALL. Notably, low PB blast cell count was significantly associated with low WBC count,

standard-risk ALL, TEL-AML1 positivity, BCR-ABL negativity, favorable prednisone treatment response, and MRD negativity. The percentages of patients who had bleeding tendency, hepatosplenomegaly and lymphadenopathy were significantly lower in the low PB blast cell count group than those in the other 2 groups.

3.4. Correlation between PB blast cell count and immunophenotypes

For the 336 patients with BCP ALL, a significantly higher percentage of CD10 was expressed in 98.1% of the low PB blast cell count group, whereas CD33 showed a lower percentage of expression in this group (Table 3). In T-ALL, a significantly higher percentage of CD13 and CD33 expression was observed in the low count group, whereas CD10 showed a higher percentage of expression in the intermediate count group $(1-29.9 \times 10^9/L)$. In both BCP ALL and T-ALL groups, a significantly higher percentage of CD45 expression was detected in the high count group.

3.5. EFS and OS

EFS and OS at 5 years in children with ALL according to patient variables were shown in Table 4. Low PB blast cell count group patients with BCP ALL had significantly superior 5-year EFS and OS compared to the high count group (Fig. 1). In contrast, the low PB blast cell count group with T-ALL had significantly worse EFS and OS than the other 2 groups (Fig. 2). BCP patients with either a low WBC count $(<4 \times 10^9/L)$ or an intermediate count $(4-99.9 \times 10^9/L)$ had significantly longer EFS ($P=.001$ and $P=.009$, respectively) and OS ($P=.000$ and $P=.004$, respectively) than those with a high count $(\geq 100 \times 10^9/L)$. A small number of children with a low WBC count had poor 5-year EFS and OS, but not significantly worse than the T-ALL with either intermediate or high WBC count. The 5-year pEFS and pOS were better for the BCP patients with MRD $< 0.01\%$ than for those with MRD $\geq 0.01\%$ (Table 4). In contrast, there was no significant difference in pEFS and pOS between subgroups similarly divided by the level of MRD at end of induction for T-ALL (Table 4).

By univariate analysis, the significant prognostic factors for inferior OS were WBC count $\geq 100 \times 10^9/L$, PB blast count $\geq 30 \times 10^9/L$, MRD $\geq 0.01\%$, BCR-ABL positivity, HR ALL, and poor prednisone response for BCP ALL. In T-ALL, both low PB blast cell count and HR ALL were found to predict worse OS outcomes in the univariate analysis. Multivariate Cox regression analysis showed that the initial WBC count and MRD at the end of induction were significant independent risk factors for BCP ALL, while risk stratification was found to be an independent prognostic factor for outcome in T-ALL (Table 5).

4. Discussion

Clinical features, biological and genetic factors, and early response to therapy are known to predict relapse in children with ALL. PB blast cell count on day 8 of prednisone therapy has previously been demonstrated as an independent prognostic factor in predicting treatment outcome in children with ALL.[17–19] However, little has been known about the impact on prognosis of PB blast cell count at diagnosis in childhood ALL. To our knowledge, this is the first study to reveal the relationship

Table 1

Baseline characteristics of 367 pediatric patients with B-cell precursor acute lymphoblastic leukemia and T-cell acute lymphoblastic leukemia.

Characteristics	Overall N (%)	BCP ALL N (%)	T-ALL N (%)
Age, yr (Median, range)	4 (0–14)	4 (0–14)	8 (1–14)
Sex			
Male	191 (52.0%)	170 (50.6%)	21 (67.7%)
Female	176 (48.0%)	166 (49.4%)	10 (32.3%)
Fever/infection	239 (65.1%)	224 (66.7%)	15 (48.4%)
Pallor	157 (42.8%)	150 (44.6%)	7 (22.6%)
Bleeding tendency	76 (20.7%)	66 (19.6%)	10 (32.3%)
Splenomegaly	184 (50.1%)	168 (50.0%)	16 (51.6%)
Hepatomegaly	218 (59.4%)	204 (60.7%)	14 (45.2%)
Lymphadenoathy	254 (69.2%)	230 (68.5%)	24 (77.4%)
CNS disease	6 (1.6%)	5 (1.5%)	1 (3.2%)
WBC count at diagnosis ($\times10^9$/L) (Median, range)	8.6 (0.5–579.0)	7.6 (0.5–579.0)	87.1 (1.3–575.7)
PB blasts count at diagnosis ($\times10^9$/L) (Median, range)	1.8 (0.0–549.7)	1.5 (0.0–549.7)	49.3 (0.0–449.5)
FAB			
L1	148 (40.3%)	132 (39.3%)	16 (51.6%)
L2	219 (59.7%)	204 (60.7%)	15 (48.4%)
Risk stratification			
Standard risk	172 (46.9%)	172 (51.2%)	0 (0.0%)
Intermediate risk	152 (41.4%)	130 (38.7%)	22 (71.0%)
High risk	43 (11.7%)	34 (10.1%)	9 (2.9%)
MLL-AF4	1 (0.3%)	1 (0.3%)	0 (0.0%)
TEL-AML1	64 (17.4%)	64 (19.0%)	0 (0.0%)
BCR-ABL	13 (3.5%)	13 (3.9%)	0 (0.0%)
E2A-PBX1	11 (3.0%)	11 (3.3%)	0 (0.0%)
Prednisone response			
Good	346 (94.3%)	319 (94.9%)	27 (87.1%)
Poor	21 (5.7%)	17 (5.1%)	4 (12.9%)
MRD on d 33			
<0.01%	213 (58.0%)	205 (61.0%)	8 (25.8%)
≥0.01%	154 (42.0%)	131 (39.0%)	23 (74.2%)

BCP ALL = B-cell precursor acute lymphoblastic leukemia, CNS = central nervous system, FAB = French-American-British, MRD = minimal residual disease, PB = peripheral blood, T-ALL = T-cell acute lymphoblastic leukemia, WBC = white blood cell.

Table 2

Clinical and laboratory features in children with acute lymphoblastic leukemia according to peripheral blood blast cell count at diagnosis.

Presenting features	PB blasts count at diagnosis ($\times10^9$/L)			*P*-value
	<1	1–29.9	≥30	
Age (yr)				.788
<1	12 (7.3%)	11 (7.8%)	6 (9.8%)	
1–9	127 (77.0%)	113 (80.1%)	45 (72.8%)	
≥10	26 (15.7%)	17 (12.1%)	10 (16.4%)	
Sex				.510
Male	81 (49.1%)	75 (53.2%)	35 (57.4%)	
Female	84 (50.9%)	66 (46.8%)	26 (42.6%)	
Fever/infection				.136
No	50 (30.3%)	51 (36.2%)	27 (44.3%)	
Yes	115 (69.7%)	90 (63.8%)	34 (55.7%)	
Pallor				.348
No	91 (55.2%)	79 (56.0%)	40 (65.6%)	
Yes	74 (44.8%)	62 (44.0%)	21 (34.4%)	
Bleeding tendency				.000
No	147 (89.1%)	106 (75.2%)	38 (62.3%)	
Yes	18 (10.9%)	35 (24.8%)	23 (37.7%)	
Splenomegaly				.000
No	109 (66.1%)	62 (44.0%)	12 (19.7%)	
Yes	56 (33.9%)	79 (56.0%)	49 (80.3%)	
Hepatomegaly				.000
No	87 (52.7%)	48 (34.0%)	14 (23.0%)	
Yes	78 (47.3%)	93 (66.0%)	47 (77.0%)	
Lymphadenoathy				.000
No	66 (40.0%)	38 (27.0%)	9 (14.8%)	
Yes	99 (60.0%)	103 (73.0%)	52 (85.2%)	
CNS disease				1.000
No	162 (98.2%)	139 (98.6%)	60 (98.4%)	
Yes	3 (1.8%)	2 (1.4%)	1 (1.6%)	
WBC count at diagnosis ($\times10^9$/L)				.000
<4	90 (54.5%)	3 (2.3%)	0 (0.0%)	
4–99.9	75 (45.5%)	137 (96.9%)	34 (55.7%)	
≥100	0 (0.0%)	1 (0.8%)	27 (44.3%)	
FAB				.905
L1	64 (38.8%)	58 (41.1%)	25 (41.0%)	
L2	101 (51.2%)	83 (58.9%)	36 (59.0%)	
Risk stratification				.000
Standard risk	92 (55.8%)	73 (51.8%)	7 (11.5%)	
Intermediate risk	60 (36.3%)	53 (37.6%)	39 (63.9%)	
High risk	13 (7.9%)	15 (10.6%)	15 (24.6%)	
Immunophenotype				.000
BCP ALL	160 (97.0%)	131 (92.9%)	45 (73.8%)	
T-ALL	5 (3.0%)	10 (7.1%)	16 (26.2%)	
MLL-AF4				.166
Negative	165 (100.0%)	141 (100.0%)	60 (98.4%)	
Positive	0 (0.0%)	0 (0.0%)	1 (1.6%)	
TEL-AML1				.015
Negative	137 (70.3%)	112 (79.4%)	53 (86.9%)	
Positive	58 (29.7%)	29 (20.6%)	8 (13.1%)	
BCR-ABL				.022
Negative	162 (98.2%)	137 (97.2%)	55 (90.2%)	
Positive	3 (1.8%)	4 (2.8%)	6 (9.8%)	
E2A-PBX1				.185
Negative	162 (98.2%)	137 (97.2%)	57 (93.4%)	
Positive	3 (1.8%)	4 (2.8%)	4 (6.6%)	
Prednisone response				.011
Good	159 (96.4%)	135 (95.7%)	52 (85.2%)	
Poor	6 (3.6%)	6 (4.3%)	9 (14.8%)	
MRD on d 33				.001
<0.01%	103 (62.4%)	88 (62.4%)	22 (36.1%)	
≥0.01%	62 (37.6%)	53 (37.6%)	39 (63.9%)	

BCP ALL = B-cell precursor acute lymphoblastic leukemia, CNS = central nervous system, FAB = French-American-British, MRD = minimal residual disease, PB = peripheral blood, T-ALL = T-cell acute lymphoblastic leukemia, WBC = white blood cell.

between initial PB blast cell count and clinical prognosis of children with ALL. To this end, clinical aspects, biological features, response to therapy, and prognostic factors of childhood ALL were investigated.

The current study confirmed our previous conclusion that WBC count at diagnosis was a significant independent risk factor for BCP ALL.[25] Several prior studies have reported the significant association of high WBC count with worse long-term survival in patients with BCP ALL, but a less favorable impact on prognosis in T-ALL.[2,14,15,26–28] Yanada et al studied the prognostic factors for survival of adult T-ALL with a median follow-up of 7.5 years.[16] They found that not only the low-WBC group, but also the high-WBC group showed a significantly worse OS than the intermediate-WBC group. Although our results failed to confirm that patients with a low WBC count had a significantly worse survival compared to those with intermediate or high count for T-ALL, there were only a small number of these cases in the present study.

To date, few studies have reported the relationship between PB blast cell count at diagnosis and survival in patients with ALL. Low PB blast cell count was statistically associated with a longer survival in adult patients with ALL according to the Finish

Leukemia Group.[29] However, Felice et al reported that initial peripheral blast count in pediatric ALL with a good prednisone treatment response was not correlated with treatment outcome.[30] Lauten et al evaluated 1935 children showing good response to initial prednisone treatment in the ALL-BFM 90 study.[31] Their results indicated that prednisone good-responders with <1000 blasts/μL at diagnosis showed a significantly

Table 3

Correlation of peripheral blood blast cell count at diagnosis and immunophenotypes in B-cell precursor acute lymphoblastic leukemia and T-cell acute lymphoblastic leukemia.

Antigen	PB blasts count at diagnosis ($\times 10^9$/L)			P-value
	<1	1–29.9	≥30	
BCP ALL				
CD10				.039
Positive	157 (98.1%)	121 (92.4%)	42 (93.3%)	
Negative	3 (1.9%)	10 (7.6%)	3 (6.7%)	
CD13				.085
Positive	73 (45.6%)	43 (32.8%)	18 (40.0%)	
Negative	87 (54.4%)	88 (67.2%)	27 (60.0%)	
CD19				-
Positive	160 (100.0%)	131 (100.0%)	45 (100.0%)	
Negative	0 (0.0%)	0 (0.0%)	0 (0.0%)	
CD20				.864
Positive	89 (55.6%)	72 (55.0%)	23 (51.1%)	
Negative	71 (44.4%)	59 (45.0%)	22 (45.9%)	
CD22				.628
Positive	158 (98.8%)	131 (100.0%)	45 (100.0%)	
Negative	2 (1.3%)	0 (0.0%)	0 (0.0%)	
CD33				.014
Positive	9 (5.6%)	8 (6.1%)	8 (20.0%)	
Negative	151 (94.4%)	123 (93.9%)	32 (80.0%)	
CD34				.820
Positive	123 (76.9%)	104 (79.4%)	34 (75.6%)	
Negative	37 (23.1%)	27 (20.6%)	11 (24.4%)	
CD45				.016
Positive	65 (40.6%)	64 (48.9%)	29 (64.4%)	
Negative	95 (59.4%)	67 (51.1%)	16 (35.6%)	
CD117				.106
Positive	9 (5.6%)	2 (1.5%)	0 (0.0%)	
Negative	151 (94.4%)	129 (98.5%)	45 (100.0%)	
HLA-DR				.695
Positive	158 (98.8%)	128 (97.7%)	45 (100.0%)	
Negative	2 (1.2%)	3 (2.3%)	0 (0.0%)	
cCD79a				.457
Positive	153 (95.6%)	125 (95.4%)	45 (100.0%)	
Negative	7 (4.4%)	6 (4.6%)	0 (0.0%)	
T-ALL				
CD2				.484
Positive	5 (100.0%)	9 (90.0%)	16 (100.0%)	
Negative	0 (0.0%)	1 (10.0%)	0 (0.0%)	
CD3				.549
Positive	2 (40.0%)	7 (70.0%)	10 (62.5%)	
Negative	3 (60.0%)	3 (30.0%)	6 (37.5%)	
CD5				-
Positive	5 (100.0%)	10 (100.0%)	16 (100.0%)	
Negative	0 (0.0%)	0 (0.0%)	0 (0.0%)	
CD7				-
Positive	5 (100.0%)	10 (100.0%)	16 (100.0%)	
Negative	0 (0.0%)	0 (0.0%)	0 (0.0%)	
CD10				.000
Positive	0 (0.0%)	7 (70.0%)	0 (0.0%)	
Negative	5 (100.0%)	3 (30.0%)	16 (100.0%)	
CD13				.022
Positive	2 (40.0%)	0 (0.0%)	0 (0.0%)	
Negative	3 (60.0%)	10 (100.0%)	16 (100.0%)	
CD33				.003
Positive	3 (60.0%)	1 (10.0%)	0 (0.0%)	
Negative	2 (40.0%)	9 (90.0%)	16 (100.0%)	
CD34				.199
Positive	3 (60.0%)	4 (40.0%)	3 (18.8%)	
Negative	2 (40.0%)	6 (60.0%)	13 (81.2%)	
CD45				.024
Positive	3 (60.0%)	9 (90.0%)	16 (100.0%)	
Negative	2 (40.0%)	1 (10.0%)	0 (0.0%)	
CD117				.484
Positive	0 (0.0%)	1 (10.0%)	0 (0.0%)	
Negative	5 (100.0%)	9 (90.0%)	16 (100.0%)	
HLA-DR				.213
Positive	3 (60.0%)	3 (30.0%)	3 (18.8%)	
Negative	2 (40.0%)	7 (70.0%)	13 (81.2%)	
cCD3				-
Positive	5 (100.0%)	10 (100.0%)	16 (100.0%)	
Negative	0 (0.0%)	0 (0.0%)	0 (0.0%)	

BCP ALL = B-cell precursor acute lymphoblastic leukemia, PB = peripheral blood, T-ALL = T-cell acute lymphoblastic leukemia.

Table 4

Event-free survival and overall survival at 5 years in children with B-cell precursor acute lymphoblastic leukemia and T-cell acute lymphoblastic leukemia according to patient characteristics.

Characteristics	No. patients (%)	EFS (%)	P-value	OS (%)	P-value
BCP ALL					
Total	336 (100.0%)	75.1 ± 3.5		83.6 ± 2.3	
Age (yr)			.642		.246
<1	28 (8.3%)	77.1 ± 9.7		80.8 ± 9.5	
1–9	267 (79.5%)	75.2 ± 3.6		85.0 ± 2.5	
≥10	41 (12.2%)	75.4 ± 6.8		77.4 ± 6.6	
Sex			.604		.476
Male	170 (50.6%)	74.3 ± 5.2		85.2 ± 3.1	
Female	166 (49.4%)	75.9 ± 3.7		82.1 ± 3.4	
Fever/infection			.056		.517
No	112 (33.3%)	71.8 ± 4.5		83.1 ± 3.7	
Yes	224 (66.7%)	76.9 ± 4.1		83.9 ± 2.9	
Pallor			.634		.862
No	186 (55.4%)	76.8 ± 4.2		84.8 ± 2.7	
Yes	150 (44.6%)	73.8 ± 4.3		82.4 ± 3.9	
Bleeding tendency			.298		.534
No	270 (80.4%)	73.2 ± 3.8		82.7 ± 2.7	
Yes	66 (19.6%)	82.5 ± 4.8		87.2 ± 4.2	
Splenomegaly			.200		.520
No	168 (50.0%)	77.8 ± 4.6		85.9 ± 2.8	
Yes	168 (50.0%)	72.5 ± 4.1		81.0 ± 3.8	
Hepatomegaly			.086		.244
No	132 (39.3%)	78.2 ± 5.6		87.7 ± 3.1	
Yes	204 (60.7%)	73.3 ± 3.6		80.9 ± 3.3	
Lymphadenoathy			.796		.599
No	106 (31.5%)	76.4 ± 4.7		81.5 ± 4.4	
Yes	230 (68.5%)	74.3 ± 4.3		84.7 ± 2.7	
CNS disease			.994		.745
No	331 (98.5%)	75.1 ± 3.2		83.6 ± 2.3	
Yes	5 (1.5%)	80.0 ± 17.9		80.0 ± 17.9	
WBC count at diagnosis ($\times 10^9$/L)			.006		.000
<4	91 (27.0%)	77.8 ± 8.2		92.3 ± 3.1	
4–99.9	230 (68.5%)	76.0 ± 3.3		82.5 ± 2.9	
≥100	15 (4.5%)	46.7 ± 12.9		53.3 ± 12.9	
PB blasts count at diagnosis ($\times 10^9$/L)			.017		.014
<1	160 (47.6%)	80.1 ± 4.6		89.7 ± 2.6	
1–29.9	131 (39.0%)	75.0 ± 4.5		81.2 ± 4.1	
≥30	45 (13.4%)	60.2 ± 8.0		70.3 ± 7.4	
FAB			.928		.231
L1	132 (39.3%)	81.5 ± 3.4		88.3 ± 2.8	
L2	204 (60.7%)	84.0 ± 2.6		80.4 ± 3.4	
Risk stratification			.000		.000
Standard risk	172 (51.2%)	78.0 ± 5.3		87.3 ± 3.0	
Intermediate risk	130 (38.7%)	78.5 ± 4.0		84.4 ± 3.7	
High risk	34 (10.1%)	44.2 ± 9.0		61.2 ± 9.0	
MLL-AF4			.077		.025
Negative	335 (99.7%)	75.5 ± 3.1		83.9 ± 2.3	
Positive	1 (0.3%)	0.0 ± 0.0		0.0 ± 0.0	
TEL-AML1			.018		.232
Negative	272 (81.0%)	72.0 ± 3.6		82.4 ± 2.6	
Positive	64 (19.0%)	88.9 ± 4.5		88.8 ± 4.5	
BCR-ABL			.042		.049
Negative	323 (96.1%)	75.8 ± 3.2		84.2 ± 2.3	
Positive	13 (3.9%)	61.5 ± 13.5		69.2 ± 12.8	
E2A-PBX1			.592		.654
Negative	325 (96.7%)	75.3 ± 3.2		83.7 ± 2.4	
Positive	11 (3.3%)	70.0 ± 14.5		80.0 ± 12.6	
Prednisone response			.002		.007
Good	319 (94.9%)	76.4 ± 3.2		84.7 ± 2.3	
Poor	17 (5.1%)	51.5 ± 12.5		62.7 ± 12.3	
MRD on d 33			.000		.000
<0.01%	205 (61.0%)	83.8 ± 3.7		90.8 ± 2.3	
≥0.01%	131 (39.0%)	61.4 ± 5.0		71.4 ± 4.8	
T-ALL					
Total	31 (100.0%)	79.5 ± 7.5		78.8 ± 7.7	
Age (yr)			.335		.346
<1	1 (3.2%)	100.0 ± 0.0		100.0 ± 0.0	
1–9	18 (58.1%)	88.9 ± 7.4		88.2 ± 7.8	
≥10	12 (38.7%)	64.2 ± 14.4		63.6 ± 14.5	
Sex			.392		.604
Male	21 (67.7%)	74.5 ± 9.9		73.7 ± 10.1	
Female	10 (32.3%)	90.0 ± 9.5		90.0 ± 9.5	

Table 4

(continued).

Characteristics	No. patients (%)	EFS (%)	P-value	OS (%)	P-value
Fever/infection			.409		.440
No	16 (51.6%)	74.5±11.0		73.3±11.4	
Yes	15 (48.4%)	84.6±10.0		84.6±10.0	
Pallor			.388		.409
No	24 (77.4%)	82.1±8.1		81.8±8.2	
Yes	7 (22.6%)	71.4±17.1		68.6±18.6	
Bleeding tendency			.888		.873
No	21 (67.7%)	80.4±8.8		80.2±8.9	
Yes	10 (32.3%)	77.1±14.4		75.0±15.3	
Splenomegaly			.261		.256
No	15 (48.4%)	72.0±12.0		69.6±12.7	
Yes	16 (51.6%)	86.7±8.8		86.7±8.8	
Hepatomegaly			.455		.456
No	17 (54.8%)	75.5±10.7		73.7±11.3	
Yes	14 (45.2%)	84.6±10.0		84.6±10.0	
Lymphadenoathy			.164		.175
No	7 (22.6%)	100.0±0.0		100.0±0.0	
Yes	24 (77.4%)	73.7±9.3		73.0±9.4	
CNS disease			.626		.622
No	30 (96.8%)	78.8±7.7		78.0±7.9	
Yes	1 (3.2%)	100.0±0.0		100.0±0.0	
WBC count at diagnosis (×10^9/L)			.438		.347
<4	2 (6.5%)	50.0±35.4		50.0±35.4	
4–99.9	16 (51.6%)	80.8±10.0		80.0±10.3	
≥100	13 (41.9%)	81.8±11.6		81.8±11.6	
PB blasts count at diagnosis (×10^9/L)			.028		.021
<1	5 (16.1%)	40.0±21.9		40.0±21.9	
1–29.9	10 (32.3%)	90.0±9.5		90.0±9.5	
≥30	16 (51.6%)	84.6±10.0		84.6±10.0	
FAB			.913		.812
L1	16 (51.6%)	80.8±10.0		80.0±10.3	
L2	15 (48.4%)	77.8±11.4		77.4±11.5	
Risk stratification			.008		.005
Standard risk					
Intermediate risk	22 (71.0%)	90.7±6.3		90.7±6.3	
High risk	9 (29.0%)	46.7±19.0		42.9±18.7	
Prednisone response			.388		.459
Good	27 (87.1%)	77.3±8.2		77.0±8.2	
Poor	4 (12.9%)	100.0±0.0		100.0±0.0	
MRD on d 33			.105		.099
<0.01%	8 (25.8%)	100.0±0.0		100.0±0.0	
≥0.01%	23 (74.2%)	71.6±9.9		70.3±10.2	

BCP ALL = B-cell precursor acute lymphoblastic leukemia, CNS = central nervous system, EFS = event-free survival, FAB = French-American-British, MRD = minimal residual disease, OS = overall survival, PB = peripheral blood, T-ALL = T-cell acute lymphoblastic leukemia, WBC = white blood cell.

better outcome than those with ≥1000 blasts/μL. However, further data analysis showed no significant differences between these 2 groups when compared within the stratified risk groups. Whereas in our study, BCP patients in the low PB blast cell count group had better survival than those in the high count group, and survival of T-ALL patients with a low PB blast cell count was inferior. However, after adjusting for potential risk factors by multivariate analysis, PB blast cell count was not an independent predictor for survival.

Donadieu et al found that PB blast cell count correlated significantly with WBC count in childhood ALL,[32] in accordance with our study. To further investigate the relationship between PB blast cell count and WBC count at diagnosis, we found that PB blast cell count correlated strongly with WBC count in the high PB blast cell count group, but not with the low count group in both BCP and T-ALL.

The tyrosine phosphatase CD45 is encoded by the PTPRC (protein-tyrosine phosphatase, receptor-type, C) gene and is selectively expressed on the surface of all nucleated hematopoietic cells. It is important for regulating antigen-receptor signaling in B and T cells by dephosphorylation of Src kinases and suppressing JAK kinases that negatively regulate cytokine receptor signaling.[33–36] Previous studies have shown that a bright CD45 expression on leukemic blasts was not only associated with an inferior outcome in BCP ALL, but also with worse prognosis in T-ALL.[37–39] This is in concordance with our observation of a significantly higher percentage of CD45 expression in the high PB blast cell count group for BCP ALL. However, on the basis of CD45 expression, it was difficult to explain the inferior survival in T-ALL patients with low blast cell count. In addition, the clinical significance of myeloid antigen expression in childhood ALL has remained controversial.[40–45] For example, Uckun et al reported that children with myeloid antigen positive (My⁺) ALL have similar treatment outcomes as My⁻ ALL patients in both BCP ALL and T-ALL.[44] Wiersma et al reported that myeloid antigen (CD13, CD33, and CD44) expression was detected in 45 of 185 children with BCP ALL and 8 of 41 patients with T-ALL.[45] However, they found that a poor response to chemotherapy was associated with myeloid antigen expression in childhood ALL. Similarly, in the current study, patients with
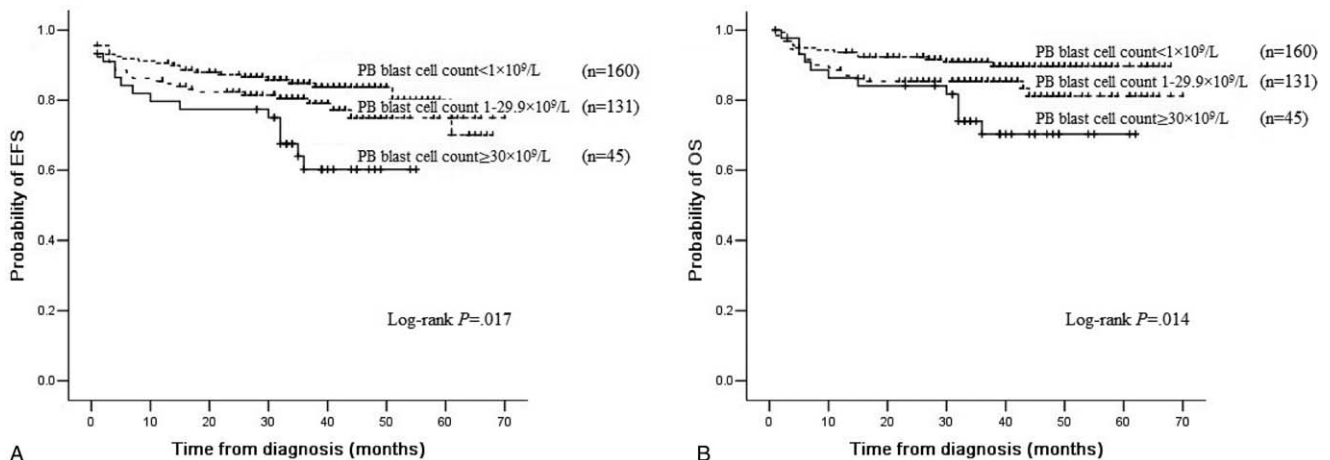

Figure 1. Kaplan–Meyer estimates of event-free survival (A) and overall survival (B) of children with B-cell precursor acute lymphoblastic leukemia according to peripheral blood blast count.
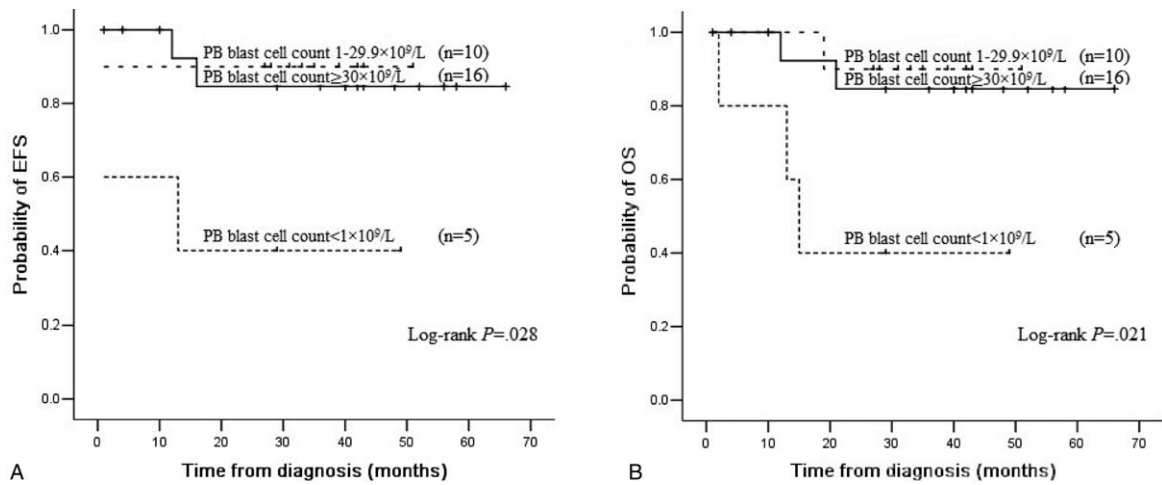
Figure 2. Kaplan–Meyer estimates of event-free survival (A) and overall survival (B) of children with T-cell acute lymphoblastic leukemia according to peripheral blood blast count.

Table 5

Multivariate analysis of prognostic factors of B-cell precursor acute lymphoblastic leukemia and T-cell acute lymphoblastic leukemia.

	Multivariate Analysis		
	Hazard ratio	95% CI	P-value
BCP ALL			
WBC count at diagnosis ($\times 10^9$/L)			
<4	1.000		
4–99.9	2.368	.996–5.631	.051
≥100	5.726	1.912–17.144	.002
PB blasts count at diagnosis ($\times 10^9$/L)			
<1	1.000		
1–29.9	1.757	.906–3.408	.510
≥30	2.949	1.380–6.301	.852
Risk stratification			
Standard risk	1.000		
Intermediate risk	1.140	.592–2.193	.093
High risk	3.921	1.901–8.086	.022
BCR-ABL			
Negative	1.000		
Positive	2.678	.961–7.459	.724
Prednisone response			
Good	1.000		
Poor	3.067	1.303–7.218	.216
MRD on d 33			
<0.01%	1.000		
≥0.01%	2.933	1.611–5.338	.000
T-ALL			
PB blasts count at diagnosis ($\times 10^9$/L)			
<1	1.000		
1–29.9	0.131	.012–1.456	.622
≥30	0.293	.049–1.763	.803
Risk stratification			
Standard risk			
Intermediate risk	1.000		
High risk	7.912	1.421–44.042	.018

BCP ALL = B-cell precursor acute lymphoblastic leukemia, CI = confidence interval, MRD = minimal residual disease, PB = peripheral blood, T-ALL = T-cell acute lymphoblastic leukemia, WBC = white blood cell.

BCP ALL in the high PB blast cell count group showed a higher frequency of CD33 expression than patients in the other 2 groups, and an inferior survival. In T-ALL, patients with low blast cell count had a higher percentage of myeloid antigens (CD13 and CD33) than those with intermediate and high count, and a significantly shorter survival. Our results, therefore, were consistent with the previous studies that myeloid antigen expression was correlated with poor outcome for BCP ALL and T-ALL.[40,41,45]

Assessment of MRD, commonly measured by flow cytometry or polymerase chain reaction analysis, has replaced conventional morphologic assessment in risk stratification.[46–49] Many previous studies have provided solid basis that the level of MRD at the completion of induction is a strong prognostic factor for the risk of relapse in BCP ALL,[14,50,51] yet there are few studies focused on MRD in T-ALL.[52–54] Parekh et al found that 32 of 33 T-ALL patients remained in continuous CR at a median follow up of 4 years despite more than 50% of patients who were MRD positive at the end of induction.[52] They concluded that MRD status at the end of induction was not strongly predictive of treatment outcome in childhood T-ALL patients, which concurs with our study. Because slower clearance of leukemic blast cells was found in T-ALL compared to BCP patients, MRD detection at a later time point (day 78 or week 12) for T-ALL patients would be more appropriate to determine risk stratification than an early evaluation.[53,54]

There were several limitations in this study. First, the age of patients was confined to 0 to 14 years because our hospital plays an important role in medical services for women and children in Southwest China. Patients older than 14 years may not be able to complete the entire chemotherapy regimen as intended. Second, the small number of patients with T-ALL limited statistical determination of factors predictive of relapse. Further large-scale multicenter studies are needed to confirm the findings in the present study.

In conclusion, the results of this study suggested that lower PB blast cell count is significantly associated with better long-term survival in children with BCP ALL, whereas it was negatively correlated with clinical outcome in T-ALL patients. PB blast cell

count is a straightforward and readily accessible test that may provide prognostic information although it was not identified as a significant independent predictor by multivariate analysis. Ideally, the combination of classical and novel parameters as prognostic factors should further improve the outcome of childhood ALL.

Acknowledgments

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