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Combined use of peripheral blood blast count and platelet count during and after induction therapy to predict prognosis in children with acute lymphoblastic leukemia

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Abstract

Several studies have reported an association between the rapidity of reduction in peripheral blood blast count or recovery of normal hematopoiesis and treatment outcome during therapy in children with acute lymphoblastic leukemia (ALL). However, little is known about the impact of both of these aspects on prognosis in pediatric ALL. Accordingly, the purpose of this study was to evaluate whether the combined use of blood blast count and platelet count could predict event-free survival (EFS) and overall survival (OS) when minimal residual disease (MRD) detection was not available.

A total of 419 patients aged 0 to 14 years diagnosed and treated for ALL between 2011 and 2015 were enrolled.

Patients with a blast count $\geq 0.1 \times 10^9$/L on day 8 exhibited significantly lower survival rates than that in those with blast counts $<0.1 \times 10^9$/L. The EFS and OS in patients with platelet count $\geq 100 \times 10^9$/L on day 33 were significantly higher than those with platelet counts $<100 \times 10^9$/L. In univariate and multivariate analyses, patients with low blast count on day 8 and high platelet count on day 33 were significantly associated with better EFS and OS. The combination of blast cell count on day 8 and platelet count on day 33 demonstrated a strong association with MRD-based risk stratification.

Complete blood count is an inexpensive, easy to perform, and reliable measurement in children with ALL. The combination of blast count and platelet count during and after induction chemotherapy was a significant and independent prognostic factor for treatment outcome in pediatric ALL.

Abbreviations: ALC = absolute lymphocyte count, ALL = acute lymphoblastic leukemia, ANC = absolute neutrophil count, Ap = average daily platelet amount increase, BFM = Berlin–Frankfurt–Münster, CCLG = Chinese Childhood Leukemia Group, CNS = central nervous system, CR = complete remission, DFS = disease-free survival, EFS = event-free survival, FAB = French-American-British, HR = high-risk, IR = intermediate-risk, MRD = minimal residual disease, OS = overall survival, SR = standard-risk, WBC = white blood cell.

Keywords: acute lymphoblastic leukaemia, children, complete blood count, minimal residual disease, prognostic factor

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common form of childhood malignancy and accounts for nearly 20% of all pediatric cancers.[1] The current survival rate for pediatric ALL has improved dramatically (>90%) in recent decades, primarily due to multiagent chemotherapy regimens and risk-adapted therapy.[1–3] However, approximately 20% of children with ALL will ultimately experience relapse,[4–6] and the outcome of relapse remain unsatisfactory.[7]

Clinical, biological, genetic, and response-based features, such as age, sex, white blood cell (WBC) count, immunophenotypic, and cytogenetic and molecular characteristics, have been reported to predict a high likelihood of relapse in children with ALL.[2–9] According to these features, risk stratification has been recommended to select more effective therapeutic regimens. Therefore, current protocols have greatly improved outcomes of children with high-risk ALL and slow response to chemotherapy using intensive therapy and mitigated the occurrence of chemotherapy-induced side effects in low-risk patients.[10,11]

Early response to treatment measured according to minimal residual disease (MRD) is currently the single most powerful prognostic factor in childhood ALL.[12–15] Furthermore, the MRD level at the end of induction is a strong predictive factor of relapse in pediatric ALL.[11,16,17] However, MRD detection is

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QD and RS contributed equally to this article.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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expensive time-consuming and requires more invasive bone marrow aspiration and highly trained technicians to conduct the test and interpret results. The majority of children with ALL who live in developing and resource-poor countries may not have access to MRD detection, which leads to lower survival rates compared with those in developed countries.[3]

Prednisone response, characterized by peripheral blood blast count, is a significant prognostic factor in children with ALL and is based on the Berlin–Frankfurt–Münster (BFM) group protocol. A blast count $<1000/\mu L$ in peripheral blood after 7 days' treatment with prednisone and 1 intrathecal dose of methotrexate has been associated with significantly better survival outcome.[18–20] In addition to considering the rapidity of reduction in peripheral blood blast levels as a predictor of treatment response, hematopoietic recovery during and after induction treatment is a significant prognostic factor for overall survival (OS) in pediatric ALL. Time to platelet recovery or platelet count after induction therapy was significantly associated with treatment outcome in acute leukemia.[21–27] Faderl et al[23] reported that time to platelet recovery (platelet count recovery to $>100 \times 10^9$/L) in 249 patients with ALL who achieved complete remission (CR) at the end of induction and was significantly associated with disease-free survival (DFS) and OS. Recent studies have also demonstrated that higher absolute lymphocyte count (ALC) is associated with improved long-term survival in children with ALL.[28–33] Rabin et al[33] found that ALL patients with ALC $>1.5 \times 10^9$/L experienced significantly better 6-year relapse-free survival and OS than those with ALC $<1.5 \times 10^9$/L.

Aside from identifying the disappearance of peripheral blood blasts during the early induction, assessment of normal hematopoietic clone recovery should also be considered to evaluate early response therapy. Nevertheless, very few studies have addressed the impact of both of these aspects on survival in patients with ALL.[33,34] As such, the purpose of this study was to assess these prognostic factors and evaluate treatment outcomes of children with ALL when they could not be monitored with MRD testing during and after chemotherapy.

2. Materials and methods

2.1. Patients

Children diagnosed with ALL between May 2011 and December 2015 at the West China Second University Hospital of Sichuan University (Sichuan, China) were enrolled. The diagnosis of ALL was based on morphological evaluation of bone marrow smears according to criteria from the French-American-British (FAB) classification[35] and immunophenotyping. The observed clinical characteristics included age, sex, fever or infection, pallor, bleeding tendency, splenomegaly, hepatomegaly, lymphadenopathy, infiltration of the central nervous system (CNS), complete blood count, FAB morphology, chromosome karyotype, fusion gene, risk stratification, prednisone response, and MRD. This study was conducted according to the Declaration of Helsinki and approved by the Ethical Review Board of Investigation in Human Beings of West China Second University Hospital. Due to the retrospective nature of the study and the use of anonymized patient data, requirements for written informed consent were waived.

2.2. Laboratory tests

Peripheral venous blood samples from the patients were collected in tubes containing potassium-EDTA as anticoagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Complete blood count was performed using an automated hematology analyzer (XE-2100, Sysmex, Kobe, Japan). Absolute neutrophil count (ANC), ALC, and blast count were calculated from differential blood cell count percentages and total WBC count on treatment days 8 and 33. A 200-cell manual WBC differential count was performed both by 2 trained technicians. All the blood films were reviewed by a supervisor or a hematopathologist before the final results were reported.

Bone marrow aspirates were collected in heparin anticoagulant tubes and sent to the laboratory for immunophenotype detection at diagnosis and MRD analysis on day 33. The staining procedure and protocol for immunophenotyping and MRD detection by flow cytometry have been described in previous studies.[36–38] Data were acquired using a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA) within 24 hours of sample collection and analyzed using BD Cell-Quest Pro software (Becton Dickinson, San Jose, CA). Cells were gated based on CD45 fluorescence intensity and side scatter. Antigen positivity was defined as $\geq$20% of blasts staining positive among total leukemic cells.

Cytogenetic studies were performed on bone marrow samples using G-banding staining techniques. Bone marrow mononuclear cells were isolated from bone marrow aspirate using density gradient centrifugation with Ficoll-Paque Premium (GE Healthcare, Madison, WI). Total RNA was extracted using a commercially available reagent kit according to the manufacturer's instructions (Trizol, Invitrogen Life Technologies, Carlsbad, CA). *BCR-ABL*, *TEL-AML1*, *E2A-PBX1*, and *MLL-AF4* fusion genes were detected by reverse transcriptase-polymerase chain reaction.

2.3. Risk stratification and treatment

According to the Chinese Children's Leukemia Group-acute lymphoblastic leukemia 2008 (CCLG-ALL 2008) protocol, patients were classified into standard risk (SR), intermediate risk (IR), and high risk (HR) groups based on age, WBC count, immunophenotype, cytogenetic features, early response treatment, and MRD measurement on day 33. All of these patients were enrolled and treated in accordance with the CCLG-ALL 2008 protocol.[36,38–40]

2.4. Definition

Complete remission (CR) was defined as the presence of $<5\%$ blasts in the bone marrow and absence of extramedullary leukemia. Relapse was defined by the reappearance of $\geq$20% blasts in bone marrow or local leukemia infiltration sites after CR. CNS involvement was defined as clinical manifestation with neurological signs and symptoms or an elevated WBC count ($\geq 5 \times 10^6$/L) in cerebrospinal fluid and lymphoblasts identified on a cytocentrifuge slide or on confirmation on imaging.

2.5. Statistical analysis

All data were collected and analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL). Categorical outcome variables were compared using the Chi-squared test or Fisher exact test, where appropriate. The optimal cut-off value for blast count on day 8 was analyzed and calculated using X-tile 3.6.1 software (Yale University, New Haven, CT).[41] Based on results from the X-tile

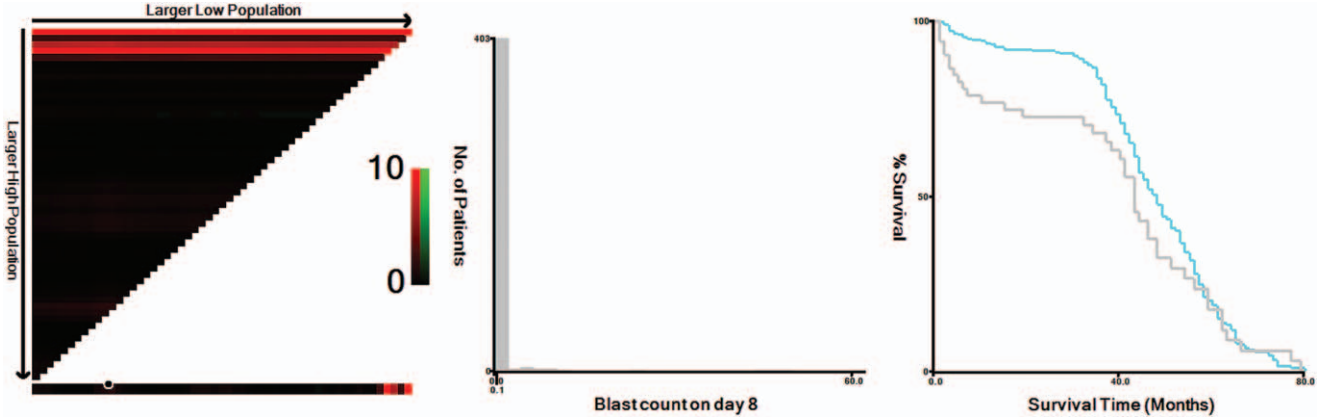

Figure 1. X-tile (Yale University, New Haven, CT) analyses of survival data of patients with acute lymphoblastic leukemia (ALL). X-tile plots (left) were randomly divided into 2 groups according to blast count. The *x*-axis of the X-tile plots represented all cut-off values from low to high (left–right), while the *y*-axis represented cut-off values from high to low (top–bottom). Red was associated with adverse survival, while green indicated direct associations. The optimal cut-off values, indicated by black/white circle (left), are shown on a histogram of the cohort (middle) and a Kaplan–Meier plot (right). The optimal cut-off value for blast count on day 8 was 0.1×10^9/L.

software, the optimal cut-off value in terms of OS was 0.1×10^9/L for blast count on day 8 (Fig. 1). The duration of event-free survival (EFS) was defined as the time from the day of diagnosis to the first negative event (failure to induce remission, relapse, or death from any cause) or to the last follow-up date. OS was determined from the diagnosis of ALL to the date of death or last follow-up. EFS and OS survival rates were estimated using the Kaplan–Meier method and comparison of survival curves assessed using the log-rank test. Potential prognostic factors were considered in a Cox proportional hazards regression model in univariate and multivariate analyses. Differences with $P < .05$ were considered to be statistically significant.

3. Results

A total of 419 pediatric patients 0 to 14 years of age were retrospectively included in this study. During a median follow-up of 41 months (range, 0–80 months), EFS and OS at 3 years for all patients were $82.2 \pm 1.9\%$ and $87.3 \pm 1.7\%$, respectively. The median age for children with ALL was 4 years (range, 3–7 years). Baseline characteristics of the patients are summarized in Table 1.

While ANC on day 33 did not exhibit a significant association with outcome, high ANC ($\geq 1.5 \times 10^9$/L) on day 8 was significantly associated with better OS ($P = .034$). Patients with high ALC on day 8 ($\geq 1.5 \times 10^9$/L) experienced poor EFS and OS, which was significantly worse than those with low ALC ($< 1.5 \times 10^9$/L) ($P = .013$ and $P = .040$, respectively). However, ALC on day 33 was not significantly associated with EFS or OS ($P = .691$ and $P = .874$, respectively). With regard to platelet count on day 8, platelet count $\geq 100 \times 10^9$/L was associated with better EFS ($P = .018$), whereas there was no significant difference in OS between subgroups divided according to platelet count ($P = .107$). EFS and OS on day 33 in patients with platelet counts $\geq 100 \times 10^9$/L were significantly higher than those with platelet count $< 100 \times 10^9$/L ($P = .001$ and $P = .002$, respectively). Patients with a blast count $\geq 0.1 \times 10^9$/L on day 8 had worse OS compared with those with blast count $< 0.1 \times 10^9$/L ($P = .000$).

We speculated that MRD testing was not available to all patients in the cohort and evaluated potential risk factors (excluding MRD and MRD-based risk stratification) that would

Table 1

Baseline characteristics of patients with acute lymphoblastic leukemia.

Characteristics	N (%)
Age, y	
<1	29 (6.9)
1–10	331 (79.0)
≥10	59 (14.1)
Sex	
Male	219 (52.3)
Female	200 (47.7)
Fever/infection	267 (63.7)
Pallor	178 (42.5)
Bleeding tendency	88 (21.0)
Splenomegaly	207 (49.4)
Hepatomegaly	245 (58.5)
Lymphadenoathy	288 (68.7)
CNS disease	6 (1.4)
FAB	
L1	166 (39.6)
L2	253 (60.4)
Karyotype	
Favorable	48 (11.5)
Intermediate	355 (84.7)
Adverse	16 (3.8)
Fusion gene	
MLL-AF4	1 (0.3)
TEL-AML1	71 (18.4)
BCR-ABL	17 (4.4)
E2A-PBX1	14 (3.6)
Risk stratification	
Standard risk	205 (48.9)
Intermediate risk	171 (40.8)
High risk	43 (10.3)
Prednisone response	
Good	398 (95.0)
Poor	21 (5.0)
MRD on day 33	
<0.01%	245 (58.8)
≥0.01%	174 (41.5)

CNS = central nervous system, FAB = French-American-British, MRD = minimal residual disease, WBC = white blood cell.

Table 2

Univariate and multivariate analysis of prognostic factors and risk of relapse in the MRD-tested cohort.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Risk stratification						
Standard risk	1.000			1.000		
Intermediate risk	1.129	0.602–2.115	.706	0.699	0.358–1.366	.295
High risk	5.537	2.924–10.486	.000	2.672	1.248–5.717	.011
WBC count at diagnosis ($\times 10^9$/L)						
<50	1.000					
≥50	1.926	1.037–3.576	.038			
ANC on day 8 ($\times 10^9$/L)						
<1.5	1.000					
≥1.5	1.923	1.036–3.571	.038			
ALC on day 8 ($\times 10^9$/L)						
<1.5	1.000					
≥1.5	1.710	1.016–2876	.043			
Blast count on day 8 ($\times 10^9$/L)						
<0.1	1.000					
≥0.1	3.412	1.797–5.493	.000			
Platelet count on day 33 ($\times 10^9$/L)						
<100	1.000					
≥100	0.403	0.219–0.739	.003			
Blast on day 8 and platelet on day 33						
$Blast^{lo}$ $Platelet^{hi}$	1.000			1.000		
$Blast^{hi}$ $Platelet^{hi}$	2.396	1.175–4.887	.016	1.268	0.573–2.805	.559
$Blast^{lo}$ $Platelet^{lo}$	1.783	0.785–4.048	.167	1.879	0.812–4.346	.141
$Blast^{hi}$ $Platelet^{lo}$	7.733	3.401–17.583	.000	4.579	1.914–10.959	.001
MRD on day 33						
<0.01%	1.000			1.000		
≥0.01%	3.039	1.751–5.272	.000	2.752	1.505–5.032	.001

ALC = absolute lymphocyte count, ANC = absolute neutrophil count, $Blast^{hi}$ = blast cell count $\geq 0.1 \times 10^9$/L, $Blast^{lo}$ = blast cell count $< 0.1 \times 10^9$/L, CI = confidence interval, HR = hazard ratio, MRD = minimal residual disease, $Platelet^{hi}$ = platelet count $\geq 100 \times 10^9$/L, $Platelet^{lo}$ = platelet count $< 100 \times 10^9$/L, WBC = white blood cell.

affect the long-term prognosis of ALL patients. Therefore, in the univariate analysis, WBC count at diagnosis, ANC, ALC and blast count on day 8, platelet count on day 33, and prednisone response strongly affected OS in ALL ($P < .05$). Multivariate Cox regression analysis revealed that blast count on day 8 and platelet count on day 33 were significant independent risk factors ($P = .000$ and $P = .009$, respectively). Initial WBC count, ANC, and ALC on day 8 were not significantly associated with survival prognosis after adjustment for the other clinical features.

According to findings, all patients were divided into 4 groups based on the combined results of blast count on day 8 and platelet count on day 33 (Table 2). Patients with low blast count on day 8 and high platelet count on day 33 demonstrated a more favorable prognosis compared with that in patients with high blast and low platelet counts (Fig. 2). Univariate and multivariate analyses were performed in the MRD-tested cohort (Table 2). In the univariate analysis, risk factors for shorter OS were WBC $\geq 50 \times 10^9$/L at diagnosis, ANC $\geq 1.5 \times 10^9$/L on day 8, and blast count $\geq 0.1 \times 10^9$/L on day 8. ALC on day 8, platelet count on day 33, combination of blast count on day 8 and platelet count on day 33, risk stratification, prednisone response, and MRD level strongly affected EFS and OS ($P < .05$). In the multivariate Cox regression model, combination of blast count on day 8 and platelet count on day 33, risk stratification, and MRD level were independently associated with treatment outcome ($P = .019$, $P = .002$, and $P = .001$, respectively) (Table 2). The remaining variables included in the analysis were not found to be independent risk factors.

The combination of blast count on day 8 and platelet count on day 33 demonstrated a strong association with MRD-based risk stratification ($P = .000$) (Table 3). Eighty-two percent of MRD SR patients had both low blast count on day 8 and high platelet count on day 33, while it was the case for 75% of IR patients and 33% of HR patients.

4. Discussion

Clinical, biological, and genetic features and early response to therapy have been used to predict the likelihood of relapse in children with ALL.[2–9] Many studies confirmed that reduction in peripheral blood blast count or recovery of normal hematopoiesis has become the most important predictor of the outcome of pediatric ALL.[21–26,31,32] However, very few published studies have addressed the impact of both of these aspects on prognosis in childhood ALL.[33,34] Results of the present study indicated that the combination of blast count on day 8 and platelet count on day 33 was an independent prognostic factor in predicting treatment outcome in children with ALL.

It is well known that early response to initial prednisone treatment is an important predictor of treatment outcome and an essential factor for stratifying patients with ALL into different risk groups. After a 7-day exposure to prednisone and 1 intrathecal dose of methotrexate, a good response to prednisone was defined as peripheral blast count <1000/μL and a poor response to prednisone as a blast count ≥1000/μL. The BFM study group indicated that prednisone response was one of the

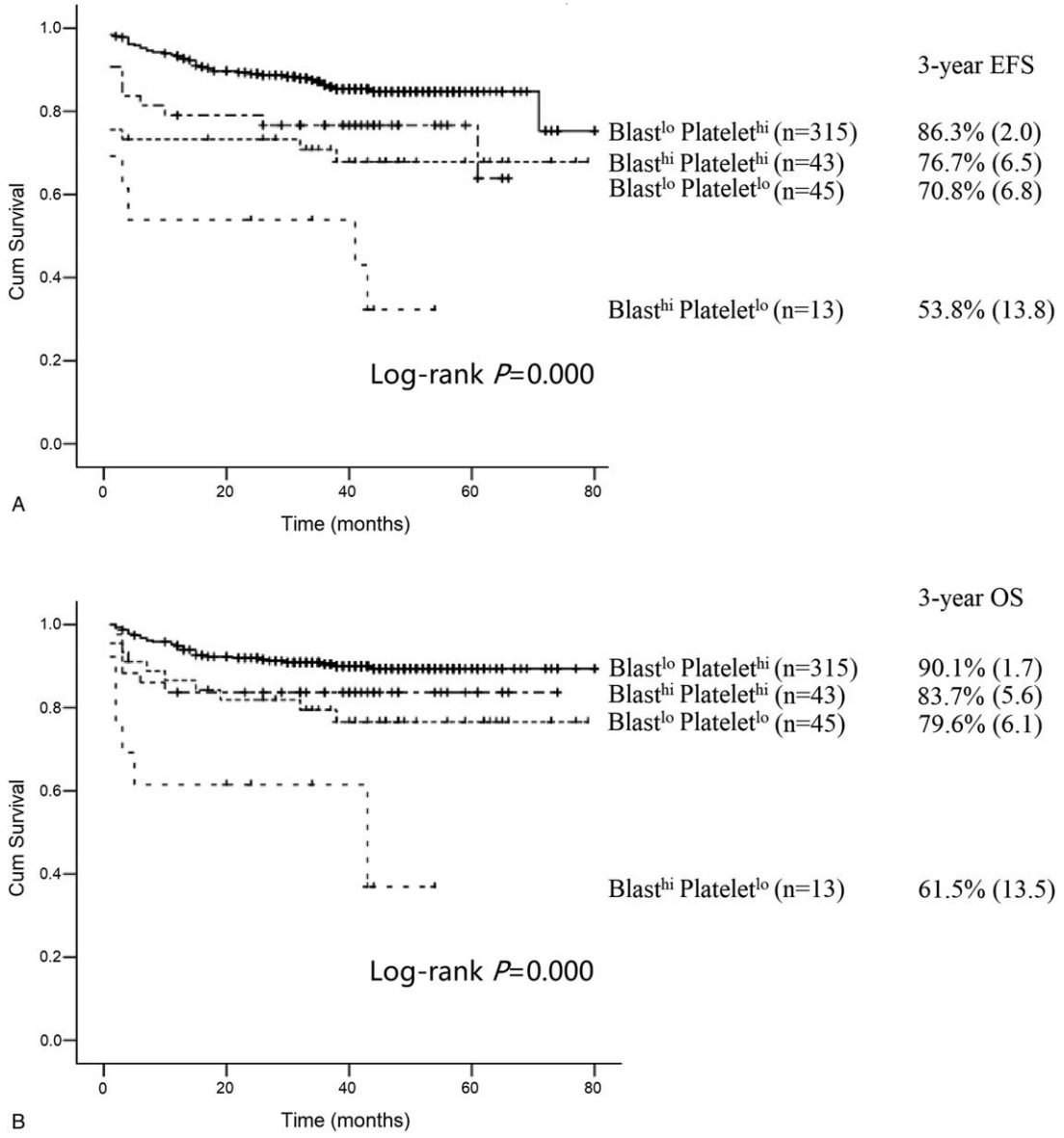

Figure 2. Kaplan–Meier estimates of event-free survival (A) and overall survival (B) of children with acute lymphoblastic leukemia according to blast count on day 8 and platelet count on day 33. Blast^{hi} = blast cell count $\geq 0.1 \times 10^9$/L; Blast^{lo} = blast cell count $< 0.1 \times 10^9$/L; Platelet^{hi} = platelet count $\geq 100 \times 10^9$/L; Platelet^{lo} = platelet count $< 100 \times 10^9$/L.

strongest predictors of relapse and survival.[42–44] Gajjar et al[45] investigated the significance of early peripheral blast cell clearance after 1week of chemotherapy among children with ALL. They found that 14% of the patients with persistent circulating leukemic blasts on day 8 exhibited a significantly higher frequency of adverse clinical features and shorter 5-year EFS. In the current study, prednisone response was significantly associated with EFS and OS in univariate analysis but was not an independent prognostic indicator in multivariate analysis. In addition, it has been reported that X-tile software could be used

Table 3

The relationship of blast count on day 8 and platelet count on day 33 and MRD-based risk stratification.

	MRD SR N (%)	MRD IR N (%)	MRD HR N (%)	*P*
Blast^{lo} Platelet^{hi}	172 (54.6)	129 (41.0)	14 (4.4)	.000
Blast^{hi} Platelet^{lo}, Blast^{lo} Platelet^{lo}, and Blast^{hi} Platelet^{hi}	32 (31.7)	41 (40.6)	28 (27.7)	

Blast^{hi} = blast cell count $\geq 0.1 \times 10^9$/L, Blast^{lo} = blast cell count $< 0.1 \times 10^9$/L, HR = high risk, IR = intermediate risk, MRD = minimal residual disease, Platelet^{hi} = platelet count $\geq 100 \times 10^9$/L, Platelet^{lo} = platelet count $< 100 \times 10^9$/L, SR = standard risk.

to calculate the cut-off points by using the minimum *P* values from the log-rank chi-squared test.[41] Based on the results from X-tile software, the optimal cut-off point was 0.1×10^9/L for blast count on day 8. Furthermore, our results indicated that blast count $\geq 0.1 \times 10^9$/L on day 8 was significantly and independently associated with inferior EFS and OS in patients with ALL.

Platelet count on treatment day 33 has been used as a marker of hematopoietic recovery and is strongly associated with treatment outcome. Faderl et al[23] reported that time to platelet recovery was found to be significantly correlated with DFS and OS in patients with ALL. Zeidler et al[26] analyzed the prognostic significance of normal blood cells during the early treatment phases of pediatric ALL. They found that platelet count at the end of induction therapy was significantly associated with survival and MRD risk group distribution. In our previous study, we evaluated the prognostic value of average daily platelet amount increase (Ap) during the recovery period in children with ALL.[24] Multivariate analysis demonstrated that Ap $>3.9 \times 10^9$/L was independently associated with a superior outcome. It was also found that there was a strong correlation between Ap and MRD on day 33. Although Ap, which is an easy and accessible test, could be used as a valuable prognostic indicator for patients with ALL, time to platelet recovery may be prolonged in some cases until platelet recovery reached $\geq 100 \times 10^9$/L before the prognosis was predicted.

MRD assessment, which is commonly measured using flow cytometry or polymerase chain reaction analysis has replaced conventional morphological assessment in risk stratification.[12–15] Our results indicated that MRD at the end of induction was one of the independent prognostic factors for determining the risk for relapse in ALL, which was in accordance with previous studies.[12–15] However, not all patients were able to undergo MRD testing due to the cost and complexity, especially in developing and resource-poor countries. In addition, the main drawback of MRD detection was based on molecular rather than functional markers of the remaining leukemic cells. Normal hematopoiesis during and after induction treatment can represent the host immunity and overcome the residual leukemic cell to prevent relapse. Both of these aspects, opposite to one another, should be considered as a whole. In an attempt to address this issue, we found an optimal solution to evaluate the early response to chemotherapy in patients with ALL. The main limitation of our study was the short-term follow-up. However, a longer follow-up period will be required to confirm the current findings.

Based on data from the current study, the combination of low blast count on day 8 and high platelet count on day 33 was found to be independently associated with a superior outcome in patients with ALL. A strong correlation between the combination of blast and platelet and MRD-based risk stratification was also found. Therefore, the combined use of blast and platelet counts during and after induction treatment for childhood ALL was a strong candidate prognostic factor for the improvement of risk stratification when MRD was not available.

Author contributions

Investigation: Hui Yang, Yuefang Wang, Lei Ye, Luyun Peng, Siqi Guo.
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Supervision: Ge Zhang.
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Writing – original draft: Qingkai Dai, Rui Shi.
Writing – review & editing: Yongmei Jiang.

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