

Diagnostic Lymphocytosis as a Favorable Prognostic Factor in Childhood Acute Myeloblastic Leukemia

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Abstract

Objective: Childhood acute myeloblastic leukemia (AML) has been associated with an unfavorable prognosis, particularly in lower-middle-income countries (LMICs). The limited resources for cytogenetic-based risk stratification in many LMICs constitute the contributing factors. To improve the outcomes of childhood AML, a better understanding of its pathogenesis, which further leads to the development of applicable risk stratification criteria, is required. Studies on adult AML linked lymphocytosis due to increased regulatory T-cells (Tregs) in the bone marrow stroma with a higher risk of remission failure and an early relapse. Since Treg population is influenced by age-dependent bacterial colonization, conducting a study on children with AML is reasonable. **Methods:** A retrospective cohort study involved 60 patients younger than 18 years with non-M3 AML, 25 of whom had diagnostic absolute lymphocyte counts (ALC₀) of less than 4.7×10^9 cells/L and 35 of whom had ALC₀ of more than 4.7×10^9 cells/L. These patients were observed for the occurrence of events, which consisted of remission failure, relapse, and death, within five years of treatment. **Results:** Patients with ALC₀ of more than 4.7×10^9 cells/L had the higher five-year event-free (EFS, 24% vs. 0%; $p = 0.01$) and overall survivals (OS, 31% vs. 10%; $p = 0.02$). Cox regression analysis demonstrated that ALC₀ of less than 4.7×10^9 cells/L was an independent prognostic factor for the lower five-year EFS (hazard ratio [HR], 3.5; 95% confidence interval [95% CI], 1.7 – 7.5; $p < 0.01$) and OS (HR, 2.1; 95% CI, 1.1 – 4.0; $p = 0.03$). **Conclusion:** In contrast to studies in adults, our study showed a correlation between diagnostic lymphocytosis and higher five-year EFS and OS.

Keywords: Acute myeloblastic leukemia- Children- Absolute lymphocyte counts- Event-free survival- Overall survival

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Introduction

Acute myeloblastic leukemia (AML) is the second most prevalent childhood hematopoietic neoplasm throughout the world. Several studies in lower-middle- (LMICs) and high-income countries (HICs) reported annual incidences of 7.1 – 10.9 per million from 1993 to 2010 [1-4] with an average global increment of 3.2% per year [5]. Despite their comparable number of new cases each year, survival gaps between LMICs and HICs remain challenging. A study in the United States, which applied allogeneic hematopoietic stem cell transplantation (HSCT) in selected patients, revealed a five-year event-free survival (EFS), overall survival (OS), and treatment-related mortality (TRM) of 62%, 71%, and

15%, respectively [6]. Nevertheless, in the absence of allogeneic HSCT, our previous study disclosed a higher TRM of 52% in the second year of treatment, which resulted in a lower EFS of 9% and OS of 16% (unpublished data). It is hypothesized that the absence of a risk-adapted treatment protocol, as a result of the lack of applicable karyotype-based risk stratification criteria, constitutes one of the contributing factors to the unfavorable outcomes of childhood AML in several LMICs.

The interaction of leukemic myeloblasts with the surrounding bone marrow stroma and their role in the pathogenesis of AML have widely been described. The leukemic myeloblasts and bone marrow mesenchymal stem

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cells (MSCs) secrete indoleamine 2,3-dioxygenase (IDO) and arginase, which induce the maturation of CD4⁺ CD25⁺ into the active CD4⁺ CD25⁺ regulatory T-cells (Tregs) and proliferation of myeloid-derived suppressor cells [7]. Moreover, CD8⁺ cytotoxic T-cells (TC) of patients with AML overexpress the inhibitory programmed cell death 1 (PD1), T-cell immunoglobulin and mucin-domain containing-3 (TIM3), and lymphocyte-activating gene 3 (LAG3) proteins [8, 9]. The simultaneous proliferation of Tregs and other suppressor cells and inhibition of TC-mediated cytotoxicity indicate the contribution of immune evasion strategies, which are demonstrated by the leukemic myeloblasts and their microenvironment, to developing progressive disease.

The dysregulation of effector and suppressor cells may affect the number of circulating lymphocytes and provide insight into the application of lymphocyte counts in risk stratification. A study on 54 children with AML in the United States associated the peripheral blood absolute lymphocyte counts on day 15 of treatment (ALC₁₅) of less than 0.4×10^9 cells/L with the lower five-year relapse-free survival (RFS) and OS [10]. This study, however, did not anticipate the occurrence of death within the first 15 days of treatment as a result of severe malnutrition [11] and infection [12, 13]. A question on the prognostic role of diagnostic ALC (ALC₀) is raised to address these issues. The studies of Bar et al. [14] and Le Jeune et al. [15], which involved adults with AML in the United States and France, respectively, found an association between ALC₀ of more than $4.5 - 4.8 \times 10^9$ cells/L and unfavorable outcomes in terms of remission failure, five-year RFS, and OS. Since childhood AML shows distinctive biological features compared to adult AML [16] and the Treg population is influenced by age-dependent bacterial colonization [17], it is obligatory to validate these findings in the population of pediatric patients.

Materials and Methods

Study design

A medical record-based retrospective cohort study was conducted on a population of patients younger than 18 years with non-M3 AML according to the French American British (FAB) classification system. These patients were admitted to the Pediatric Hematology-Oncology Division of Dr. Sardjito General Hospital from 2011 – 2019. The diagnosis of AML was confirmed by the presence of myeloid-lineage blasts, which were stained with Sudan Black B and expressed cytoplasmic myeloperoxidase (MPO), in more than 20% of bone marrow nucleated cells [18]. Patients with Down syndrome, secondary AML, and mixed phenotypic leukemia were excluded from our study. Our study protocol has been approved by the Medical and Health Research Ethics Committee of Universitas Gadjah Mada and Dr. Sardjito General Hospital.

Basic hematological examination

The diagnostic white blood cell (WBC), peripheral blood lymphocyte, and blast counts were obtained during the initial hospital admission. An XN-1000 (Sysmex,

Kobe, Japan) and a Cell-Dyn Ruby (Abbott, Chicago, IL) hematology analyzers were used to assess the complete blood counts, while microscopic examination of the Wright-stained (Merck, Darmstadt, Germany) peripheral blood smears was performed to confirm the percentage of lymphocytes and blasts. ALC and the absolute blast counts were determined by multiplying WBC with the percentage of lymphocytes and blasts, respectively.

Treatment protocols

Patients with a confirmed diagnosis of non-M3 AML received the National Pilot Protocol of Indonesian Childhood AML (2011 – 2016), the modified low-intensity protocol (2016 – 2018), and the modified International Society of Pediatric Oncology – Pediatric Oncology in Developing Countries Committee (SIOP PODC) protocol (2018 – 2019). The first remission-induction phase of these protocols consisted of intravenous daunorubicin (National Pilot Protocol and modified low-intensity protocol, 50 mg/m²/day on days 1, 3, and 5; modified SIOP PODC protocol, 25 mg/m²/day on days 1, 3, and 5) and cytarabine (National Pilot Protocol, 100 mg/m²/12 hours on days 1 – 5; modified low-intensity and SIOP PODC protocols, 10 mg/m²/12 hours on days 1 – 10). The age-adjusted dose of intrathecal methotrexate was administered on the first day of the National Pilot Protocol. In the absence of hyperleukocytosis, subcutaneous granulocyte-colony stimulating factor (5 µg/kg/day on days 1 – 10) was given by the modified low-intensity and SIOP PODC protocols. The modified SIOP PODC protocol also inserted a pre-induction phase of intravenous etoposide (37.5 mg/m²/day on days 1 – 7), oral 6-mercaptopurine (50 mg/m²/day on days 1 – 21), and prednisone (40 mg/m²/day on days 1 – 14).

The second remission-induction phase with the same regimens was commenced on day 29 in patients receiving the National Pilot Protocol and the modified low-intensity protocol, irrespective of their remission status. In contrast, patients in the modified SIOP PODC protocol were stratified at the end of the first remission-induction phase. Patients showing complete (CR) or partial remission (PR) status received the same regimens. At the same time, the higher dose of daunorubicin (50 mg/m²/day on days 1, 3, and 5) and cytarabine (100 mg/m²/12 hours on days 1 – 7) were given to those who failed to achieve CR or PR. The high-dose cytarabine (National Pilot Protocol, 1 g/m²/day; modified low-intensity protocol, 2 – 3 g/m²/12 hours; modified SIOP PODC protocol, 1.5 g/m²/12 hours) constituted the backbone of the consolidation phase.

Treatment outcomes and statistical analysis

Each eligible patient was observed for events that consisted of remission failure, relapse, and death within five years of treatment. CR was defined as reducing bone marrow myeloid-lineage blasts toward less than 5% at the end of the first remission-induction phase. In comparison, patients with bone marrow blast counts of 6 – 15% were classified as PR and considered as achieving remission by the study of Fleming et al. [19]. Patients with CR and

PR status later showed bone marrow blast counts of more than 20% were classified as relapse.

A linear regression analysis was performed to determine whether ALC_0 correlates with the diagnostic blast counts and WBC. Kaplan-Meier survival analysis and log-rank test were performed to compare the EFS and OS between patients with ALC_0 of more than 4.7×10^9 cells/L and those with ALC_0 of less than 4.7×10^9 cells/L. Cox regression analysis was then performed to determine the hazard ratio (HR) of remission failure, relapse, and death within five years of treatment in patients with ALC_0 of less than 4.7×10^9 cells/L. All collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 27 software (IBM, Armonk, NY).

Results

Patient characteristics

Sixty-nine patients with accessible medical record data were enrolled in our study. Nine patients were excluded because of the previous diagnosis of myelodysplastic syndrome (n = 1), chronic myeloid leukemia (n = 1), and the presence of the morphological, cytochemical, and immunophenotypic features of mixed phenotypic

leukemia (n = 7). Our study, therefore, analyzed 60 patients consisting of 25 patients with ALC_0 of less than 4.7×10^9 cells/L and 35 patients with ALC_0 of more than 4.7×10^9 cells/L. Of these 60 patients, 34 (57%) were male, 25 (42%) were older than ten years, and 18 (30%) were morphologically classified as AML-M4. In terms of treatment protocols, 51 (85%) patients received intravenous daunorubicin and cytarabine at the beginning of treatment (National Pilot Protocol, n = 45; the modified low-intensity protocol, n = 6), while nine (15%) patients received the pre-induction cytostatics according to the modified SIOP PODC protocol (Table 1). Ten patients were classified as drop-outs in the pre-induction and induction phases (n = 5) and after the achievement of remission (n = 5). These patients were included in the analysis and considered as experiencing no event until the last date of contact. There was no significant difference between patients with CR and PR on the five-year EFS (p = 0.88; data not shown) and OS (p = 0.57; data not shown).

Correlation of ALC_0 with the diagnostic blast counts and WBC

The descriptive analysis of diagnostic complete blood counts revealed the median blast counts and WBC of 12.9

Table 1. Patient Characteristics

Characteristics	$ALC_0 < 4.7 \times 10^9$ cells/L n = 25	$ALC_0 \geq 4.7 \times 10^9$ cells/L n = 35	Total n = 60
Sex			
Male (%)	14 (56.0)	20 (57.1)	34 (56.7)
Female (%)	11 (44.0)	15 (42.9)	26 (43.3)
Median age at diagnosis in years (IQR)	9.9 (6.7 – 14.2)	7.6 (2.2 – 11.8)	8.8 (3.4 – 13.0)
Age < 10 years (%)	13 (52.0)	22 (62.9)	35 (58.3)
Age \geq 10 years (%)	12 (48.0)	13 (37.1)	25 (41.7)
FAB morphological subtypes			
M0 (%)	1 (4.0)	2 (5.7)	3 (5.0)
M1 (%)	4 (16.0)	5 (14.3)	9 (15.0)
M2 (%)	5 (20.0)	7 (20.0)	12 (20.0)
M4 (%)	7 (28.0)	11 (31.4)	18 (30.0)
M5 (%)	3 (12.0)	6 (17.1)	9 (15.0)
M6 (%)	1 (4.0)	1 (2.9)	2 (3.3)
M7 (%)	4 (16.0)	3 (8.6)	7 (11.7)
Median diagnostic blast counts in 10^9 cells/L (IQR)	7.9 (0.5 – 23.7)	20.2 (8.4 – 91.2)	12.9 (3.5 – 43.9)
Blasts < 19×10^9 cells/L (%)	19 (76.0)	17 (48.6)	36 (60.0)
Blasts $\geq 19 \times 10^9$ cells/L (%)	6 (24.0)	18 (51.4)	24 (40.0)
Median diagnostic WBC in 10^9 cells/L (IQR)	13.8 (5.3 – 36.5)	46.6 (26.5 – 133.6)	30.2 (13.5 – 70.0)
WBC < 50×10^9 cells/L (%)	21 (84.0)	18 (51.4)	39 (65.0)
WBC $\geq 50 \times 10^9$ cells/L (%)	4 (16.0)	17 (48.6)	21 (35.0)
Treatment protocols			
National Pilot Protocol (%)	19 (76.0)	26 (74.3)	45 (75.0)
Modified low intensity (%)	2 (8.0)	4 (11.4)	6 (10.0)
Modified SIOP PODC (%)	4 (16.0)	5 (14.3)	9 (15.0)

ALC_0 , diagnostic absolute lymphocyte counts; FAB, French American British; IQR, interquartile range; SIOP PODC, International Society of Pediatric Oncology – Pediatric Oncology in Developing Countries Committee; WBC, white blood cell counts

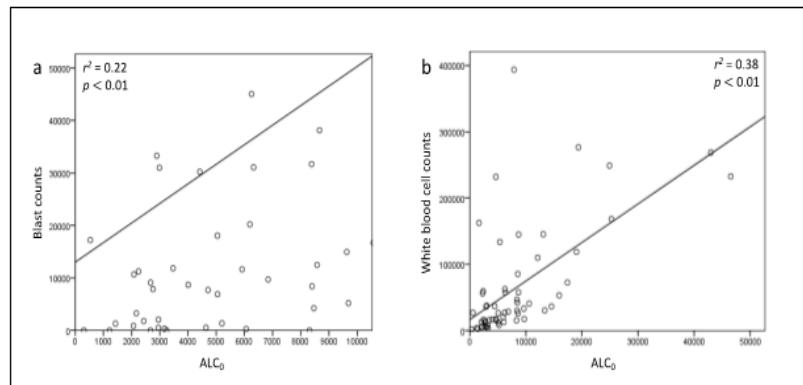


Figure 1. Correlation of ALC_0 with the Diagnostic Blast Counts (a) and WBC (b)

(interquartile range [IQR], 3.5 – 43.9) and 30.2 (IQR, 13.5 – 70.0) $\times 10^9$ cells/L, respectively. Twenty-four (40%) patients were classified as having blast counts of more than 19×10^9 cells/L, while 21 (35%) patients had a WBC of more than 50×10^9 cells/L (Table 1). Linear regression analysis demonstrated a weak correlation of ALC_0 with the blast counts ($r^2 = 0.22$; $p < 0.01$; Figure 1a) and WBC ($r^2 = 0.38$; $p < 0.01$; Figure 1b).

ALC_0 -based treatment outcomes

Forty-five (75%) of 60 patients experienced their first events within five years of treatment. Four (7%) patients failed to achieve remission, 17 (28%) patients developed a relapsed disease, and 24 (40%) patients died. In a survival analysis, patients with ALC_0 of more than 4.7×10^9 cells/L had a higher EFS (24% vs. 0%; $p = 0.01$; Figure 2). Moreover, a univariate Cox regression analysis showed that patients with ALC_0 of less than 4.7×10^9 cells/L had

a higher risk of remission failure, relapse, and death (HR, 2.1; 95% confidence interval [CI], 1.2 – 3.8; Table 2). The multivariate analysis, which also involved the diagnostic blast counts and treatment protocols, revealed the role of ALC_0 of less than 4.7×10^9 cells/L as an independent prognostic factor for these events (HR, 3.5; 95% CI, 1.7 – 7.5; Table 2).

Thirty-nine (65%) of 60 patients died of progressive disease and the adverse effects of cytostatics within five years of treatment, irrespective of their remission and relapse status. The descriptive analysis revealed the median time of death of 2.4 (IQR, 0.6 – 8.5) months after the initiation of treatment. Kaplan-Meier survival analysis and log-rank test showed that 90% of patients with ALC_0 of less than 4.7×10^9 cells/L and 69% of patients with ALC_0 of more than 4.7×10^9 cells/L died during these five years ($p = 0.02$; Figure 3). A univariate Cox regression analysis showed an increased risk of death in patients with

Table 2. Cox Regression Analysis for the Five-year Event-free Survival

Variables	Univariate HR (95% CI)	p	Multivariate HR (95% CI)	p
ALC_0				
< 4.7×10^9 cells/L	2.1 (1.2 – 3.8)	0.02	3.5 (1.7 – 7.5)	0.01
$\geq 4.7 \times 10^9$ cells/L	1		1	
Sex				
Male	0.7 (0.4 – 1.3)	0.32		
Female	1			
Age at diagnosis				
< 10 years	1			
≥ 10 years	1.1 (0.6 – 2.0)	0.77		
Diagnostic blast counts				
< 19×10^9 cells/L	1		1	
$\geq 19 \times 10^9$ cells/L	1.5 (0.8 – 2.6)	0.21	2.7 (1.3 – 5.7)	0.01
Diagnostic WBC				
< 50×10^9 cells/L	1			
$\geq 50 \times 10^9$ cells/L	1.0 (0.6 – 1.9)	0.91		
Treatment protocols				
National Pilot Protocol	1		1	
Modified low intensity	1.0 (0.3 – 2.7)	0.96	1.4 (0.5 – 4.1)	0.54
Modified SIOP PODC	1.7 (0.7 – 3.8)	0.23	1.6 (0.7 – 3.6)	0.3

ALC_0 , diagnostic absolute lymphocyte counts; CI, confidence interval; HR, hazard ratio; SIOP PODC, International Society of Pediatric Oncology – Pediatric Oncology in Developing Countries Committee; WBC, white blood cell counts

Table 3. Cox Regression Analysis for the Five-year Overall Survival

Variables	Univariate HR (95% CI)	p	Multivariate HR (95% CI)	p
ALC₀				
< 4.7 x 10 ⁹ cells/L	2.1 (1.1 – 4.0)	0.03	2.1 (1.1 – 4.0)	0.03
≥ 4.7 x 10 ⁹ cells/L	1		1	
Sex				
Male	0.7 (0.4 – 1.3)	0.23	0.8 (0.4 – 1.5)	0.46
Female	1		1	
Age at diagnosis				
10 years	1			
10 years	0.9 (0.5 – 1.8)	0.81		
Diagnostic blast counts				
< 19 x 10 ⁹ cells/L	1			
≥ 19 x 10 ⁹ cells/L	1.4 (0.7 – 2.6)	0.32		
Diagnostic WBC				
< 50 x 10 ⁹ cells/L	1			
≥ 50 x 10 ⁹ cells/L	0.7 (0.4 – 1.5)	0.38		
Treatment protocols				
National Pilot Protocol	1		1	
Modified low intensity	0.6 (0.2 – 2.1)	0.47	0.6 (0.2 – 2.1)	0.46
Modified SIOP PODC	2.4 (1.0 – 5.5)	0.05	2.0 (0.8 – 5.0)	0.13

ALC₀, diagnostic absolute lymphocyte counts; CI, confidence interval; HR, hazard ratio; SIOP PODC, International Society of Pediatric Oncology – Pediatric Oncology in Developing Countries Committee; WBC, white blood cell counts

ALC₀ of less than 4.7 x 10⁹ cells/L (HR, 2.1; 95% CI, 1.1 – 4.0; Table 3). This association also reached a statistical significance when the multivariate analysis was performed (HR, 2.4; 95% CI, 1.1 – 5.2; Table 3).

Discussion

Our study found a weak but statistically significant correlation of ALC₀ with the diagnostic blast counts and WBC. There was also an association of ALC₀ of more than 4.7 x 10⁹ cells/L with the higher five-year EFS and OS. Our study, therefore, showed the conflicting roles of lymphocytes in the pathogenesis of childhood AML, in which lymphocytosis was linked to an increasing leukemic cell burden and favorable outcomes. While there are limited reports on the correlation of ALC₀ with blast counts and WBC, our findings contrasted with those of adult AML, which associated remission failure, an earlier onset of relapse, the lower five-year RFS, and OS with ALC₀ of more than 4.5 – 4.8 x 10⁹ cells/L [14, 15]. Despite different cut-off points of ALC and time of examination, the results of our study were similar to those involving children with cancers. In a study involving 54 children with AML in the United States, patients with ALC15 of less than 0.4 x 10⁹ cells/L had a higher risk of relapse and death within five years [10], while another study on 100 Peruvian children with musculoskeletal tumors reported that patients with ALC₀ of less than 1 x 10⁹ cells/L and ALC15 of less than 0.8 x 10⁹ cells/L showed a lower five-year OS [20].

The association of lymphocytosis with the higher blast counts and WBC indicated the predominant role of Tregs at the time of diagnosis. Several previous studies

correlated an increasing number of these suppressor cells with the colonization of nasopharyngeal otopathogens [17] that was more prevalent in children [21]. Our findings, however, conflicted with the previous reports on IDO-induced immune suppression. Folgiero et al. [22] reported that none of 37 children with AML constitutively expressed IDO protein and 49% of patients did not upregulate IDO expression in response to interferon (IFN)- γ . The low expression levels of IDO in children with AML might be specific to certain cytogenetic features of leukemic blasts [22], which were not routinely examined in our patients. Our findings and others [23] might indicate the predominant role of arginase, which was secreted by the leukemic blasts, irrespective of the ages and cytogenetic features. Nevertheless, since the observed events (remission failure, relapse, and death) occurred after the initiation of treatment protocols, our findings might also provide insight into the synergistic effects of TC- and natural killer (NK) cell-mediated cytotoxicity and cytostatic treatment in childhood AML. In a population of children with acute lymphoblastic leukemia (ALL), Bhattacharya et al. [24] reported the cytostatic-induced suppression of forkhead box P3 (FoxP3) expression and interleukin (IL)-10 synthesis by Tregs, which might further augment the TC- and NK cell-mediated cytotoxicity [25].

Our study found that 40% of patients experienced death as their first event, with a median of nine weeks after the initiation of treatment protocols. Despite the application of three different treatment protocols, our study showed that these patients died after achieving CR, which might indicate the treatment-related causes of death. Infection constitutes one of the significant

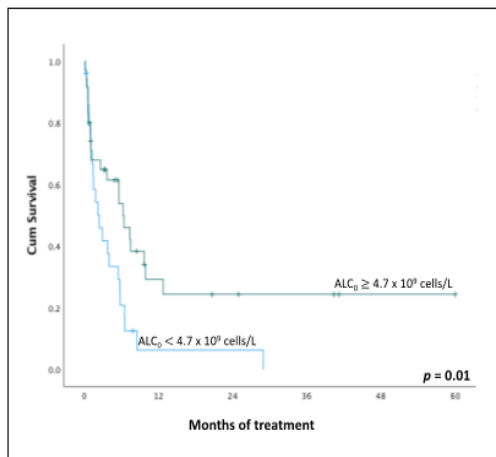


Figure 2. Five-year EFS of Patients with ALC_0 of more than 4.7×10^9 Cells/L (green line) and Less than 4.7×10^9 Cells/L (blue line). ALC_0 , Diagnostic Absolute Lymphocyte Counts

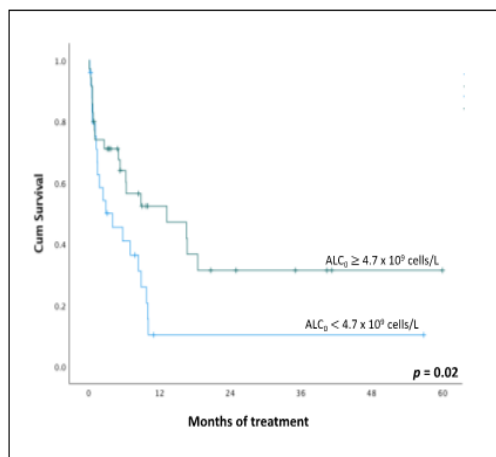


Figure 3. Five-year OS of Patients with ALC_0 of more than 4.7×10^9 Cells/L (green line) and Less than 4.7×10^9 Cells/L (blue line). ALC_0 , Diagnostic Absolute Lymphocyte Counts

challenges in the management of children with cancers in several LMICs. Our previous studies revealed that 43% of deaths in children with ALL [26] and 52% of deaths in children with AML (unpublished data) were preceded by clinically and microbiologically documented infections. Our results were similar to those reported in several studies which involved adults with AML. In those studies, the administration of high-dose cytarabine during the consolidation phase of treatment increased the risk of bacteremia and fungemia [27–29]. Despite lacked supportive data, it is reasonable to correlate ALC_0 of less than 4.7×10^9 cells/L with a higher risk of infection. Several studies involving children [30] and adults [31–33] with solid tumors revealed that patients with grade III lymphopenia had a higher risk of febrile neutropenia during treatment than those with grade I–II lymphopenia.

As a conclusion, our study showed the association of ALC_0 of more than 4.7×10^9 cells/L with the higher five-year EFS and OS. These findings provide insight into the application of diagnostic lymphocyte counts in the risk stratification of childhood AML in pediatric oncology

centers with limited resources, where cytogenetic and molecular analyses are not routinely performed. The heterogeneous nature of treatment protocols applied and treatment adherence might affect the Kaplan-Meier survival analysis. Nevertheless, the multivariate Cox regression analysis, which involved treatment protocols, demonstrated the independent role of ALC_0 as a prognostic factor of childhood AML. Further studies are required to confirm the dysregulation of immune effector and regulatory cells.

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Funding Statement

Not applicable

Approval

Not applicable

Conflict of Interest

Nothing to declare

Ethical declaration

The protocol of this study has been approved by the Medical and Health Research Ethics Committee of Universitas Gadjah Mada and Dr. Sardjito General Hospital.

Authors' contribution

BA, RT, and SS designed the study, analyzed the data, and prepared the manuscript; CC and IA collected the data; ES performed survival analysis.

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