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Absolute Neutrophil Count as Predictor Hematopoietic Recovery in Acute Lymphoblastic Leukemia in Remission Induction Phase Chemotherapy

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ABSTRACT

Introduction: The main treatment for leukemia is chemotherapy therapy, divided into the induction of remission, consolidation, and maintenance therapy. Early response to remission-induced therapy is an important prognostic factor in ALL. This study aimed to assess the relationship between absolute neutrophil count (ANC) and bone marrow improvement after undergoing induction phase chemotherapy which was characterized by the occurrence of remission.
Methods: Acute Lymphoblastic Leukemia diagnosed based on CBC, peripheral smear, bone marrow morphology, and flow cytometry. Nineteen samples from children with Acute Lymphoblastic Leukemia were evaluated for blast count and absolute neutrophil count (ANC) on day 0, ANC on 3rd week, and blast count and ANC on the 6th week of remission induction therapy. We compared the results of outcomes after remission induction therapy.
Results: The ANC value at the 3rd week and 6th week of the chemotherapy phase was positively correlated with remission with the r=0.687 and with a p-value=0.001.
Conclusion: The trend of increasing ANC values was moderately positively correlated with the incidence of remission.

Keywords: Absolute Neutrophil Count, Acute Lymphoblastic Leukemia, Remission.
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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. This disease accounts for a quarter of all childhood cancers and 72% of all childhood leukemia cases, with the peak incidence of ALL occurring between the ages of 2 and 5 years.[1] In Indonesia, childhood cancer occurs at the age of 0-14 years[2], with a prevalence amounting to 87% of all leukemia cases at Sanglah Hospital Denpasar.[3] In a study conducted by Putri Tampubolon et al. at H. Adam Malik Hospital, Medan found 70% of ALL cases.[4]

Over the last 50 years, many new modalities for the diagnosis and treatment of leukemia have been growing, leading to increased survival in patients with ALL. The main treatment for leukemia is chemotherapy therapy, which suppresses the bone marrow to produce cancer cells and prevents the formation of new cancer cells. Chemotherapy in ALL patients is divided into the induction of remission, consolidation, or intensification and maintenance therapy. The goal of remission induction therapy is to induce complete remission. Early response to remission induction therapy is one of the most important prognostic factors in ALL.[5,6]

Chemotherapy causes inflammation due to cell damage, neutrophil maturation, and cytokine release.[7] Cytokines such as interleukin-6 (IL-6) will regulate hematopoiesis, inflammation and stimulate the production and mobilization of neutrophils in the bone marrow[8] against anemia, bleeding, and infection. Therefore, extensive blood count monitoring is required to assess hemopoietic recovery.[9]

Severe neutropenia is defined as an absolute neutrophil count (ANC) lower than 500 cells/mm3 and is common in post-chemotherapy leukemia patients. This makes the patient more susceptible to life-threatening infections.[10,11] Hemopoietic recovery can be assessed by an absolute neutrophil count (ANC). This value is obtained by multiplying the number of leukocytes by the percentage of neutrophils and the band. Absolute neutrophil count (ANC) has a sensitivity of >96% to predict bone marrow recovery after chemotherapy.[9]

Based on the description above, this study was conducted to assess the relationship between absolute neutrophil count (ANC) and bone marrow improvement after undergoing induction phase chemotherapy which was characterized by remission.

MATERIAL AND METHODS

This study was a retrospective cohort study conducted at the Clinical Pathology Department Faculty of Medicine Universitas Sumatera Utara/General Hospital Haji Adam Malik, Medan, Indonesia from July to September 2021. This study included all new pediatric

patients with anemia, leukocytosis, and or thrombocytopenia based on complete blood count within study period. The morphological examination was carried out on peripheral smear and bone marrow samples with Giemsa stain. Acute Lymphoblastic Leukemia is diagnosed based on blast count ≥ 20% according to WHO classification. Inclusion criteria: All patients with acute lymphoblastic leukemia who underwent a second bone marrow aspiration after the induction phase of therapy at the end of week 6, aged <18 years old, and willing to be involved in research. Exclusion criteria for this study: previous history of acute leukemia, previous history of malignancy, previous history of chemotherapy treatment, and patients with incomplete medical record data.

Nineteen samples from children with Acute Lymphoblastic Leukemia were evaluated for blast count and absolute neutrophil count (ANC) on day 0, ANC on the 3rd week, and blast count and ANC on the 6th week of remission induction therapy. A complete blood count examination was performed using the automatic cell counting Sysmex XN-1000i to obtain the leukocyte count, neutrophil count, and ANC. We compared the results of outcomes after induction therapy remission.

Ethical approval for this study was obtained from The Research Ethics Commission of The Faculty of Medicine Universitas Sumatera Utara. All the subjects' parents in this study have been approved to join and signed the informed consent form. All data were analyzed using SPSS version 25. After performing the normality test, we performed comparative analysis (Mann Whitney-U test and Kruskal-Wallis test) and correlative analysis (Spearman correlation test). P-value < 0.05 was significant. There was no data missing in this study.

RESULTS

Total samples of 19 pediatric patients who have acute lymphoblastic leukemia from June 2021 to August 2021 at H. Adam Malik Hospital Medan was included in this study (no patient was excluded). Table 1 shows the characteristics of acute lymphoblastic leukemia patients. The median age was found to be 9 years. The bone marrow aspirate morphology examination showed that 9 people (47.4%) were boys and 10 people (52.6%) were girls. Clinical symptoms found were pale (42.1%), bleeding (36.8%), and organomegaly (15.8%). Anemia was found in 13 people (68.4%), thrombocytopenia in 18 people (94.7%), and hyperleukocytosis in 4 people (21.1%).

This table also shows that the median leukocyte count in patients with ALL was 49720 /µL, the median hemoglobin level was 7.2 g/dL, and the median platelet count was 24,000 /µL. Evaluation of the induction phase of chemotherapy found 17 people (89.5%) in remission and 2 people (10.5%) no remission with a median number of blasts in the bone marrow of 1.5%.

Furthermore, there is no significant difference (p-value>0.05) in hemoglobin levels, leukocyte counts, platelet counts, and ANC before chemotherapy with the clinical symptoms of patients (Table 2).

The ANC of acute lymphoblastic leukemia patients increased after the induction phase of chemotherapy, with

Table 1. Patient Characteristics.

Variable	n (%)	Median (min-max)
Demographic		
Age (year)		9 (1-16)
<5	6 (31.6)	
5-10	7 (36.8)	
>10	6 (31.6)	
Gender		
Boy	9 (47.4%)	
Girl	10 (52.6%)	
Clinical symptoms		
Fever		
Yes	7 (36.8)	
No	12 (63.2)	
Pale		
Yes	8 (42.1)	
No	11 (57.9)	
Bleeding		
Yes	7 (36.8)	
No	12 (63.2)	
Organomegaly		
Yes	3 (15.8)	
No	16 (84.2)	
Laboratory test		
Anemia (Hb <10 g/dl)		
Yes	13 (68.4)	
No	6 (31.6)	
Hyperleukocytosis (>100.000/mm^3)		
Yes	4 (21.1)	
No	15 (78.9)	
Thrombocytopenia (<50.000/mm^3)		
Yes	18 (94.7)	
No	1 (5.3)	
Hb		7.2 (3.2 – 14.3)
Leukocyte		49720 (2030 – 743390)
Platelet		24000 (9000 – 199000)
BMA Evaluation of the induction phase		
Remission	17 (89.5)	
Not Remission	2 (10.5)	
Blast Count		1.5 (0-12.5)

Notes: Hb, haemoglobin; BMA, bone marrow aspiration, Min-max, minimal-maximum

Table 2. Association between Clinical and Hematological Profile of Patients with Acute Lymphoblastic Leukemia.

Variable	Hb		Leukocyte		Platelet		ANC	
	Mean Rank	P-value*	Mean Rank	P-value*	Mean Rank	P-value*	Mean Rank	P-value**
Age (year)								
<5	10.58		8.67		8.42		8.00	
5-10	9.29	0.91	10.71	0.78	13.07	0.19	11.86	0.46
>10	10.25		10.50		8.00		9.83	
Gender								
Boy	9.5	0.71	11.19	0.41	10.3	0.81	10.67	0.62
Girl	10.45				9.7		9.4	
Fever								
Yes	10.57	0.73	11.86	0.27	10	1.00	12	0.24
No	9.67		8.92		10		8.83	
Pale								
Yes	8.69	0.38	11.50	0.32	8.94	0.48	11.38	0.36
No	10.95		8.91		10.77		9	
Bleeding								
Yes	13	0.31	11.33	0.65	14.5	0.13	13.67	0.22
No	9.44		9.75		9.16		9.31	
Organomegaly								
Yes	8.29	0.31	10.14	0.93	12.57	0.13	9.57	0.80
No	11		9.52		8.50		10.25	

*Mann Whitney test (CI 95%), **Kruskal-Wallis test (CI 95%). Hb, hemoglobin; ANC, absolute neutrophil count

a median ANC value of 2.77 at week 0 (before chemotherapy), a median ANC value of 3.33 at 3rd week of chemotherapy, and a median ANC value of 3.87 at the end of the induction phase (Table 3).

The correlation of ANC value to the evaluation of therapy at the end of the induction phase showed that the ANC value before chemotherapy was negatively correlated with the incidence of remission with r -0.205 but not statistically significant with a p-value of 0.200. The ANC value at the third week during chemotherapy was positively correlated with the incidence of remission with r 0.687 and statistically significant with a p-value of 0.001. While the ANC value at the end of the chemotherapy induction phase was also found to have a positive correlation with the incidence of remission with r 0.687 and statistically significant with a p-value of 0.001 (Table 4).

Table 3. Differences in Median ANC Values at Week 0, Week 3, and End of Induction Phase.

Variable	Median	Min – Max
ANC		
Week 0	2.77	0 – 22.93
3rd Weeks	3.33	0.26 – 7.94
6th Weeks	3.87	0.10 – 31.8

Table 4. Correlation of ANC Value with Evaluation of therapy at the end of the induction phase.

Variable	BMA Evaluation of the induction phase	
	Remission	Blast Count
ANC (≥500/mm³)		
Week 0		
R	-0.205	-0.319
p value	0.200	0.092
3rd week		
r	0.687**	0.390*
p value	0.001	0.049
End of Induction Phase		
r	0.687**	0.347
p value	0.001	0.073

*Spearman correlation test (CI 95%; 1-tailed). **Spearman correlation test (CI 99%, 1-tailed). BMA, bone marrow aspiration; ANC, absolute neutrophil count

DISCUSSION

Acute lymphoblastic leukemia (ALL) mainly affects children, with a peak incidence between 2 and 3 years of age. The frequency decreases until it reaches a low point around age 25 to 50 years, after which it increases to reach a second, but small, peak at age over 80 years.[12] The main treatment for leukemia is chemotherapy therapy, which suppresses the bone marrow to produce cancer cells and prevent the formation of new cancer cells. Chemotherapy used in the induction phase of remission usually causes severe bone marrow suppression. This critical period requires critical management, supportive therapy, and regular monitoring.[5]

In this study, the age of patients with acute lymphoblastic leukemia at the time of diagnosis was found with a median of 9 years and an age range of 1-16 years.

A previous study by Patrick Brown et al.[13] showed that the median age of ALL patients when diagnosed was 15 years, while Perdani R[14] had a median of 6.58 with an age range of 0-17.8 years. This difference is due to the prevalence of childhood cancer in Indonesia occurring at the age of 0-14 years, according to the 2013 Basic Health Research data.

The trend of increasing ANC values was moderately positively correlated with the incidence of remission. This parameter could be used to describe bone marrow recovery in pediatric patients with acute lymphoblastic leukemia. However, research on the relationship between ANC in describing the incidence of remission in the late phase of induction chemotherapy requires a longer time so that the sample obtained is larger.

All patients in this study were diagnosed with acute lymphoblastic leukemia, and to establish the diagnosis of acute lymphoblastic leukemia, a morphological examination of bone marrow aspirates was performed, and there were 9 (47.4%) males and 10 (52.6%) females. This is in accordance with the study by Tarigan A et al., which showed no difference in the number of male and female patients.[3]

Clinical symptoms found were pallor (42.1%), bleeding (36.8%), and organomegaly (15.8%), similar to the study by Shawahna et al.[15] where anemia was the most common clinical symptom. Fever was found in 7 people (36.8%), according to a study by Ozdemir et al.[16] which found a higher incidence of fever during therapy at the time of relapse than when initially starting ALL therapy.

Hyperleukocytosis (leukocytes>100x10^3/μL) was found in 21.1%. Hyperleukocytosis in ALL is closely related to early morbidity and mortality, where children with hyperleukocytosis >200 x10^3 /μL have a worse event-free survival (EFS) than children with hyperleukocytosis 100-200 x 10^3 /μL.[17] This becomes important in determining risk stratification in patients with acute leukemia, especially in ALL, where the state of leukocytosis (leukocytes >50 x 10^3 /μL), patients age over ten years, and male sex have a worse prognosis than patients who do not. have this condition, or what we know as High-Risk.[18-20]

Thrombocytopenia (<50,000 / mm3) was found in 18 people (94.7%) according to the study by Wilson et al. and is associated with bleeding events in acute leukemia. However, the incidence of bleeding is more common in patients with relapsed acute leukemia compared to de novo acute leukemia.[21]

In this study, 17 out of 19 people achieved remission in the induction phase (89.5%). Research from several developing countries also shows the incidence of remission ranging from 80%.[18,19,22] The ANC value before chemotherapy was negatively correlated with the incidence of remission with r -0.205, but not statistically significant with a p-value of 0.200. This shows that a low ANC value is correlated with a high incidence of no remission condition. This is similar to a study by Perdani R., where a low ANC (ANC 500) was correlated with a lower incidence of remission due to severe infections that prevent remission at the end of the induction phase. associated with infectious states and febrile neutropenia in the chemotherapy phase.[23,24]

The ANC value at the third week during chemotherapy and at the end of the chemotherapy induction phase was positively correlated with the incidence of remission with the same value of r 0.687 and statistically significant with a p-value of 0.001. This shows that a high ANC value is correlated with a high incidence of remission. This is similar to the study conducted by Rauf SE and Perdani R.[5,14]

In this study, we found that the increase in ANC values and the induction phase of chemotherapy followed by bone marrow remission can be used for monitoring during chemotherapy. This is in line with the research conducted by Grunnan and Rosthøj (2019), which showed that ANC recovery started from week 3 to week 5 of the induction phase. This is in accordance with a study conducted by Rauf SE., 2015 and Perdani R., 2018.[5,14] Short period of sample collection time and a small number of participants became the limitation in this study. This study also did not performed adjusted analysis for variables which might confound the result, such as sex, race, the severity of the patient's condition, types and doses of chemotherapy or steroid used in this study. We hope that this study can be explored more in the future with a longer study duration and bigger sample size. To decrease the possibility of study bias, a randomized technique sampling and a more detailed patient specification can be done in the future.

CONCLUSIONS

We found that the trend of increasing ANC values was moderately positively correlated with the incidence of remission at the end of the induction chemotherapy phase, so perhaps this parameter could be used to describe bone marrow recovery in pediatric patients with acute lymphoblastic leukemia. However, research on the relationship between ANC in describing the incidence of remission in the late phase of induction chemotherapy requires a longer time so that the sample obtained is larger.

DISCLOSURE

Ethical approvement

The Research Ethics Commission The Faculty of Medicine Universitas Sumatera Utara/General Hospital Haji Adam Malik Medan, Indonesia, has approved this study with ethical clearance number 706/KEP/USU/2021. All patients have approved of joining this study, and all patients' parents had signed the informed consent.

Conflict of interest

There was no conflict of interest in this study

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Author contribution

Conceptualization and methodology, MRN, PCT; data collection, MRN,PCT,ISN; analysis and writing MRN,PCT,ISN; review, MRN. All authors have read and agreed to publish the manuscript.

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