Survival rate of children acute lymphoblastic leukemia at the end of therapy protocol with minimal residual disease (MRD) in Prof I G.N.G. Ngoerah General Hospital Denpasar

Dewa Gde Windu Sanjaya1*, Ketut Ariawati1, Putu Junara Putra1, I Putu Gede Karyana1, Eka Gunawijaya1, I Made Gde Dwi Lingga Utama1

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, occurring in about 3 out of 4 cases of leukemia in the pediatric population and is responsible for the most malignancy-related deaths in the pediatric age group.1 This malignancy has a peak incidence between the ages of 2 and 5 years and more likely to occur in male than female.2 The incidence of ALL in the United States is approximately 30 cases per one million population of children less than 20 years old, with the peak incidence occurring at 3 to 5 years of age. The incidence of ALL in Indonesia is estimated to be around 3000 new cases in children each year, where the number of event free survival (EFS) is around 50% far below developed countries which reach 80-90%.3,4,5

The de novo management of the ALL generally consists of a series of therapeutic phases: induction of remission, consolidation, intensification and maintenance. The duration and combination of drugs in each phase of treatment may vary between clinical protocols, but can largely be simplified with the goal of initial therapy through induction therapy for eradication. Although therapeutic regimens still differ among treatment protocols, some major medications are always given because they are considered effective.6 Due to the adoption of risk-adjusted therapies and increased supportive care, the 5-year survival rate for children with ALL has increased significantly from 57 to 92%. Much of the improvement in survival rates represents the positive benefits of classifying patients into risk groups based on prognostic factors, and adjusting treatment intensity according to risk groups, in national and multinational clinical trials.7

Minimal residual disease (MRD) is when leukemia cells in the bone marrow are found at very low levels even though the patient is in complete remission (<5% of leukemia cells in the bone marrow sample). Acute lymphoblastic leukemia is responsible for the most malignancy-related deaths in the pediatric age group. It is the most common malignancy in children, with the peak incidence occurring at 3 to 5 years of age. The incidence of ALL in the United States is approximately 30 cases per one million population of children less than 20 years old, with the peak incidence occurring at 3 to 5 years of age. The incidence of ALL in Indonesia is estimated to be around 3000 new cases in children each year, where the number of event free survival (EFS) is around 50% far below developed countries which reach 80-90%. Much of the improvement in survival rates represents the positive benefits of classifying patients into risk groups based on prognostic factors, and adjusting treatment intensity according to risk groups, in national and multinational clinical trials.

Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Although the survival rate of pediatric patients with ALL is currently improving, cases of relapse still occur. Minimal residual disease (MRD) status is a prognostic factor that plays an important role in recurrence of ALL patients who have been through induction therapy.

Aim: To determine the survival rate in pediatric patients with ALL based on negative MRD examination is better than positive MRD at the end of therapy.

Method: A retrospective cohort study with inclusion criteria including pediatric patients aged 1 to 18 years who were diagnosed with ALL in the period of 2017-2019 at Sanglah General Hospital, Denpasar. Subject's recruitment was initiated when the minimum number of subjects was fulfilled.

Result: From 32 research subjects, the mean age was 5.1 years, the majority obtained MRD+ results, the dominant sex was male, the average leukocyte was 28.33 thousand, most of the subjects had no mediastinal masses, and generally experienced remissions. MRD levels were found to be significantly lower in remitting patients than patients who did not achieve remission (p value <0.05). The Kaplan-Meier estimates for event-free survival on MRD status (negative versus positive) were 84.6% and 42.1% (P = 0.030).

Conclusion: There was a significant relation between MRD levels and the survival rate to achieve remission and MRD levels are significantly lower in remitting patients than patients who do not achieve remission.

Keywords: acute lymphoblastic leukemia, minimal residual disease, remission, survival rate.


*Child Health Department, Faculty of Medicine, Universitas Udayana/RSUP I.G.N.G Ngoerah.

+ Corresponding author:
Dewa Gde Windu Sanjaya;
Child Health Department, Faculty of Medicine, Universitas Udayana/RSUP I.G.N.G Ngoerah.
windsanjaya666@gmail.com

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is the first malignancy in which early response assessment to therapy with monitoring of MRD has been shown to be a fundamental tool to guide treatment choice. The most standard methods for MRD studies in ALL are multi-parametric flow cytometry (MFC) and amplification methods with polymerase chain reaction (PCR). The technology improves MRD detection in ALL patients.

The Prof. IGNG Ngoerah General Hospital is one of the referral center hospitals in Bali and provinces outside Bali, including for pediatric ALL cases. With various previous studies on the benefits of MRD examination, the study of the survival rate of pediatric ALL patients using the MRD examination method at the end of therapy is an important and interesting research to be carried out, so that it can contribute to improving service and management of pediatric ALL patients.

METHODS

This study is a retrospective survival cohort study that aims to determine the survival rate of ALL patients with MRD + and MRD- status after undergoing therapy using the pediatric ALL chemotherapy protocol in 2013 at Prof. IGNG Ngoerah General Hospital in the 2017-2019 period with ethical clearance (No: 399/UN14.2.2.VII.14/LT/2023). The search used secondary data obtained from medical record data, hospitalization registers, and interviews with patients’ families. The exclusion criteria was children with ALL that do not pass the induction phase, pass the induction phase of week 6 but do not achieve remission with bone marrow examination, or not carried out MRD examination. Subjects were taken consecutively and stopped once the minimum number of subjects was reached. The validity of MRD test validity has been tested previously in research at RSAB Harapan Kita, Jakarta. Minimal residual disease results were obtained as a sensitive predictor factor that can detect 1 blast cell among 10,000 normal cells (0.01%), so that it can be used to improve the induction remission status in ALL.

The subjects were pediatric patients aged 1-18 years diagnosed with ALL who had undergone induction phase therapy after week 6, and had carried out MRD examinations in the period recorded since 2017 until the number of samples was met at Prof. IGNG Ngoerah General Hospital Denpasar which met the inclusion and exclusion criteria. Each subject is then grouped into 2 categories, namely MRD positive and MRD negative. Secondary data monitoring then proceeded to the consolidation, intensification and maintenance phases until the entire therapy protocol was completed for 110 weeks. In subjects who successfully passed the entire therapy process for 110 weeks, at the end of the phase an bone marrow aspiration was carried out to determine the status of the subject’s final category. The final category of observed subjects is divided into two, namely: remission, no remission (relapse / death).

RESULTS

This study included 36 child subjects diagnosed with ALL who had undergone induction phase therapy after week 6 and carried out MRD examination. A total of 4 subjects met the exclusion criteria because they did not achieve remission with BMA examination, so only 32 subjects were included as research subjects. In general, MRD examination carried out obtained MRD + results on 19 subjects (59.4%). The majority of subjects were male in 17 subjects (53.1%). Most subjects had no mediastinal mass (93.8%). The results of the Kolmogorov-Smirnov distribution test show age values and leukocytes are abnormally distributed so that they are used secondary data obtained from medical record data, hospitalization registers, and interviews with patients’ families. The exclusion criteria was children with ALL that do not pass the induction phase, pass the induction phase of week 6 but do not achieve remission with bone marrow examination, or not carried out MRD examination. Subjects were taken consecutively and stopped once the minimum number of subjects was reached. The validity of MRD test validity has been tested previously in research at RSAB Harapan Kita, Jakarta. Minimal residual disease results were obtained as a sensitive predictor factor that can detect 1 blast cell among 10,000 normal cells (0.01%), so that it can be used to improve the induction remission status in ALL.

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The Kaplan-Meier analysis method was used to compare the survival probabilities of two groups of positive MRD and negative MRD. The results of Log Rank test analysis show that there is a significant relationship between MRD levels and survival rates to achieve remission (p value <0.05). When comparing 2 groups of patients based on MRD status at end-induction, 19 MRD-positive patients at month 12 had significantly lower EFS than 13 MRD-negative patients (84.6% versus 42.61; P=0.030). Kaplan-Meier and Cox Regression as a follow-up research data analysis tool whose variable outcome is the length of time until the end point under study occurs. The results of cox regression analysis showed MRD is a strong prognostic factor for event-free survival where MRD negative patients have a higher likelihood of achieving remission than patients with positive MRD (adjusted hazard ratio 0.42; p <0.05).

DISCUSSION

The analysis was conducted on 36 child subjects who met the inclusion and exclusion criteria in the period January 2017 to December 2019. The median age in this study was 5.1 (5-8 years). This finding is in line with previous research that states the peak incidence of ALL is 2 and 5 years of age with 60% of cases occurring in individuals under the age of 20 years. The incidence of ALL in the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Age *</td>
<td>5.1 (3.98)</td>
</tr>
<tr>
<td>Minimal Residual Disease (MRD)</td>
<td>13 (40.6%)</td>
</tr>
<tr>
<td>MRD-</td>
<td>19 (59.4%)</td>
</tr>
<tr>
<td>MRD+</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (53.1%)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (46.9%)</td>
</tr>
<tr>
<td>Leucocyte*</td>
<td>28.33 (27.04)</td>
</tr>
<tr>
<td>Mediastinal Mass</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>2 (6.3%)</td>
</tr>
<tr>
<td>Not Present</td>
<td>30 (93.8%)</td>
</tr>
<tr>
<td>Remission</td>
<td></td>
</tr>
<tr>
<td>No Remission</td>
<td>14 (43.8%)</td>
</tr>
<tr>
<td>Remission</td>
<td>18 (56.3%)</td>
</tr>
</tbody>
</table>

*abnormal distribution of data and presented in median (interquartile range)
The presence of somatic mutations in the X-linked chromosome histone H3K27me3 demethylase ubiquitously transcribed X (UTX) causes more frequent manifestations of ALL in males than females. More specific analysis revealed that UTX tends to escape X chromosome inactivation in women and normal T cells, so UTX can work as a tumor suppressor. Given the role of UTX as a tumor suppressor, inactivation of only one copy of UTX in men will contribute to tumor development and conversely female cells will be protected from losing a single copy of UTX because they still express UTX from both alleles.¹³

The results of this study showed that 46.9% of T cell ALL patients suffered from leukocytosis, even 9.4% of them had hyperleukocytosis. These results are in line with research by Kong which found 58% of pediatric patients with a high risk of ALL experiencing leukocytosis, even 18% of them had leukocytosis levels of 100 - 200 x 10⁹ / L. The researchers’ findings are also in line with the results by Lee and Cho who state leukocytosis as a general presentation of ALL findings and correlate with the patient’s prognostic outcomes.⁶

Acute lymphoblastic leukemia is a result of a malignant transformation process of lymphocyte progenitor cells in B and T lineages. When an oncogene is activated by a mutation, the encoded protein is structurally modified so as to increase transformation activity, maintain the active state, transmit signals continuously through tyrosine and threonine-cinaline binding. These signals cause cell growth to continue including leukocyte growth until it reaches leukocytosis.¹⁰

Based on a literature review, children with ALL especially T cell types are often found with mediastinal masses and then progress to progressive leukemia rapidly.¹¹ Based on the results of this study, most children with ALL did not have a mediastinal mass (93.8%). These results are not in line with Attarbaschi in 2002 who found 53% of patients with ALL accompanied by mediastinal mass.¹² The results of the study are not in line with this theory can be caused by cases of mixed phenotype ALL, which appears with mediastinal masses are categorized as rare. Acute leukemia with a mediastinal mass represents a very small subset of acute leukemias that are not easily determined by either lymphoid or myeloid lineage. Acute leukemia with a mediastinal mass usually occurs in only one population in one lineage, after antigen expression builds up and crosses multiple lineages.¹³

The outcome of ALL patient therapy is still influenced by several prognostic factors, one of which is MRD status. Minimal residual disease status is a prognostic factor that plays an important role in relapse in ALL patients who have been induced.² Based on a literature review, high MRD rates (>1%) correlated strongly with poorer therapeutic outcomes, considering 5-year event-free survival in patients with negative MRD status was 94.6% versus 76.1% in patients with positive MRD status.¹⁴ The findings obtained in this study support this statement, namely found a significant relationship between MRD levels and survival rates to achieve remission (p=0.030). In a comparison of 2 groups of ALL patients with MRD status at the end of induction, MRD positive patients had lower event-free survival compared to MRD negative patients within 2 years of monitoring (42.61 versus 84.6%). Detection by MRD during induction and consolidation-remission therapy to date is arguably the most sensitive method for evaluating treatment response in childhood leukemia. The MRD assessment technique allows the detection of one leukemia cell among 10⁴ to 10⁵ normal cells, which shows a 100-fold increase in sensitivity compared to conventional bone marrow cytomorphology.¹⁴

Another study that supports the researchers’ data is the findings of Gandemer in 2014 who identified sex factors and MRD as having a significant relationship associated with 5-year survival in a multivariate analysis. A minimal residual disease of ≤0.01 represents a very small subset of acute leukemias that are not easily determined by either lymphoid or myeloid lineage. Acute leukemia with a mediastinal mass usually occurs in only one population in one lineage, after antigen expression builds up and crosses multiple lineages.¹³

The United States also shows a peak incidence at the age of 3 to 5 years.³ The high incidence in childhood is associated with various factors such as exposure to postnatal diagnostic radiation, exposure or use of herbicides during pregnancy, variant polymorphisms in some genes, as well as specific chromosomal abnormalities such as in identical twins.⁶

The review is in line with the results of a study conducted by researchers found male sex dominance in patients diagnosed with ALL, with ratio of 1:2.5: 1. T-cell ALL is an aggressive form of leukemia that is often diagnosed in children, with a pattern of sex distribution that is often unbalanced, especially in men. The tendency of L-cell ALL among boys is associated with specific mutations targeting X chromosome genes.¹³ The presence of somatic mutations in the X-linked chromosome histone H3K27me3 demethylase ubiquitously transcribed X (UTX) causes more frequent manifestations of ALL in males than females. More specific analysis revealed that UTX tends to escape X chromosome inactivation in women and normal T cells, so UTX can work as a tumor suppressor. Given the role of UTX as a tumor suppressor, inactivation of only one copy of UTX in men will contribute to tumor development and conversely female cells will be protected from losing a single copy of UTX because they still express UTX from both alleles.³
CONCLUSION

In conclusion, a significant relation was found between MRD levels and survival rates to achieve remission and MRD levels were significantly lower in remission patients than patients who did not achieve remission (p value <0.05) in our study. Suggestions for future research are expected to include a larger number of subjects and a longer observation period. This is considered important to be able to provide a better picture of survival in ALL patients with positive and negative MRD results.

CONFLICT OF INTEREST

None declared.

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All authors are equally contributing to the study from the conceptual framework, data gathering, data analysis until presenting the results through publication.

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