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# Diagnostic Accuracy of Bone Marrow Morphology to Determine Remission in Acute Lymphoblastic Leukemia Children: The Role of Minimal Residual Disease



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## ABSTRACT

**Background:** Relapse in acute lymphoblastic leukemia (ALL) patients are still widely found even though most patients experience remission at the end of the induction phase. Inaccurate determination of remission status due to the remaining leukemic cells that cannot be detected by bone marrow morphology examination is thought to play a considerable role in the occurrence of relapse.

**Objective:** To measure the accuracy of the bone marrow morphology in determining remission at the end of the induction phase of ALL.

**Methods:** Diagnostic tests were carried out in pediatric ward and laboratory of Sanglah Hospital and Dharmais Cancer Hospital Jakarta in January 2017 to March 2019. Bone marrow morphology and minimal residual disease (MRD) test was performed on ALL children

at the end of the induction phase. Minimal residual disease is the gold standard.

**Results:** Forty subjects with a median age of 5 years were included in this study. The median duration of induction phase was 75 days. Thirty-eight subjects (95%) had complete remission by bone marrow morphology, while only 16 (40%) subjects had complete remission by MRD. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of bone marrow morphology in this study were 100%, 8.3%, 42.1% and 100% respectively. Area under the curve (AUC) value of bone marrow morphology was 0.521 (95% CI 0.338 - 0.704).

**Conclusion:** Bone marrow morphology has high sensitivity but low specificity and PPV in determining remission at the end of the induction phase of children with ALL.

**Keywords:** bone marrow morphology, leukemia, children, minimal residual disease, diagnostic

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## INTRODUCTION

Relapse in acute lymphoblastic leukemia (ALL) patients is common. Some patients relapse within five years after diagnosis even though most patients experience remission at the end of the induction phase.<sup>1</sup> Many factors influence relapse in ALL patients, one of which is the success of risk stratification-based therapy.<sup>2</sup> Determination of the remaining blast cells in bone marrow supports the success of risk stratification-based therapy. Standard-risk ALL patients who do not experience remission at the end of the induction phase, must switch to high-risk protocols and get re-induction therapy.<sup>3</sup> An error in determining the remission status due to the remaining leukemic cells that cannot be detected by the bone marrow morphology examination is thought to play a role in the occurrence of relapse.

ALL is the most common malignancy in children, reaching one third of all childhood cancers and 75-80% of hematopoietic malignancies in children. ALL incidence varies in various parts

of the world, with an incidence of 3-4 cases per 100,000 children aged less than 15 years.<sup>1,4</sup> Study in Sanglah Hospital Denpasar has seen an increase in ALL incidence of 56 new cases in 2007-2011 to 84 new cases in 2011-2015. This increase is thought to be due to better diagnostic tools and referral system in Indonesia, especially in Bali.<sup>5,6</sup>

More than 95% of ALL patients achieve complete remission after receiving induction phase therapy characterized by less than 5% of the remaining blast cells in the bone marrow smear.<sup>7</sup> Oudot, et al. also found only 3.8% of patients failed to achieve remission at the end of the induction phase.<sup>8</sup> The study by Simanjanong, et al. showed that 50% of ALL patients experienced complete remission and 29% relapsed during 1997-2008 at Dharmais Cancer Hospital Jakarta. This study also found that the risk of mortality was increased to 3.35 times in ALL patients who failed to get remission compared to remission patients.<sup>9</sup> Errors in determining the remaining blast cells at the end of the induction

phase are thought to be one of the factors that play a role in this phenomenon.

Bone marrow morphology is still widely used in determining the amount of residual cancer cells in post-induction chemotherapy patients for the past few decades. Bone marrow morphology calculating the percentage of blast cells on the bone marrow smear using a light microscope. This examination is cheap, easy and simple, but very dependent on the operator and only able to detect one in 100 bone marrow mononuclear cells.<sup>10,11</sup>

Minimal residual disease (MRD) examination is used to detect the remaining cancer cells that cannot be detected using conventional morphological examination methods. The MRD method that currently most widely used is flow cytometry. Flow cytometry could detect abnormal immunophenotypes in leukemic cells. This method could detect cancer cells with a threshold reaching less than  $1 \times 10^4$  (<0.01%) of normal bone marrow cells. Minimal residual disease is also known to be strong and independent predictor in assessing survival and relapse in ALL patients.<sup>12,13</sup>

There are no studies that have tested the accuracy of bone marrow morphology in determining remission at the end induction phase using MRD examination as the gold standard. Previous studies only compare bone marrow morphology and MRD results. This research aims to evaluate whether the bone marrow morphology is accurate enough to determine the remaining blast cells at the end of the induction phase.

## MATERIAL AND METHODS

This study is a diagnostic test to determine the accuracy of bone marrow morphology examination in determining remission at the end induction phase in ALL children. The gold standard used is minimal residual disease. This study was done on January 2017 until March 2019 at pediatric ward Sanglah Hospital Denpasar, Clinical Pathology Laboratory of Sanglah Hospital Denpasar, and Clinical Pathology Laboratory of Dharmais Cancer Hospital Jakarta.

The target population was pediatric patients aged 0-18 years old suffer from ALL in the end of induction phase of chemotherapy. The inclusion criterias were pediatric patient aged 0-18 years old that diagnosed as ALL by pediatric hematology oncologist and got chemotherapy regimen based on Indonesian ALL High Risk 2013 or Indonesian ALL Standard Risk 2013 Protocols. Patients with incomplete data and ALL L3 (Burkitt Lymphoma) were excluded.

Subjects were consecutively enrolled until complete the required sample size. The minimum

subjects required in this study is 31 subjects. ALL patients who reach the end of the induction phase will perform bone marrow aspiration for bone marrow morphology and minimal residual disease examination. Bone marrow samples for morphology and MRD are taken at the same time with the same needle.

The bone marrow morphology examination was carried out by the Clinical Pathology Laboratory of Sanglah Hospital Denpasar and interpreted by at least two clinical pathology specialists who had the same competence. Minimal residual disease is carried out by the Clinical Pathology Laboratory of Dharmais Cancer Hospital Jakarta. The specimen transportation was handled as applicable standard operating procedures.

Bone marrow morphology is an examination of bone marrow smear by Giemsa staining to count the number of blast cells using a light microscope. Complete remission achieved if less than 5% of blast cells are obtained. Minimal residual disease is an examination that detects the small numbers of leukemic cells that remain in the bone marrow during chemotherapy using flow cytometry (FACSCalibur®). Remission achieved if the results are less than 0,01%.

Subject characteristics, bone marrow morphology results and MRD results were recorded and analyzed using SPSS 20.0 software. Descriptive data is presented in frequency distribution, percentage and mean. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of bone marrow morphology were obtained using 2x2 table analysis.

This study was performed under supervision of Hematology Oncology Division, Department of Child Health Medical Faculty of Udayana University-Sanglah Hospital Denpasar and approved by ethical clearance 1191/UN.14.2/KEP/2017 from the Ethics Committee of Faculty of Medicine, Udayana University-Sanglah General Hospital, Bali-Indonesia.

## RESULTS

During the study period there were 59 children diagnosed with ALL. Twelve children died before reaching the end of the induction phase, three children were lost to follow up, one child with L3 morphological results, and three children could not be examined by MRD, so there were 40 pediatric patients who met the inclusion criteria and did not meet exclusion criteria.

Of 40 subjects, 23 (57.5%) subjects were men with a median age of 5 years. The age range of the subject is between 1 year 6 months to 15 years 2 months. Most of the subjects with mild-moderate

**Table 1** Subjects characteristics

Variables	n= 40 f (%)
<b>Age, year, median (min - max)</b>	5 (1 - 15)
- <1 year	0 (0)
- 1 - <10 year	32 (80)
- > 10 year	8 (20)
<b>Sex</b>	
- Male	21 (52,5)
- Female	19 (47,5)
<b>Nutritional status</b>	
- Well-nourished	14 (35)
- Mild-moderate malnutrition	17 (42,5)
- Severe malnutrition	1 (2,5)
- Overweight	6 (15)
- Obesity	2 (5)
<b>Risk stratification</b>	
- Standard risk	20 (50)
- High risk	20 (50)
<b>FAB classification</b>	
- L1	6 (15)
- L2	34 (85)
<b>Induction phase duration, day, median (min - max)</b>	65 (50 - 125)
<b>Blast response</b>	
- Good response	32 (80)
- Poor response	8 (20)
<b>Bone marrow lymphoblast percentage, median (min - max)</b>	2 (1 - 5)

Notes: Abbreviations: FAB: French-British-America.

**Table 2** Bone marrow morphology and minimal residual disease

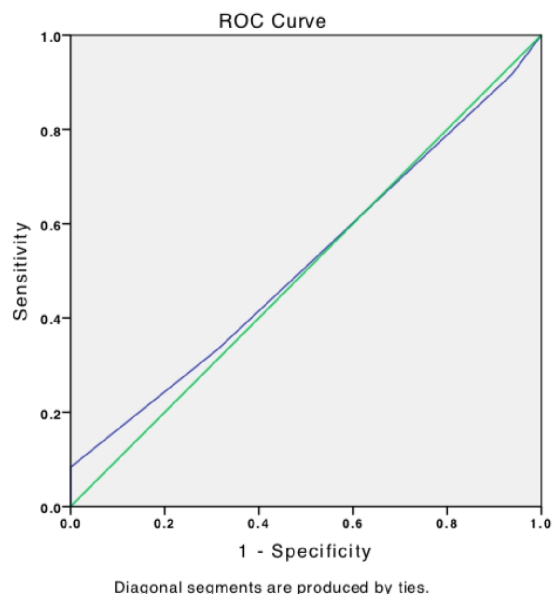
		Minimal Residual Disease		
		Remission	No remission	Total
Bone marrow morphology	Remission	16	22	38
	No remission	0	2	2
	Total	16	24	40

**Table 3** Diagnostic accuracy of bone marrow morphology

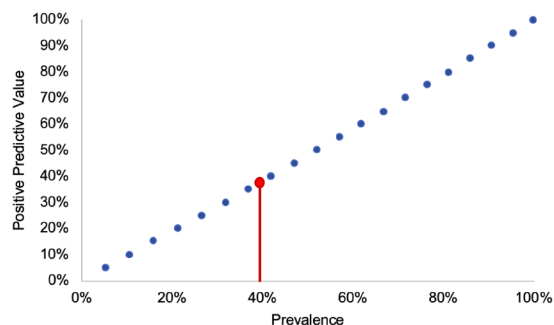
	Sn (%)	Sp (%)	PPV (%)	NPV (%)	LR+	LR-	Acc (%)	PTP
Bone marrow morphology	100	8,3	42,1	100	1,09	0	45	0.42

Notes: Abbreviations: Sn: Sensitivity; Sp: Specificity; PPV: positive predictive value; NPV: negative predictive value, LR+: positive likelihood ratio; LR-: negative likelihood ratio, Acc: accuracy, PTP: post-test probability

malnutrition (40%). Twenty people were classified as standard-risk and the remaining 20 (50%) subjects were classified as high-risk. The median



**Figure 1** Receiver operating characteristic (ROC) curve of bone marrow morphology



**Figure 2** The effect of prevalence on the positive predictive value

duration of the induction phase in this study was 75 days with most (80%) subjects having a good blast response during the first week of the induction phase. Most (95%) subjects had complete remission in bone marrow morphology, only two (5%) subjects not achieved remission. The median lymphoblast detected in bone marrow morphology was 2%. Most (60%) subjects not achieved remission and only 16 (40%) subjects achieved remission by MRD examination. Details of the subject characteristics are shown in Table 1.

Complete remission by bone marrow morphology is determined by the number of residual blasts in the bone marrow. A cut-off point of 5% is still used in the worldwide guidelines for leukemia as the optimal cut-off point for complete remission. Of the total 38 subjects achieved remission according to bone marrow morphology, only 16 (42.1%) subjects were achieved remission according to MRD and the remaining 22 (57.9%) subjects were not remission. There were two subjects who were not achieved remission based on bone marrow

morphology and both were also declared not achieved remission according to MRD (Table 2).

This study shows that bone marrow morphology has a very high sensitivity value (100%) but the specificity value is very low (8.3%). The positive predictive value of bone marrow morphology was only 42.1% with positive likelihood ratio only 1.09 indicating poor diagnostic value in determining remission of ALL patients at the end of the induction phase (Table 3). The AUC value of bone marrow morphology was only 0.521 (95% CI 0.338 - 0.704), indicating poor diagnostic value. (Figure 1). Prevalence in this study was 40% with PPV 42.1%. The effect of prevalence on the positive predictive value was shown in Figure 2.

## DISCUSSION

In this study, bone marrow morphology has a 100% sensitivity and 8.3% specificity at 5% cut-off points of blast as complete remission markers. There are no previous studies that study the diagnostic value of bone marrow morphology with MRD as the gold standard for determining remission. Several previous studies only compared the results of remissions based on morphology and MRD. The study by Andriastuti in Jakarta found remission discordance based on bone marrow morphology and MRD was 15.2%.<sup>14</sup> Wongprajun and Auewarakul in Thailand found remission discordance based on bone marrow morphology and MRD reached 51% with a 0.07 kappa test result which showed a very low agreement.<sup>15</sup>

This study showed bone marrow morphology sensitivity for determining complete remission in ALL patients was 100%. This result shows that bone marrow morphology can detect all ALL patients who achieved complete remission accurately. On the other hand, bone marrow morphology specificity is very low at 8%. These results indicate the ability of bone marrow morphology to determine ALL patients who not achieved remission is very poor. Determination of patients who do not achieved remission at the end of the induction phase is very important especially in determining the need for chemotherapy re-induction.

Determination of remission by calculating the percentage of bone marrow blast cells through conventional morphological examination has been used for decades, but this examination has several limitations. The main limitation of the bone marrow morphology is the low ability to detect the remaining leukemic cell. This examination only could detect one cell in 100 bone marrow mononuclear cells. The ability to detect these blast cells also depends on experience, ability and operator

interpretation.<sup>10,11</sup> Lymphoblast are also often detected incorrectly as normal blood cells which are morphologically very similar to blast cells, which are precursors of immature B cells whose numbers increase due to bone marrow regeneration.<sup>16</sup> A study by Birkhead, Salt and Jackson found a low agreement between observers in determining remission with bone marrow morphology.<sup>17</sup>

The use of bone marrow morphology as the gold standard for determining remission at the end of the induction phase has been used for the past several decades throughout the world, including at Sanglah Hospital Denpasar. Some ALL patients still experience relapse even though most patients experience remission at the end of the induction phase according to bone marrow morphology.<sup>17</sup> During the last 20 years, MRD examination was developed that was better to detect the remaining leukemic cells. Minimal residual disease could detect by flow cytometry and polymerase chain reaction (PCR).<sup>18</sup> Pui and Campana proposed a new definition of complete remission in the form of finding bone marrow blast cells <0.01% identified by MRD.<sup>19</sup> Minimal residual disease is considered to be 100 times more sensitive than bone marrow morphology because it can detect one blast cell among 100,000 normal cells.<sup>12</sup> Some previous studies have found that MRD is considered better in determining remission and relapse in ALL than bone marrow morphology. Gupta et al. recommends the use of MRD at the end of the induction phase to determine the failure of induction and determine the outcome of the patient.<sup>16</sup>

The UKALL 2003 study showed ALL patients who achieved remission based on morphology at the end of induction therapy, but with MRD indicating not remission results had poor outcomes, similar to those of patients with morphologic induction failure. Based on these results, the United Kingdom (UK) working group proposed a new definition of remission failure in ALL patients: >5% residual blast, measured by either morphology or MRD.<sup>20</sup>

ALL protocol in Indonesia still uses therapy based on bone marrow morphology. Patients need to be re-induction if the bone marrow morphology shows no remission.<sup>3</sup> This study shows that bone marrow morphology is not good at determining remission at the end of the induction phase of ALL patients, so that it can be a consideration of the need for MRD-guided therapy in Indonesia. Several previous studies have tried MRD-guided therapy in ALL patients. Randomized controlled trial by Vora et al. in 533 high-risk ALL patients showing MRD-guided therapy could be beneficial in reducing the risk of relapse but there were no



significant differences in overall survival rates.<sup>21</sup> MRD-guided therapies have also been tried in acute myeloid leukemia (AML) and myelodysplasia syndrome with very satisfactory results.<sup>22</sup>

## STUDY LIMITATION

This study did not analyse the agreement between Clinical Pathologist who examined the bone marrow morphology.

## CONCLUSION

Bone marrow morphology has high sensitivity but low specificity and PPV in determining remission at the end of the induction phase of children with ALL.

## AUTHOR CONTRIBUTION

All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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## DISCLOSURE

The author reports no conflicts of interest in this work.

## REFERENCES

1. Terwilinger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J*. 2017; 7: e577.
2. Shen S, Cai J, Chen J, Xue H, Pan C, Gao Y, et al. Long-term results of the risk-stratified treatment of childhood acute lymphoblastic leukemia in China. *Hematol Oncol*. 2018; 36: 679-688.
3. UKK Hematologi Onkologi IDAI. Panduan Protokol Pengobatan Leukemia Limfoblastik Akut Anak-2013. Jakarta: Ikatan Dokter Anak Indonesia; 2013.
4. Sousa DW, Ferreira FV, Felix FH, Lopes MV. Acute lymphoblastic leukemia in children and adolescents: prognostic factors and analysis of survival. *Rev Bras Hematol Hemoter*. 2015; 37: 223-229.
5. Widyanti PA, Ariawati K, Subanada IB. Karakteristik anak dengan leukemia limfoblastik akut di RS Sanglah Denpasar. 2012.
6. Tarigan AT, Ariawati K, Widnyana AA. Prevalens dan karakteristik anak dengan leukemia limfoblastik akut tahun 2011-2015. 2016.
7. Schrappe M, Hunger SP, Pui CH, Saha V, Baruchel A, Conter V. Outcomes after induction failure in childhood acute lymphoblastic leukemia. *N Engl J Med*. 2012; 366: 1371-81.
8. Oudot C, Auclerc MF, Levy V, Porcher R, Piguet C, Perel Y. Prognostic factors for leukemic induction failure in children with acute lymphoblastic leukemia and outcome after salvage therapy: The FRALLE 93 Study. *J Clin Oncol*. 2008; 26: 1496-1504.
9. Simanjorang C, Kodim N, Tehuteru E. Perbedaan kesintasan 5 tahun pasien leukemia limfoblastik akut dan leukemia mieloblastik akut pada anak di Rumah Sakit Kanker Dharmais, Jakarta, 1997-2008. *Indones J Cancer*. 2013; 7: 15-21.
10. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Beau MM. The 2016 revision to the World Health Organization classification of myeloid neoplasm and acute leukemia. 2016; 127: 2391-2405.
11. Islam A. Manual of Bone Marrow Examination. Philadelphia: Trafford; 2013.
12. Van Dongen JJ, Velden VH, Bruggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: a need for sensitive, fast and standardized technologies. *Blood*; 2015: 125; 3996-4009.
13. Rocha JM, Xavier SG, Souza ME, Assumpcao JG, Murao M, Oliviera BM. Current strategies for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. *Mediterr J Hematol Infect Dis*. 2016; 8: 1-12.
14. Andriastuti, M. Respons Steroid sebagai Faktor Prognostik Kesintasan Leukemia Limfoblastik Akut pada Anak: Tinjauan Khusus pada Penilaian Imunofenotiping, Sitogenetik, Molekular, dan Minimal Residual Disease (*disertasi*). Jakarta: Universitas Indonesia; 2015.
15. Wongprajun S, Auewarakul CU. A method comparison study of flow Cctometry and cytomorphology to determine the percentages of blasts in patients with acute leukemia after induction and consolidation chemotherapy. *J Med Assoc Thai*. 2010; 93: 157-64.
16. Gupta S, Devidas M, Loh ML, Raetz EA, Chen S, Wang C, et al. Flow-cytometric vs morphologic assesment remission in childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group (COG). *Leukemia*. 2018; 32: 1370-9.
17. Permatasari E, Windiastuti E. Survival and prognostic factors of childhood acute lymphoblastic leukemia. *Pediatr Indones*. 2009; 49: 365-71.
18. Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. *Lancet*. 1998; 351: 550-4.
19. Pui CH, Campana D. New definition of remission in childhood acute lymphoblastic leukemia. *Leukemia*. 2000; 14: 783-5.
20. O'Connor D, Moorman AV, Wade R, Hancock J, Tan RM, Bartram J. Use of minimal residual disease assesment to redefine induction failure in pediatric acute lymphoblastic leukemia. *J Clin Oncol*. 2017; 35: 660-7.
21. Vora A, Goulden N, Mitchell C, Hancock J, Hough R, Rowntree C. Augmented post-remission therapy for a minimal residual disease high-risk sub-group of children and young people with clinical standard-risk and intermediate-risk acute lymphoblastic leukaemia (UKALL 2003): A Randomised Controlled Trial. *Lancet Oncol*. 2014; 15: 809-18.
22. Platzbecker U, Middeke JM, Sockel K, Herbst R, Wolf D, Baldus CD, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-Label, multicentre, phase 2 trial. *Lancet Oncol*. 2018; 19: 1668-79.



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