Prognostic factors affecting remissions based on minimal residual disease (MRD) examination in paediatric acute lymphoblastic leukaemia patients after induction phase treatment

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Abstract

Introduction: Acute lymphoblastic leukaemia (ALL) is the commonest cancer in children. Remission in the induction phase plays an important role in prognosis. Minimal residual disease (MRD) examination can detect cancer cell residues not detectable by conventional morphological methods.

Objectives: To identify prognostic factors affecting ALL remissions at the end of the induction phase of chemotherapy treatment using MRD examination as the paediatric gold standard.

Method: This is a retrospective cohort study. Forty-six children diagnosed with ALL aged 1 to 18 years in the paediatric ward of Sanglah General Hospital, Bali, Indonesia were enrolled in this study from 2017 to 2019. Data with categorical variables were analysed using the Chi-Square test. A p-value <0.05 was considered statistically significant.

Results: A total of 46 subjects, aged 1 to 10 years, was included in this study. Twenty-five (54.3%) patients experienced relapse, 21 (45.7%) of them achieving remission by MRD examination at the end of the induction phase. Bivariate test results showed a significant difference in therapeutic response based on L1 type (p = 0.028) and platelet levels (p = 0.017). Multivariate test results showed a platelet level >30,000/ cu mm caused remission in ALL patients (CI 95% 1.486-28.326, p=0.013). The L1 type was significantly associated with treatment response (95% CI 0.013-0.617, p = 0.014).

Conclusions: Platelet levels >30.000/cu mm and L1 type of leukaemia are prognostic factors for remissions based on examination of MRD in paediatric patients with ALL after induction phase.

(Key words: Acute lymphoblastic leukaemia, Prognostic factor, Minimal residual disease)

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Introduction

Acute lymphoblastic leukaemia (ALL) is the commonest lymphoid progenitor cell disease malignancy in children and represents 75–80% of acute leukaemia in this age group[1,2]. A study in Sanglah General Hospital, Bali, Indonesia, has seen an increase in ALL incidence, 56 new cases in 2007-2011 compared to 84 new cases in 2011-2015. This increase is thought to be due to better diagnostic tools and referral system in Indonesia, especially Bali[3,4]. ALL treatment consists of several phases, one of which is the induction phase[5,6]. Remission in induction phase plays an important role in prognosis of ALL patients[7]. Simanjorang C, *et al*[8] showed that 50% of ALL patients experienced complete remission and 29% relapsed during 1997-2008 at Dharmais Cancer Hospital, Jakarta, Indonesia.

Minimal Residual Disease (MRD) examination is used to detect the remaining cancer cells that cannot be detected using conventional morphological examination methods. Peripheral blood or bone marrow samples can be analysed with either polymerase chain reaction (PCR) or flow cytometry. This could detect cancer cells with a threshold less than $1x10^4$ (<0.01%) of normal bone marrow cells. MRD examination is a strong and independent predictor in assessing survival and relapse in ALL patients[9-11]. The potential value of a prognostic factor in ALL is providing information on the risk of relapse so that treatment can be modified to minimise chemotherapy toxicity without compromising survival[12]. Many prognostic factors can affect remission in ALL patients such as age, sex, leucocyte level, haemoglobin, initial platelet count at time of diagnosis, immunophenotypes, cytogenetics, peripheral blood blast cell levels on 8th day of therapy, French-American-British (FAB) classification and nutritional status[13,14]. There are limited studies about prognostic factors in children

with ALL in Sanglah General Hospital using MRD to determine the occurrence of remission after induction phase chemotherapy treatment.

Objectives

To identify prognostic factors affecting ALL remission at the end of the induction phase of chemotherapy treatment using MRD examination as the paediatric gold standard.

Method

This study was done from 2017 to 2019 in the paediatric ward and clinical pathology laboratory of Sanglah General Hospital, Bali, Indonesia, and clinical pathology laboratory of Dharmais Cancer Hospital, Jakarta, Indonesia. The MRD examination detects the small numbers of leukaemic cells that remain in the bone marrow during chemotherapy using flow cytometry (FACSCalibur®), carried out by the clinical pathology laboratory of Dharmais Cancer Hospital, Jakarta. Remission is achieved if the results are less than 0.01%.

The target population was paediatric patients aged 1-18 years with ALL at the end of the induction phase of chemotherapy. The inclusion criteria were paediatric patients aged 0-18 years, diagnosed as ALL. Patients with incomplete data and ALL L3 (Burkitt Lymphoma) were excluded.

Subjects were consecutively enrolled until they completed the required sample size. The minimum subjects required in this study was 46. Medical records of ALL patients were assessed to collect MRD examination data. Subjects were then divided into remission and relapse. Subject identity and data variables needed were obtained and recorded in the research form.

Ethical issues: This study was performed under the supervision of the Haematology-Oncology Division, Department of Child Health, Medical Faculty of Udayana University-Sanglah Hospital Denpasar and approved by the Ethics Committee of the Faculty of Medicine, Udayana University-Sanglah General Hospital, Bali, Indonesia (No. 2305/UN14.2.2.VII.14/LP/2019).

Statistical analysis: Data with categorical variables were analysed using the bivariate test (Chi-Square). Those with a significant result ($p<0.05$) were subjected to multivariate logistic regression analysis. Results were interpreted from the multivariate analysis by calculating the probability of occurrence of each variable, which was significant ($p<0.05$). Study results were recorded and analysed using SPSS 20.0 software (SPSS Inc., Chicago, IL).

Results

During the study period, there were 51 children diagnosed with ALL and underwent the MRD examination. Five children had incomplete medical records. Thus, there were 46 paediatric patients who met the inclusion criteria. Of the 46 patients, 28 (60.9%) were males and 18 (39.1%) were females. The patient's characteristics are summarized in Table 1.

Table 1: ***Subject characteristics according to therapeutic response (n=46)***

Variable	Total number	Therapeutic response	
		Remission *(n=21)*	**Relapse *(n=25)***
Age (years) - n (%)			
1-10	36 (78.3)	16 (44.4)	20 (55.6)
>10	10 (21.7)	05 (50.0)	05 (50.0)
Sex - n (%)			
Male	28 (60.9)	11 (39.3)	17 (60.7)
Female	18 (39.1)	10 (55.6)	08 (44.4)
Leucocyte count - n (%)			
≥ 50.000/ cu mm	09 (19.6)	06 (66.7)	03 (33.3)
< 50.000/ cu mm	37 (80.4)	15 (40.5)	22 (59.5)
Nutritional status - n (%)			
Not obese	38 (82.6)	17 (44.7)	21 (55.3)
Obese	08 (17.4)	04 (50.0)	04 (50.0)
FAB classification - n (%)			
L1	10 (21.7)	08 (80.0)	02 (20.0)
L2	36 (78.3)	13 (36.1)	23 (63.9)
Haemoglobin level - n (%)			
< 8 g/dl	22 (47.8)	10 (45.5)	12 (54.5)
> 8 g/dl	24 (52.2)	11 (45.8)	13 (54.2)
Platelet count - n (%)			
> 30.000/ cu mm	22 (47.8)	10 (45.5)	12 (54.5)
≤ 30.000/cu mm	24 (52.2)	11 (45.8)	13 (54.2)

FAB: French-British-American

Of the 46 patients, 21 (45.7%) had a remission, and 25 (54.3%) had a relapse based on MRD examination at the end of remission induction therapy. Bivariate analysis in Table 2 showed that L1 type and platelet count were significantly associated with a therapeutic response (p = 0.028 and p = 0.017 respectively). Relapse tended to occur in patients with haemoglobin levels ≥ 8 g/dl and obese, but these results were not statistically significant (p > 0.05).

Table 2: ***Bivariate analysis result***

Variable	Therapeutic response		p-value
	Remission *(n = 21)*	**Relapse *(n = 25)***	
Nutritional status - n (%)			
Not obese	17 (44.7)	21 (55.3)	1.000
Obese	04 (50.0)	04 (50.0)	
FAB classification - n (%)			
L1	08 (80.0)	02 (20.0)	0.028
L2	13 (36.1)	23 (63.9)	
Haemoglobin level - n (%)			
< 8 g/dl	10 (45.5)	12 (54.5)	0.979
> 8 g/dl	11 (45.8)	13 (54.2)	
Platelet count - n (%)			
> 30.000/cu mm	15 (62.5)	09 (37.5)	0.017
< 30.000/cu mm	06 (27.3)	16 (72.7)	

FAB: French-British-American

Multivariate analysis result using multiple logistic regression, presented in Table 3, showed that remissions were significantly more in patients whose platelet counts were >30.000/cu mm on early diagnosis, compared to those with platelet counts <30,000/ cu mm (Exp (B) 6.488, 95% CI 1.486-28.326, p = 0.013). L1 type was a statistically significant prognostic factor for therapeutic response (Exp (B) 0.019, 95% CI 0.013-0.617, p = 0.014).

Table 3: ***Multivariate analysis result***

Variable	B	Exp (B)	p-value	95% CI
Platelet count	1.870	6.488	0.013	1.486-28.326
FAB Classification	-2.400	0.091	0.014	0.013-0.617

FAB: French-British-American; Constanta: -0.180

The variables that affected remissions were platelet levels and L1 type. The strength of the greatest association was a platelet count >30,000 / cu mm (OR = 6.49). The application of the equation obtained is to predict the probability of a patient experiencing remission. The probability is calculated based on the results of the multivariate test, and the results of the patient's greatest probability of remission (54.5%) are patients with platelet levels > 30,000 / cu mm with the L1 type.

Discussion

This study included 46 ALL patients with 36 (78.3%) aged 1-10 years. A study by Sousa DW, *et al*[1]. showed similar results with a mean age at diagnosis of 6.3 ± 0.5 years. Age at diagnosis is an important prognostic factor for ALL survival in paediatric patients[1]. The highest peak event-free survival (EFS) was found in children diagnosed in the age range 1-9 years, then decreasing with increasing age[1,15]. Children <1 year of age at diagnosis had the lowest survival[16]. The poor prognosis seen in infants and adolescents with ALL compared to patients aged 1-10 years is associated with several conditions. ALL in children <1-year-old at diagnosis is associated with high leucocyte counts and the Mixed Lineage Leukaemia (MLL) gene re-arrangement system in up to 80% on chromosome 11q23. The existence of the MLL gene reset system causes infants to receive more intensive therapy than other age groups, even with agents that are rarely used, such as high doses of cytarabine[17,18]. Older ages at diagnosis of ALL are also known to be associated with a worse prognosis. Children diagnosed with ALL at the age of 10-12 years had more T cell leukaemia, high leucocyte counts, a lower incidence of beneficial chromosomal abnormalities (high hyperdiploidy and TEL/AML1 fusion), and a higher incidence for the Philadelphia chromosome [t (9; 22)]. Adolescents were also at high risk for complications due to therapy such as osteonecrosis, pancreatitis, and deep vein thrombosis, which can also affect prognosis[17,19]. In our study, remissions were more common in the age group >10 years, although there was no significant

difference between the 1-10-year and >10-year age groups.

Gender is often an independent prognostic factor in ALL patients. In our study, 28 (60.9%) patients with ALL were males, and remissions tended to occur in girls, although this was not statistically significant. Sousa DW, *et al*[1] found that 65.8% were male with a male to female ratio of 1.9: 1 and Permatasari E, *et al*[15] found that 63.9% of ALL patients were male with a male to female ratio of 1.7: 1, but there was no difference in survival rates between male and female patients.

The number of leucocytes at diagnosis is an independent prognostic factor based on various recent studies. Since 1996, based on guidelines developed by The Cancer Therapy Evaluation Programme (CTEP) of the National Cancer Institute (NCI), a leucocyte count of 50,000/cu mm is usually used as the threshold to classify patients as either high risk or standard risk[19]. A total of 37 (80.4%) patients in this study had a leucocyte count <50,000/ cu mm when diagnosed with ALL. Sousa DW, *et al*[1] found that 21% of patients had a leucocyte count >50,000/cu mm. Patients with severe leucocytosis at diagnosis were associated with larger tumour mass, mediastinal enlargement, hepatosplenomegaly and significant lymphadenopathy. These findings also correlated with translocation of t (4;11) and t (9;22) chromosomes which had a worse prognosis. Patients with a leucocyte count >50,000/ cu mm had a risk of relapse 589 times compared to those with aa leucocyte count <50,000/ cu mm[1]. Research by Rahim P, *et al*[20] showed that a leucocyte count of 50,000-100,000/cu mm was associated with a higher incidence of remission after induction therapy. Different results were found in our study. The remission incidence was more in patients with leucocyte counts <50,000/ cu mm compared to patients who had leucocyte counts ≥ 50,000/mm^3, but this result was not statistically significant.

In our study, the majority of patients who experienced remission had haemoglobin (Hb) levels <8 g/dl, but this difference was not statistically significant. Rahim P, *et al*[20] found the incidence of remission as high as 100% in patients with Hb levels <5 g/dl. Study by Perdani RRW, *et al*[21] at Kariadi Hospital on 55 paediatric patients with ALL also did not show a significant relationship between Hb levels and remission, and remissions were more common in children with anaemia. The difference in prognosis based on Hb levels is thought to be because the lower Hb levels (Hb <8g /dl) are more frequently found in types of leukaemia with better outcomes (TEL/AML1, hyperdiploidy). The more aggressive types of leukaemia (T cell leukaemia and BCR-ABL) are associated with higher Hb levels. T-cell leukaemia patients with lower Hb levels have better outcomes[22].

Thrombocytopenia is the most common cause of bleeding and also relapse in ALL patients. This study showed the remissions tended to occur in patients with platelet counts >30,000/cu mm and this was statistically significant. Perdani RRW, *et al*[21] performed a study on 55 patients with ALL showing that as many as 56% of patients had thrombocytopenia (<150,000/cu mm) and this was significantly associated with a lower remission rate than normal platelet levels or thrombocytosis. It can be seen that there are differences in the platelet cut-offs used in different studies, thus allowing for differences in results.

French-American-British (FAB) classification of ALL cases is based on morphology, immunophenotypes, and cytogenetic features. FAB differentiates ALL into L1, L2, and L3 types[15]. Leukaemia with L2 type is known to have a higher relapse incidence and a worse prognosis compared with the other morphologies. L2 type blast cells were more resistant to anti-cancer treatments than L1 type blast cells[23]. In this study, 78.3% had the L2 type. The remission was more common in patients with a L1 type ALL classification, and it was statistically significant. Sousa DW, *et al*[1] also showed that 83% of ALL patients have an L1 classification and the remaining 17% have an L2 classification. A study conducted at Dharmais Cancer Hospital showed that 80.3% of patients with L1 status experienced remission, which was not significantly different from the L2 subtype (80%)[20].

Obesity is often associated with a higher risk of relapse because the chemotherapy doses received were inadequate. Obesity was also associated with an increased risk of cancer due to the role of adipocytes in metabolic function, immune system, and endocrine physiology, each of which contributes to the carcinogenesis process[24]. In this study, as many as 8 (17.4%) cases were obese. Patients who were not obese were more likely to experience remission than those who were obese, but this result was not statistically significant. Sari TT, *et al*[24] demonstrated that only 2 out of 12 children (6.1%) with ALL were categorized as obese. A study by Athifah A, *et al*[25] of 45 ALL children in Surabaya also showed that there was no correlation between nutritional status and remission of ALL patients at the end of the induction phase. Saenz AM, *et al*[26] at Johns Hopkins All Children's Hospital involving 181 children with ALL also did not show a significant association between obesity status and mortality due to the small sample size. However, their meta-analysis showed an increased risk of mortality in overweight patients or obese patients.

Their study also showed an increased risk of relapse in obese patients aged ≥10 years (HR = 2.89, 95% CI = 0.89-9.36), but not in children younger than 10 years. Our study only distinguishes subject nutritional status as obese and non-obese. The other nutritional status, such as malnourished (underweight, stunting, and wasting) and normal-weight children, may be considered in the next study as they also have a different prognosis in ALL therapy.

Conclusions

In this study, platelet count >30,000/cu mm found on early diagnosis and L1 type morphology were the prognostic factors for remission based on MRD in ALL paediatric patients after induction phase treatment. It was also found that factors such as haemoglobin level <8.00 g/dL and non-obese nutritional status on early diagnosis cannot be used as the prognostic factor for remission in ALL paediatric patients.

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