

Relationship between Bax and Bcl-2 Protein Expression and Outcome of Induction Phase Chemotherapy in Pediatric Acute Lymphoblastic Leukemia

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Abstract

Background: Overexpression of the antiapoptotic protein Bcl-2 causes apoptosis to stop and conversely the increased expression of the proapoptotic protein Bax makes lymphoblasts easy to destroy. The Bax/Bcl-2 ratio plays a role in the balance of apoptosis, immortality, resistance, and outcome of chemotherapy. We analyzed the relationship between the Bax/Bcl-2 ratio and the outcome of induction phase chemotherapy in pediatric Acute Lymphoblastic Leukemia (ALL). **Methods:** This research was conducted with a prospective observational study on pediatric ALL aged 1-18 years who were newly diagnosed based on bone marrow aspiration (morphology and immunophenotyping) at Dr. Soetomo General Hospital, Surabaya on October 2020 to March 2021. Expression of Bcl-2, Bax, and Bax/Bcl-2 protein ratio was measured by the flow cytometry method from lymphoblast on bone marrow aspirate samples before and after induction phase chemotherapy according to the 2018 Childhood ALL Indonesian Protocol. The outcomes evaluated were survival and remission rate (lymphoblasts in the bone marrow less than 5%). We used the Mann-Whitney U test and Wilcoxon Signed Rank test to analyze the differences between protein expression with $p < 0.05$ for a two-tailed test. **Results:** We included 17/26 pediatric ALL, consisting of 88% male, 94% LLA-L1, 76% B cell ALL and 24% T cell ALL. Mean expression of Bax, Bcl-2, and Bax/Bcl-2 protein ratio before chemotherapy among pediatric ALL who alive (N=11) and dead (N=6) were not significantly different ($p > 0.05$). All children who completed the induction phase of chemotherapy went into remission. Bax and Bcl-2 expression before and after chemotherapy showed no difference ($p > 0.05$). The Bax/Bcl-2 ratio increased from 1.74(SD 1.846) to 6.17(4.139) with $p = 0.021$. **Conclusion:** Expression of Bax, Bcl-2, and Bax/Bcl-2 protein ratio at the beginning of diagnosis did not affect the survival of pediatric ALL after the induction phase of chemotherapy. The Bax/Bcl-2 protein ratio increased 3.5 times in pediatric ALL with remission outcomes, indicating proapoptotic dominance.

Keywords: Pediatric acute lymphoblastic leukemia- Bax- Bcl-2- apoptosis chemotherapy- survival and remission rate

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, with survival reaching 70-90% despite chemotherapy failure and relapse (Pui et al., 2008; 1998; Lanzkowsky, 2011; Kapoor and Singh, 2018). Data in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, about 223 children underwent chemotherapy with the 2006 Children ALL Indonesian Protocol between 2006 and 2011. The death occurred in 22% of children before chemotherapy with a 5-years free survival rate of only 36%. The outcome of post-induction chemotherapy remission with the 2013 Children ALL Indonesian protocol was only 46.4% of the 143 children diagnosed with ALL between 2013-2014. Failure to

achieve remission, relapse, and death are still challenges in the field of oncology (Pui et al., 2008; Lanzkowsky, 2011; Kapoor and Singh, 2018).

The imbalance between proapoptotic and antiapoptotic proteins is associated with remission in pediatric ALL (Hassan et al., 2014). The permeability transition pore (Pt.pore) of mitochondria regulated intrinsic pathway apoptosis. Bax and Bcl-2 proteins managed the opening and closing of Pt.pore. The Bcl-2 protein normally binds to the mitochondrial Pt.pore so that the Pt.pore close, contrary to the action of Bax. The opening of Pt.pore stimulates the release of Apaf-1, which activates caspase-9 as an initiator of apoptosis. Lymphoblast susceptibility to apoptosis was influenced by proapoptotic protein expression (Bax, p53, p21, Bcl-10, Bak, Bid, Bad, Bim, Bik, Blk, Apaf-1, and

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caspase-9) and antiapoptotic proteins expression (Bcl-2, Mdm-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, BAG, MCL-1, and Hsp-70) (Hogarth and Hall, 1999; Liu et al., 2002; Llambi and Green, 2011; Yogosawa and Yoshida, 2018; Singh et al., 2018; Khanzadeh et al., 2018; Ghasemi et al., 2018).

Overexpression of Bcl-2 in cancer caused Pt.pore to close and stop the apoptosis, and cells become immortal. Conversely, an increase in proapoptosis of Bax makes lymphoblasts easy to destroy (Hogarth and Hall, 1999; Liu et al., 2002; Tzifi et al., 2012; Hassan et al., 2014; Trino et al., 2016; Singh et al., 2018; Ghasemi et al., 2018; Kapoor and Singh, 2018). The Bax/Bcl-2 protein ratio has also played a role in the balance of apoptosis, immortality, resistance to chemotherapy, remission, and survival outcomes (Taylor et al., 2008; Llambi and Green, 2011; Kapoor and Singh, 2018; Singh et al., 2018; Ghasemi et al., 2018). The purpose of this study was to analyze the relationship between Bax and Bcl-2 protein expression and the outcome of induction phase chemotherapy in pediatric ALL.

Materials and Methods

The research was a prospective observational study on pediatric ALL aged 1-18 years newly diagnosed at the Division of Hematology-Oncology, Department of Child Health, Dr. Soetomo General Academic Hospital, Surabaya, East Java on October 2020 to March 2021. Research ethics were obtained from the Health Research Ethics Committee of Clinical Research Unit (CRU) on Dr. Soetomo General Academic Hospital, Surabaya, and a letter of exemption Ref. No.: 0116/LOE/301.4.2/IX/2020. All research subjects must obtain written consent from parents by signing the consent form. This research will disguise the identity of the subject by giving a code. Researchers only used data for research and publication purposes.

The minimum sample size was calculated based on the estimation formula for proportion data. Remission rates of ALL chemotherapy in the proapoptotic expression dominant group were 94%. Remission rates of ALL chemotherapy in the antiapoptotic expression dominant group were 85% (Singh et al., 2018). The error rate (α) was 5%, the standard for a two-tailed test was 1.96. The difference in the desired proportion was 20%. The number of samples required was at least 11 pediatric ALL.

We reported age, gender, hepatomegaly, splenomegaly, and lymphadenopathy. Hemoglobin levels were grouped into hemoglobin less than 8 g/dL and more than 10 g/dL. Thrombocyte counts were classified as thrombocytopenia if the platelets count was less than $150 \times 10^3/\mu\text{L}$. Leukocyte counts were grouped into leukocytes less than $4 \times 10^3/\mu\text{L}$, $4-20 \times 10^3/\mu\text{L}$, and more than $20 \times 10^3/\mu\text{L}$. Neutrophil counts were classified as neutropenia if the neutrophil count was less than $0.5 \times 10^3/\mu\text{L}$.

The criteria for the diagnosis of ALL were the finding of lymphoblasts in the bone marrow aspiration (BMA) staining of 20% of the 200 nucleated cells examined. The reading of the bone marrow smear was carried out by a different researcher from the researcher who took the bone

marrow sample. There were three types of childhood ALL based on lymphoblast morphology. They were ALL-L-1, ALL-L-2, and ALL-L-3 according to the French American British (FAB) classification. Children with ALL-L-3 or less than one year of age were excluded from this study because they were given chemotherapy with different treatment regimens. Immunophenotyping was used to evaluate the molecular characteristics of lymphoblasts using flow cytometry of 1 ml bone marrow aspirate samples using FACS Calibur with lymphoid markers (CD45), B lymphocyte markers (CD19+ and CD22+), and T lymphocyte markers (CD3+ and CD7+). Examination of Bax and Bcl-2 expression was carried out on samples that had been confirmed positive for B cell ALL and T cell ALL based on immunophenotyping results.

Childhood ALL were treated using the 2018 Children ALL Indonesian Protocol developed by the Working Group of Hematology-Oncology of the Indonesian Pediatric Society. There were two risk classifications of chemotherapy, Standard Risk ALL (ALL-SR) and High-Risk ALL (ALL-HR). The criteria for ALL HR were (1) age above ten years old, (2) leukocyte count at diagnosis more than $50 \times 10^3/\mu\text{L}$, (3) the presence of a mediastinal tumor, (4) T cell ALL (5) central nervous system metastases. Children who do not have any of the above criteria were classified as ALL-SR. Children with B cells ALL fall into the ALL-SR category. Children with ALL HR have a worse prognosis so they receive chemotherapy with different doses of cytotoxic agents.

After the diagnosis was proven, the chemotherapy regimen according to the 2018 Children ALL Indonesian Protocol was immediately given. Induction phase chemotherapy takes seven weeks for both ALL-SR and ALL-HR. The chemotherapy regimens for ALL-SR were (1). Vincristine 1.5 mg/m² intravenously on days 8, 15, 22, 29, 36, and 43 (2). Intrathecal Methotrexate 12 mg in combination with Dexamethasone 1 mg on days 8, 22, and 36 (3). Daunorubicin 25 mg/m² intravenously on days 22 and 29, (4). Prednisone 60 mg/m² for seven days and then 40 mg/m² orally in three divided doses from day 8 to day 42 then gradually tapered over seven days and (5) L-Asparaginase 7,500 units/m² intravenously on days for 36, 38, 40, 42, 44 and 46. The chemotherapy regimens for ALL-HR were (1). Vincristine 1.5 mg/m² intravenously on days 8, 15, 22, 29, 36, and 43, (2). Intrathecal Methotrexate 12 mg in combination with Dexamethasone 1 mg on days 8, 22, and 36, (3). Daunorubicin 30 mg/m² intravenously on days 8, 15, 22 and 29, (4). Dexamethasone 6 mg/m² orally daily from day 1 to day 42 then tapered off over seven days and (5) L-asparaginase 7,500 units/m² intravenously on days 29, 32, 35, 38, 41, 45, 48 and 51.

The outcomes obtained were survival and remission rate. After they finish the induction phase of chemotherapy, all children will undergo bone marrow aspiration. They got remission if the lymphoblasts were less than 5% of the 100 nucleated cells examined on bone marrow aspirate staining. We did not evaluate minimal residual disease (MRD) as an outcome of chemotherapy because of the limitation of the facility at our institution. Chemotherapy was continued with a consolidation phase after the induction phase was complete.

The expression of Bax and Bcl-2 proteins was examined by the flow cytometry method of bone marrow aspirate samples in EDTA tubes, using the FACS Calibur machine. Bone marrow aspirate samples required were 0.5-1 ml taken before and after the induction phase of chemotherapy. We used reagents Anti-Bax Antibody (B-9): sc-7480 and Anti-Bcl-2 Antibody (C-2): sc-7382 manufactured by Santa Cruz Biotechnology, Inc. Oregon, USA. The protein expression was examined at least before 6 hours from the bone marrow sample was taken. Expression of Bax and Bcl-2 protein were in percentages. We calculated the Bax/Bcl-2 protein ratio to determine the predominance of proapoptotic or antiapoptosis. The Bax/Bcl-2 ratio of more than 1 indicated the dominance of proapoptotic protein. The Bax/Bcl-2 ratio of less than 1 refers to the predominance of antiapoptotic protein.

We used the Statistical Package for the Social Sciences (SPSS 18.0) software for further analysis. Differences in protein expression before and after chemotherapy were analyzed by the Wilcoxon Signed Rank Test. Differences in protein expression based on survival were analyzed using the Mann-Whitney U Test. All tests used a confidence level of $p < 0.05$ in the two-tailed test.

Results

The number of children with suspected leukemia examined was 37 children. Twenty children were excluded consisting of 4 not expressing lymphoblasts, 7 with myeloid lineage immunophenotyping, 6 children not having baseline data, 2 children not having final data, one child refusing chemotherapy. We compared 11 pediatric ALL who lived until the end of the induction phase of chemotherapy, and 6 children who died during the study.

The mortality rate for pediatric ALL aged 1-10 years was almost the same as for ALL children over 10 years old, but 6 of the children who died were boys (Table 1). The mortality rate in children with hepatomegaly was 83.3% and in splenomegaly was 66.7%. Children with anemia died about 66.7%, but all pediatric ALL who died had thrombocytopenia. The mortality rate spread evenly based on the classification of leukocytes but more deaths in the neutropenic state (83.3%).

The number of children with ALL-L1 was 94.1% and one with ALL-L2 (Table 2). The death occurred in six children with ALL-L1. The death case in B cell ALL was 38.5%, and in T cell ALL was 33.3%, but the proportion of deaths was not a significant difference. The mortality

Table 1. Clinical and Laboratory Characteristics of Pediatric ALL at Diagnosis or before Induction Phase Chemotherapy based on Survival and Death Outcomes

Age group	Outcome		P
	Alive N=11 (%)	Died N=6 (%)	
Age			
1-10 years old	9 (81.8)	5 (83.3)	1.000 ^a
>10 years old	2 (18.2)	1 (16.7)	
Boys	9 (81.8)	6 (100)	0.515 ^a
Hepatomegaly	8 (72.7)	5 (83.3)	1.000 ^b
Splenomegaly	2 (18.2)	4 (66.7)	0.109 ^b
Lymphadenopathy	1 (9.1)	3 (50)	0.099 ^b
Hemoglobin levels			
Hb < 8 g/dl	3 (27.3)	4 (66.7)	0.162 ^a
Hb > 8 g/dL	8 (72.7)	2 (33.3)	
Trombocyte count			
<150 x10 ³ /μL	6 (54.5)	6 (100)	0.102 ^a
>150 x10 ³ /μL	5 (45.5)	0	
Leukocyte count			
<4 x10 ³ /μL	5 (45.4)	2 (33.3)	1.000 ^c
4-20 x10 ³ /μL	3 (27.3)	2 (33.3)	
>20 x10 ³ /μL	3 (27.3)	2 (33.3)	
Neutrophil count			
<500/μL	5 (45.5)	5 (83.3)	0.304 ^b
>500/μL	6 (54.5)	1 (16.7)	

^a, Fisher exact test; ^b, Chi-square test; ^c, Kplmogorof-Smirnov Z test

Table 2. Classification of Pediatric ALL Diagnoses

ALL Classification	Outcome		P ^a
	Alive N=11	Died (N=6)	
ALL type			
LLA-L1, n (%)	10 (90.9)	6 (100)	1
LLA-L2, n (%)	1 (9.1)	0	
Immunophenotyping result			
B-cell ALL, n (%)	8 (72.7)	5 (83.3)	1
T-cell ALL, n (%)	3 (27.3)	1 (16.7)	
Risk classification			
Standard Risk ALL, n (%)	6 (54.5)	1 (16.7)	0.304
High Risk ALL, n (%)	5 (45.5)	5 (83.3)	

^a, Fisher exact test

Table 3. Differences in the Mean Protein Expression of Bax, Bcl-2 and Bax/Bcl-2 Ratio before and after Chemotherapy and Death among Pediatric ALL

Protein type	Outcome (N=17)			P ^b
	Alive (N=11) Mean (SD)		Died (N=6) Mean (SD)	
	Before Mean (SD)	After Mean (SD)		
Bax expression	22.8 (25.86)	37.1 (16.55)	14.2 (6.55)	0.763
Bcl2 expression	22.6 (21.83)	9.9 (9.99)	22.9 (27.26)	0.366
Bax/Bcl-2 expression	1.74 (1.846)	6.17 (4.139)	3.88 (4.663)	0.763

^{*}, Wilcoxon-Signed Rank test between before and after induction phase chemotherapy; ^b, Mann-Whitney U test between the living (before) and the dead

Table 4. Differences in *Bax*, *Bcl-2*, and *Bax/Bcl-2* Ratio Protein Expression before Induction Phase Chemotherapy in Pediatric ALL

Clinical characteristic	Outcome (n=17)		
	Bax Mean (SD)	Bcl-2 Mean (SD)	Bax/Bcl Ratio Mean (SD)
Age			
1-10 years old	20.9 (23.16)	23.8 (24.62)	2.70 (3.444)
>10 years old	14.4 (7.37)	17.2 (15.70)	1.49 (1.145)
Gender			
Boys	18.3 (20.85)	19.2 (18.16)	2.59 (3.317)
Girls	30.7 (28.87)	49.1 (47.80)	1.73 (2.273)
Hepatomegaly			
Presence	21.4 (23.86)	23.0 (25.90)	2.92 (3.497)
Absence	14.4 (8.33)	21.5 (11.87)	1.08 (1.066)
Splenomegaly			
Presence	14.9 (6.72)	25.3 (26.07)	3.66 (4.775)
Absence	22.4 (25.96)	21.2 (22.39)	1.85 (1.841)
Lymphadenopathy			
Presence	12.4 (6.75)	16.9 (22.14)	3.41 (4.030)
Absence	21.4 (23.98)	24.4 (23.87)	2.21 (2.992)
Hemoglobin level			
Hb < 8 g/dL	24.6 (29.51)	21.6 (25.18)	3.5 (1.81)
Hb >8 g/dL	16.4 (13.69)	23.5 (22.75)	1.8 (2.79)
Trombocyte count			
<150 x10 ³ /μL	19.5 (23.15)	19.1 (20.27)	3.0 (3.59)
>150 x10 ³ /μL	19.1 (20.27)	31.1 (29.35)	1.2 (1.31)
Leukocyte count			
<4 x10 ³ /μL	28.3 (31.41)	28.5 (28.66)	3.1 (3.82)
4-20 x10 ³ /μL	10.1 (1.73)	25.8 (24.89)	1.1 (1.39)
>20 x10 ³ /μL	17.6 (7.96)	11.4 (6.26)	2.9 (3.61)
Neutrophil count			
<500/μL	24.0 (16.76)	28.3 (27.79)	2.54 (3.301)
>500/μL	13.7 (7.11)	14.6 (11.15)	2.41 (3.225)

the statistical test used is the Mann Whitney U test, except for the number of leukocytes using the Kolmogorov-Smirnov Test.

rate in ALL-HR was lower than in the ALL-SR group with p=304. The causes of death of the six children were infection (3), hemorrhage (1), pneumonia (1), and tumor lysis syndrome (1).

Table 5. Differences in *Bax*, *Bcl-2*, and *Bax/Bcl-2* Ratio Protein Expression before Chemotherapy Based on the Classification of Pediatric ALL Diagnosis

Diagnoses classification	Outcome (n=17)		
	Bax Mean (SD)	Bcl-2 Mean (SD)	Bax/Bcl Ratio Mean (SD)
ALL type			
ALL-L1 (N=16)	20.7 (21.49)	23.2 (23.65)	2.63 (3.219)
ALL-L2 (N=1)	4.2	0.703	0.31
Immunophenotyping result			
B-cell ALL (N=13)	19.6 (24.35)	25.9 (25.54)	2.72 (3.616)
T-cell ALL (N=4)	20.0 (4.88)	12.2 (4.94)	1.75 (0.501)
Risk classification			
Standard risk ALL (N=7)	13.4 (72.9)	14.5 (10.53)	1.49 (1.210)
High risk ALL (N=10)	24.2 (26.64)	28.3 (27.95)	3.19 (3.942)

The statistical test used is the Mann Whitney U test

Bax and *Bcl-2* protein expression before chemotherapy in pediatric ALL who survived and completed chemotherapy was higher than those who died but was not significant different. The *Bax/Bcl-2* ratio in children who survived was lower than children who died, about 1.74 (SD 1.846) compared to 3.88 (SD 4.663) but not significantly different (p=0.763). The data can be seen in Table 3. Eleven children who survived were in remission. *Bax* protein expression increased from 22.8% to 37.1% after induction phase chemotherapy but was not statistically significant (p=0.062). *Bcl-2* protein expression decreased from 22.6% to 9.9% but not significant (p=0.075). On the other hand, the *Bax/Bcl-2* ratio after chemotherapy was higher than before chemotherapy, namely 6.17 compared to the previous 1.74 and statistically significant (p=0.021).

Differences in clinical parameters of pediatric ALL before chemotherapy can be seen in table 4. The *Bax*, *Bcl-2*, and *Bax/Bcl-2* protein ratio expression in children aged 1-10 years were higher than those over 10 years old but not statistically different. Organomegaly (hepatomegaly, splenomegaly, and lymphadenopathy) did not affect the difference in protein expression. The *Bax*, *Bcl-2*, and *Bax/Bcl-2* protein ratio expressions were not significantly different based on the classification of hemoglobin levels, leukocyte counts, platelet counts, and neutrophil counts. All clinical and laboratory parameters had p>0.05.

Expression of *Bax*, *Bcl-2*, and *Bax/Bcl-2* ratio before induction phase chemotherapy in ALL-L1 was higher than in ALL-L2 but not statistically significant (p>0.05). *Bax* expression in B-cell ALL was lower than T-cell ALL and the opposite of *Bcl-2* expression but p>0.05. The *Bax/Bcl-2* ratio was not significantly different between B-cell ALL and T-cell ALL, although the mean *Bax/Bcl-2* ratio in B-cell ALL was higher (p>0.05). Expression of *Bax*, *Bcl-2*, and *Bax/Bcl-2* ratios between High-Risk ALL and Standard-Risk ALL were not statistically significant (p>0.05). The data can be seen in Table 5.

Discussion

The development in pediatric ALL management over the last decade has succeeded in improving chemotherapy outcomes, although the mortality rate is still relatively

high. The highest incidence and mortality of leukemia was in Asia (Namayandeh et al., 2020) and the predominant in boys (Butt et al., 2017; Singh et al., 2018; Jaime-Pérez et al., 2018; Kakaje et al., 2020; Namayandeh et al., 2020). The proportion of ALL children aged less than ten years reached 86%, greater than other age groups (Hossain et al., 2014; Jaime-Pérez et al., 2018; Kakaje et al., 2020).

ALL-L1 cases (93%) were dominant compared to ALL-L2, as in other studies (Narayan et al., 2007; Kakaje et al., 2020). The number of B-cell ALL was 71%, as previously reported (Addeo et al., 2005; Singh et al., 2018; Huang et al., 2021; Kakaje et al., 2020; Hossain et al., 2014) and also in adult ALL (Kanagal-Shamanna et al., 2017). Based on risk stratification, ten children were Standard Risk ALL, and seven were High Risk ALL. The risk classification of Children's Oncology Group (COG) was based on age, leukocyte count, cytogenetics, bone marrow response on day 14, and minimal residual disease (MRD) (Schultz et al., 2007).

About 22% of children died before completing the induction phase of chemotherapy. Addeo et al. (2005) reported that children with ALL who did not respond to induction phase chemotherapy occurred in 40.3% of cases. As many as 39% of cancer deaths of children aged 0-14 years are associated with leukemia (Namayandeh et al., 2020), especially ALL in boys and T-cell ALL (Kakaje et al., 2020).

The expression of apoptosis-related genes was associated with variable clinical outcomes in hematological malignancies. Chemotherapy-induced apoptosis was mediated by the receptor (extrinsic pathway) and the mitochondrial (intrinsic pathway) (Kapoor et al., 2018). The role of proapoptotic and antiapoptotic proteins were the basis for understanding chemotherapy outcomes (Kaparou et al., 2013). Apoptotic dysregulation and balance of lymphoblast survival were the keys to leukemia pathogenesis, chemotherapy success, survival, and relapse.

The main proteins in the intrinsic pathway of apoptosis were Bax and Bcl-2 protein. At the beginning of diagnosis, the mean Bcl-2 protein expression was 27.5%, lower than Aref (2004), with a median of 73.2% and 80% in Coustan-Smith (1996). Bcl-2 lymphoblast expression was lower in pediatric ALL with splenomegaly but higher in hepatomegaly (Narayan et al., 2007). However, in this study, the mean expression of Bcl-2 protein at the beginning of diagnosis was higher than in children with organomegaly, although it was not significant. The Bcl-2 lymphoblasts expression was higher in ALL children with high Hemoglobin levels and blast cells in the bone marrow (Narayan et al., 2007), but Bcl-2 was inversely proportional to leukocytes (Kaparou et al., 2013).

The mean expression of Bcl-2 in B-cell ALL was twice higher than in T-cell ALL (25.9% vs. 12.2%). However, Aref (2004) reported a much higher median expression of Bcl-2 in ALL T cells, which was 68% versus 27%. Most of the subjects were B-cell ALL. The higher expression of Bcl-2 in T-cell ALL was the reason T-cell ALL has high initial leukocytes with a worse prognosis. Hogarth and Hall (1999) reported that Bcl-2 was not associated with baseline leukocyte count at the time of diagnosis of ALL.

Bax protein expression increased from 22.8% to 37.1% after induction phase chemotherapy but was not statistically significant. The expression of Bax lymphoblasts was higher in pediatric ALL with splenomegaly (Narayan et al., 2007). At the beginning of diagnosis, Bax was inversely related to Bcl-2 and directly proportional to the Bax/Bcl-2 ratio (Kaparou et al., 2013). Bax protein expression after chemotherapy was higher in pediatric ALL with initial hemoglobin above 8 g/dL. High Bax correlates with a good prognosis in AML, whereas increased Bcl-2 expression is a poor prognostic factor in lymphoma and chronic lymphocytic leukemia (Hogarth and Hall, 1999).

The mean of Bcl-2 protein expression before chemotherapy was 22.5%, and after chemotherapy was 9.9%. Bcl-2 protein expression at diagnosis correlated with response to induction chemotherapy but not with the outcome (Aref et al., 2004). However, Coustan-Smith (1996) found no association between Bcl-2 and disease aggressiveness or chemotherapy resistance. Antiapoptotic proteins were more dominant than proapoptotic in 77% of pediatric ALL before diagnosis (Singh et al., 2018). Mutation of the Bcl-2 gene increases lymphocytes in the blood (Butt et al., 2017). In ALL patients who responded to chemotherapy, Bcl-2 protein expression was lower than the unresponsive group, namely 60.2% vs. 92.2% (Aref et al., 2004).

The Bcl-2 protein expression before chemotherapy in pediatric ALL who lived and who died was not significantly different (22.6% vs. 22.9%). Bcl-2 expression was higher in B-cell ALL than in T-cell ALL (Narayan et al., 2007). Bcl-2 expression in children who remained in remission for the next year decreased from 79.2% at initial diagnosis to 9.1% in the paired test (Aref et al., 2004). The expression of Bcl-2, Bax, and Bax/Bcl-2 ratios were not affected by prognostic classification, gender, and initial leukocyte count (Hogarth and Hall, 1999). Prokop et al., (2000) reported that Bax expression and Bax/Bcl-2 ratio decreased between new and relapsed cases.

The Bax/Bcl-2 ratio increased from 1.74 to 6.17 after the induction phase of chemotherapy. The Bax/Bcl-2 protein ratio in High-Risk ALL was lower than that of Standard-Risk ALL; 1.5 compared to 3.2. Children over 10 years in the High-Risk group have a higher Bax/Bcl-2 ratio than younger ages (Kaparou et al., 2013). High-Risk ALL patients have a lower Bax/Bcl-2 ratio at the time of remission than moderate-risk ALL patients (Kaparou et al., 2013). Bcl-2, HRK, TNF genes were associated with L-asparaginase resistance. Overexpression of Bcl-2 was also associated with poor outcomes in pediatric ALL (Holleman et al., 2006). Although high Bax protein expression was associated with an increased risk of recurrence (Hogarth and Hall, 1999), other investigators suggested a Bax/Bcl-2 ratio better than Bax or Bcl-2 alone as a prognostic indicator in ALL (Prokop et al., 2000).

The selected outcome was remission and non-remission, but the evaluation of all pediatric ALL who completed chemotherapy was in remission. Chemotherapy would increase the apoptotic index of lymphoblasts after induction (Singh et al., 2018). Failure of apoptotic will result in poor ALL outcomes such as death, failure to

achieve remission, and a high risk of recurrence. The expression of Bax, Bcl-2 and Bax/Bcl-2 ratio did not appear as the main prognostic factors in pediatric ALL. However, high Bax protein expression at diagnosis was associated with an increased likelihood of relapse (Hogarth and Hall, 1999).

The Bax, Bcl-2, and Bax/Bcl-2 ratio protein expression at the beginning of diagnosis did not affect the survival of pediatric ALL after induction phase chemotherapy. In ALL children who survived and reached remission, the expression of Bax and Bcl-2 did not show significant changes, but the Bax/Bcl-2 ratio increased 3.5 times after induction phase chemotherapy. Proapoptotic proteins dominate over antiapoptotic proteins. Future research needs to examine the apoptosis rate as one of the outcomes besides remission rates, mortality, and risk of recurrence.

In conclusion, expression of Bax and Bcl-2 proteins at diagnosis was not associated with survival in the induction phase of pediatric ALL. In children who achieved remission after the induction phase of chemotherapy, Bax protein expression was not different. Bax/Bcl-2 protein ratio after the induction phase of chemotherapy increased four times compared with before the chemotherapy induction phase. Proapoptotic protein was more dominant than antiapoptotic proteins in pediatric ALL, who achieved complete remission.

Author Contribution Statement

Andi Cahyadi: Conception and design of study, Drafting the manuscript, Acquisition of data, Analyzing and interpretation of the data, Literature search.

I Dewa Gede Ugrasena: Conception and design of study, Drafting the manuscript, Analyzing and interpretation of the data, Literature search.

Mia Ratwita Andarsini: Conception and design of study, Drafting the manuscript, Analyzing and interpretation of the data, Literature search.

Maria Christina Shanty Larasati: Conception and design of study, Analyzing and interpretation of the data, Literature search.

Aryati Aryati: Conception and design of study, Analyzing and interpretation of the data, Literature search.

Diah Kusuma Arumsari: Drafting the manuscript, Acquisition of data,

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the title of Consultant Hematology Oncology of Children.

Approval

It is part of an approved student thesis.

Ethical Declaration

Research ethics were obtained from the Health Research Ethics Committee of Clinical Research Unit (CRU) on Dr. Soetomo General Academic Hospital, Surabaya, and a letter of exemption Ref. No.: 0116/LOE/301.4.2/IX/2020.

Conflict of Interest

All authors have disclosed no conflicts of interest.

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