

Role of cytogenetic profiles as prognostic factors for complete remission after induction phase in acute myeloblastic leukemia

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Abstract

Background Risk stratification for acute myeloid leukemia (AML) in children is a must in treatment strategy. This stratification is based on cytogenetic profiles, which are needed to determine proper management to gain better outcomes and reduce side effects of treatment. There is no such risk stratification available in Indonesia until now.

Objective To evaluate the association between cytogenetic profiles of t(8,21) and inv(16) mutations with the complete response to induction phase of chemotherapy in pediatric AML.

Methods A prospective study was conducted between year 2018 and 2020, involving children with AML from 4 pediatric oncology centers in Jakarta. Subjects were evaluated for cytogenetic profiles, especially t(8,21) and inv(16), as the favorable predictors for AML. Bone marrow remission was evaluated after 2 cycles of induction phase. The results were evaluated for remission rate and survival analysis.

Results Karyotype data of 18 subjects were obtained. Translocation t(8;21) detected in 1 subject, and inv(16) mutation in 4 subjects. These two variables had no significant correlation with complete remission after induction phase. Nevertheless, favorable group had more tendencies to achieved remission than unfavorable group. Complete remission achieved in 61% subjects, 90% of them had a relapse period with an average time 43 weeks. The relapse period in favorable group was shorter than in unfavorable group (34 weeks and 44 weeks, respectively).

Conclusion This study shows that cytogenetic profiles of t(8;21) and inv(16) mutation can not be used as prognostic factors for complete remission after induction phase of chemotherapy in pediatric AML. [Paediatr Indones. 2021;61:343-9 ; DOI: 10.14238/pi61.6.2021.343-9].

Keywords: acute myeloid leukemia; t(8;21); inv(16); pediatric AML; cytogenetic

Leukemia is the most common cancer in children, accounts for a third of all cancer types. Approximately, 15-20% of acute leukemia cases in children are acute myeloid leukemia (AML). In childhood, AML incidence increases with age, with 5 cases per 1 million in children aged 5-9 years, 9 cases per 1 million in children aged 15-19 years, and 37 cases per 100.000 in adults aged ≥ 20 years.¹

Risk-based treatment protocol is needed to improve pediatric AML outcomes. It has been applied in developed countries to decrease treatment-related death as an adverse effect of chemotherapy. Patients will be classified into low-risk or high-risk group based on clinical characteristics and cytogenetic profiles in each patient. Treatment will be given depending on the risk groups. As a result, pediatric AML outcomes has improved significantly in the past decade, with complete remission and overall survival rates 80-90%

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Submitted October 19, 2021. Accepted November 18, 2021.

and 64-78%, respectively.² Unfortunately, pediatric AML outcomes in Indonesia is still poor, with complete remission rate of 32%, and overall survival in Dr. Cipto Mangunkusumo Hospital only 10% in year 2011. We do not have risk-based treatment protocol in Indonesia until now, since cytogenetic and molecular testing is not available for practical purposes.

Prognostic factors to determine the risk stratification group can be assessed mainly by cytogenetic profiles (karyotype and gene mutation). According to *World Health Organization* (WHO) criteria, AML with core binding factor (CBF)-associated translocation is considered favorable cytogenetic subgroup. The CBF-AML includes 2 major subtypes of favorable group, translocation t(8;21)(q22;q22), and inversion of chromosome 16 (inv(16)(p13q22)), which together account for 25% of pediatric de novo AML patients.³ *Berlin-Frankfurt-Munster* (BFM) study groups and *Medical Research Council* (MRC) have confirmed the overall survival (OS) rate of t(8;21) and inv(16) mutation in childhood CBF AML were 91% and 92%, respectively.⁴

This study was conducted to evaluate the role of karyotype abnormalities t(8,21) and inv(16) as favorable prognostic factors of complete remission after induction phase chemotherapy in pediatric AML.

Methods

A prospective cohort study was conducted at 4 following pediatric oncology centers in Jakarta: Dr. Cipto Mangunkusumo Hospital (CMH), Gatot Soebroto Army Hospital, Dharmais Cancer Hospital, and Harapan Kita Women and Child Hospital, between year 2018 and 2020. Institutional review board approval was obtained from the CMH Board and Ethics Committee of Faculty of Medicine, Universitas Indonesia Medical School.

The inclusion criteria were the following: children aged <18 years with de novo AML who were diagnosed by morphological and leukemia immunophenotyping; underwent induction phase of chemotherapy under national AML protocol and agreed to sign the study informed consent. The exclusion criteria were: AML L3 subtype, AML in Down syndrome, or secondary AML as a result of chemotherapy (treatment related leukemia).

Consecutive sampling method was performed until number of subjects required was met or until 12 months of study period.

The national AML protocol consisted of cytarabine and daunorubicin given for 5 days. De novo AML referred to AML in patients with no clinical history of prior myelodysplastic syndrome (MDS), myeloproliferative disorder, or exposure to potentially leukemogenic therapies or agents.

Favorable risk group included subjects with chromosomal abnormality, such as t(8;21)(q22;q22), inv(16)(p13;q22) or t(16;16)(p13;q22), and t(15;17)(q22;q12). Unfavorable risk group included subjects with monosomy 7, FLT3-ITD mutation, t(6;11)(q27;q23), t(10;11)(p12;q23), t(10;11)(p11.2;q23), t(6;9)(p23;q34), t(8;16)(p11;p13), t(16;21)(q24;q22), t(5;11)(q35;p15.5), inv(16)(p13.3q24.3), t(11;15)(p15;q35), t(3;5)(q25;q34).²

Patient who met the inclusion criteria underwent bone marrow puncture (BMP) procedure. Blood specimens from BMP were used for morphological, immunophenotyping, karyotyping, and molecular examination. Patient who met the inclusion criteria underwent bone marrow puncture (BMP) procedure. Blood specimens from BMP were used for morphological, immunophenotyping, karyotyping, and molecular examination. Karyotyping test was performed in the Laboratory of Biomolecular and Cytogenetics Internal Medicine of CMH, with culture preparation using synchronized method and later analyzed microscopically. Abnormalities found from the microscopic examination were then photographed and printed. Meanwhile immunophenotyping was performed in the Clinical Pathology Laboratory of Dharmais Cancer Hospital, using 4 color flowcytometry. Results were then evaluated with FACS Cento equipment using Diva program.

Clinical data of patients were obtained from medical records. Blood specimens from BMP required for each examination were 2 mL for morphological and immunophenotyping, 3 mL for karyotyping, and 2 mL for molecular examination. If the specimens were not sufficient, the sample would be retaken from the peripheral blood.

Subjects received the first induction phase chemotherapy, which was then followed by a second BMP within 1-4 weeks, according to patient's clinical conditions. Afterward, subjects received the second

induction phase of chemotherapy and continued underwent re-examination of response to therapy within 1-4 weeks. If the 2nd BMP result (after the first induction phase) was not in remission, then the response to therapy would be assessed on the 3rd BMP (performed after the second induction phase). Subjects' data was collected and then processed until the 3rd BMP was done. Response to treatment was evaluated based on morphological examination after the induction phase. Remission was defined as blasts cell < 5% in bone marrow.

The association of cytogenetic mutation and analysed using Fisher's exact test. All statistical analyses were performed using the IBM SPSS software version 20.0 (IBM Corp., Armonk, NY, USA); a P value of < 0.05 were considered indicative of statistical significance.

Results

This study was performed for 18 months, between November 2018 to March 2020. Eighteen patients were obtained as subjects for this study from 4 pediatric oncology centers. The study was discontinued due to the COVID-19 pandemic.

The most common AML types in this study were AML M1 (7 subjects) followed by AML M2 (6 subjects). The most common symptoms and signs found in this study were paleness (12/18), fever (8/18), hepatomegaly (6/18), and splenomegaly (6/18) (Table 1).

Of those subjects, only one had a karyotype with translocation t(8;21). Fisher's exact test showed no significant correlation between the presence of translocation t(8;21) and the achievement of post-induction remission (P=0.38) (Table 2). Inversion of chromosome 16 was detected in 4 subjects.

Bivariate analysis using Fisher's exact test showed no significant correlation between inv(16) karyotype and the incidence of remission after the induction phase (P=0.10). Nevertheless, inv(16) subjects were more likely to achieve remission than subjects who were not inv(16) (4/4 vs. 7/14 subjects) (Table 3).

Median overall survival (OS) in this study was 51 (range 20-165) weeks (95%CI 40.9 to 61.1) (Figure 1a) and median disease-free survival (DFS) was 43 (range 10-165) weeks (95%CI 38.8 to 47.1) (Figure 1b). Median overall survival (OS) of favorable group was 51 (range 20-79) weeks (95%CI 41.3 to 60.6) compared to unfavorable group 49 (range 32-165) weeks (95%CI 34.3 to 63.6). Median disease-free survival (DFS) of

Table 1. Baseline characteristics of subjects

Characteristics	N=18
Age	
2-10 years	8
> 10 years	10
Sex	
Boy	9
Girl	9
AML morphological subtype	
AML M1	7
AML M2	6
AML M4	1
AML M5	2
AML M6	2
Clinical manifestations	
Fever	8
Bleeding	4
Paleness	12
Decreased appetite	5
Weight loss	4
Malaise	3
Hepatomegaly	6
Splenomegaly	6
Lymphadenopathy	2
Gingival hypertrophy	0
Skin lesion	1
Joint pain	3

Table 2. Bivariate analysis of t(8;21), inv(16), and risk groups (N=18)

Variables	Remission	No remission	Total	P value
t(8;21), n				0.389
Positive	0	1	1	
Negative	11	6	17	
Inv(16), n				0.10
Positive	4	0	4	
Negative	7	7	14	
Risk group, n				0.11
Favorable	6	1	7	
Unfavorable	5	6	11	

Table 3. Karyotype results of 18 subjects

No	Subtype	Karyotype	Age, yr	Fav/Unfav	Remission*
1	M1	44,XY,-18,-19[2]/46,XY,+6,-12,inv(16)(p13q22)/46,XY[9]	8	Fav	R
2	M1	43,X,-2,-14[1]/ 45,XY,-6[1]/ 45,XY,rob(13;21)(q10;q10)[3]/ 45,XY,del(6q),-14[1]/ 46,XY,inv(16)(p13q22)[1]/ 47,XY,-11,+14,+19[1]/46,XY[12]	17	Fav	R
3	M5	43,XY,-10,inv(16)(p13q22),-17,-19[1]/45,XY,-18[2]/ 46,XY,inv(16)(p13q22)[4]/46,XY[13]	1	Fav	R
4	M2	43,XX,-10,-12,rob(14;22)(q10;q10),inv16(p13q22)[1]/44,XX,-9,-21[2]/45,XX,rob(4;22)(q10;q10)[1]/46,XX,inv(16)(p13q22)[3]/46,XX[14]	13	Fav	R
5	M2	45,XY,-20[2]/46,XY,t(8;21)(q22;q22)[2]/46,XY[13]	9	Fav	NR
6	M1	45,XX,-20[1]/46,XX[16]	11	Fav	R
7	M1	45,XX,rob(14;22)(q10;q10)[2]/46,XX[8]	14	Fav	R
8	M6	45,XY,-21[1]/45,XY,-11,-15,+18,+mar[1]/46,XY,rob(14;22)(q10;q10)[1]/46,XY[9]	8	Unfav	NR
9	M5	44,XX,del(7q),-9,-15,[1]/45,XX,-20[2]/46,XX,del(7q)[1]/46,XX[10]/47,XX,del(4q),+21[1]	9	Unfav	R
10	M1	43,XX,-3,-9,-11,rob(14;22)(q10;q10)[1]/43,XX,-11,-18,-19,-20,+22[1]/43,XX,-13,-14,-20[1]/45,X[2]/46,XX[10]	13	Unfav	R
11	M2	44,XX,-5,-8[2]/ 45,XX,rob(14;22)(q10;q10)[2]/ 46,XX[13]	4	Unfav	R
12	M2	44,X,del(8q)-22[2]/45,X,del(8q)[5]/46,XX[13]	14	Unfav	R
13	M1	45,XY,-9[1]/45,XY,del(6q),-21[1]/45,X,t(9;22)(q34;q11),+13[1]/46,XY,-7,t(9:22)(q34;q11,+18(1)/46,XY[12]	14	Unfav	NR
14	M2	39,XY,-4,-6,-12,-16,-19,-20,-22[1]/41,XY,-3,-4,-18,-21, -22[1]/46,XY[12]/48,XY,-12,+16,+2mar[1]	7	Unfav	R
15	M4	45,XX,-9,-18,+22(2)/46,X,+22(1)/46,XY(8)	6	Unfav	NR
16	M6	45,XY,rob(13;21)(q10;q10)[3]/46,XY[14]	10	Unfav	NR
17	M1	44,XY,-9,-20[3]/46,XY[4]	1	Unfav	NR
18	M1	42,XX,-7,-14,-17,-20[1]/44,XX,-13,-21[1]/46,XX,+11,-17[1]/46,XX[12]/47,XX,+11[1]	9	Unfav	NR

*R= remission; NR= not remission

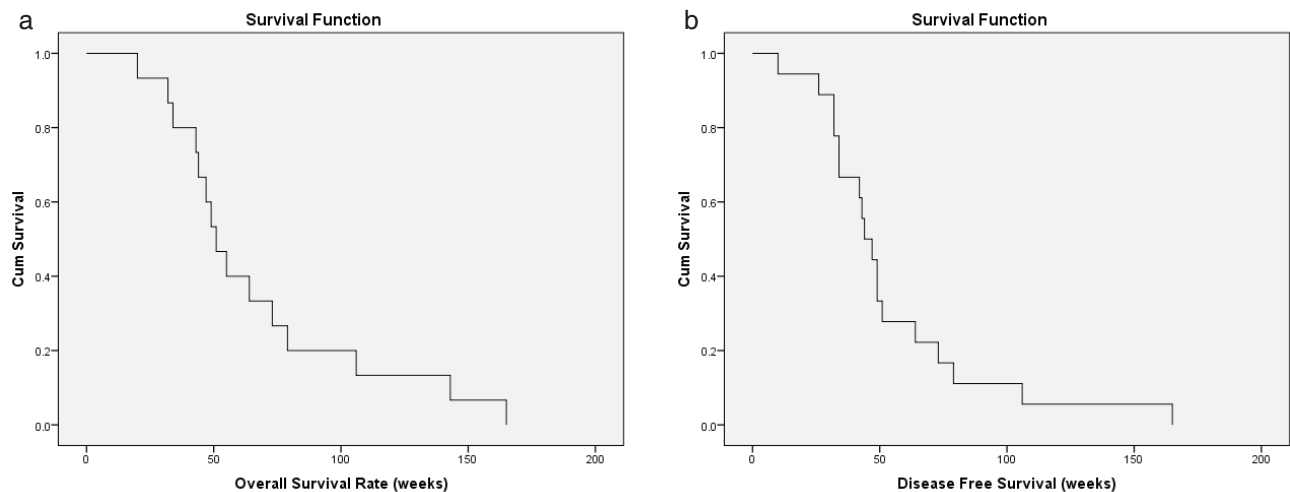


Figure 1. Survival rate of 18 children with AML [a. Overall, b. Disease-free]

favorable and unfavorable group were 34 (range 10-64) weeks (95%CI 28.8 to 39.1), and 44 (range 31-165) weeks (95% CI 38.6 to 49.3), respectively. There was

no statistical different of survival rate between favorable and unfavorable groups (P value of DFS and OS were 0.161 and 0.120, respectively) (Figure 2).

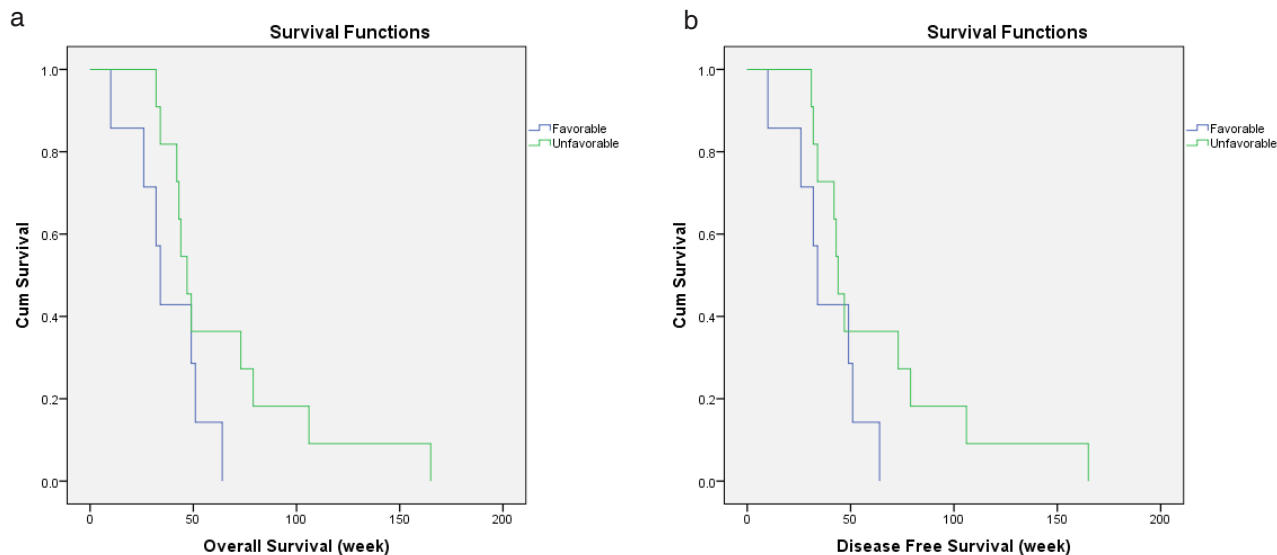


Figure 2. Survival rate of 18 children with AML between favorable and unfavorable group [a. Overall , b. Disease-free]

Discussion

Approximately 70% of pediatric AML patients have cytogenetic aberrations at the time of diagnosis. The most common chromosomal abnormalities found were $t(8;21)$ and $inv(16)$. Translocation of $t(8;21)$ were found variably. Incidence of $t(8;21)$ in pediatric AML patients were 15% in Saudi Arabia,^{4,5} 15% in China,⁶ 24% in Japan,⁷ 26-29% in India.^{8,9} A study stated that $t(8;21)$ expression in Asian children tends to be higher compared to Europe and North America.⁸ In our study $t(8;21)$ only found in 1 of 18 subjects. This result is lower than reports from both Asian and European countries. Translocation $t(8;21)$ as one of the favorable cytogenetic group was reported commonly found at a younger age. In our study, we found lower $t(8;21)$ expression although most subjects were less than 10-years of age. Other factors which may influence the findings are ethnic or environmental factors that may differ from other countries.^{4,6}

Incidence of $inv(16)$ in pediatric AML was reported to be less frequent than $t(8;21)$, which was 5% in India,⁸ 3.5% in Japan,⁷ 1% in China,⁶ while studies in Europe and the United States reported between 4-9%.^{6,10} In our study, $inv(16)$ mutation was

found in 4 out of 18 subjects. Those findings were different compared to other reports from both Asian and European countries. This result is an important finding and needs further investigation because higher number of favorable group increases the advantage of therapy management. Age, geographical, and ethnic differences are reported to affect karyotype patterns and it is suspected that these differences are also related to exposure to certain chemicals or environmental substances.⁶

Approximately 7/18 subjects in this study were in the favorable risk karyotype group. The small number of subjects with favorable karyotypes in this study can explain inferior response to induction therapy in pediatric AML patients in Indonesia. Complete remission rate in our study was 61%, which was much lower than complete remission rate reporteds in AML patients less than 15 years of age in Vietnam which was 82.6%,¹¹ or 92% in Saudi Arabia.^{4,11} Karyotype data in Saudi Arabia with 19% higher rate of $t(8;21)$ expression compared to this study might explain the difference of remission rate result.

The findings of $t(8;21)$ and $inv(16)$ in our study did not show significant relation towards induction phase therapy outcomes. Expression of $inv(16)$ in our study was not proven to be a prognostic factor for remission to induction phase. Nevertheless,

it seemed that 4 out of 4 subjects with inv(16) achieved remission while in non-inv(16) only 7 out of 14 achieved remissions (Table 3). This showed a consistent tendency with the study hypothesis and literatures which stated that inv(16) is a good prognostic factor for AML.

Our study showed that t(8;21) was not proven as a prognostic factor for remission in induction phase of AML. A meta-analysis shows the influence of ethnic and racial factors in the prognosis of AML with t(8;21) expression.⁴ White ethnicity in pediatric AML have better prognosis compared to the non-whites and the effect of racial or ethnic differences is significantly seen when accompanied by KIT mutations. Genetic abnormalities finding such as chromosome 9 deletion or the addition of chromosome 4 lower the prognosis rate. None of our subjects in this study had those cytogenetic abnormality.

The complete remission rate after induction phase chemotherapy in this study was 61%. This rate was lower than other countries, both developed countries and countries with the similar level of pediatric cancer services, such as Vietnam and Pakistan. Complete remission rate in Vietnam was 82.6%,¹¹ Pakistan was 80%,¹² and Saudi Arabia was 86%.⁵ Nevertheless, the remission rate in this study was higher than the data of pediatric AML in RSCM in year 2010 which was 32%.¹³ This improvement of remission rate may be caused by protocol changes after year 2011, when the National AML protocol was started to use. Low remission rate in this study may suggest that the chemotherapy regimen that is being used in the National AML protocol is not adequately enough. The National AML protocol consists of 5 days of treatment, while the treatment protocol in other countries generally consists of 7 days of treatment, with the same drug regimen: cytarabine and daunorubicin. Based on this results, we suggest to improve the strength of existing national protocol to achieve a higher remission rate.

The survival rate in this study is also lower than other countries. The 1-year OS in this study was 47%, lower than the outcomes in other Asian countries such as China (5-year OS was 69% in year 2011)¹⁴ and Saudi Arabia (58.8% in year 2016).¹⁵ The outcomes of this study was worse considering that the OS for 1 year was already low and the recurrence rate was high. A previous study shows that 3-year OS of

all AML patients was $22.6 \pm 5.4\%$, while prognosis of cytogenetic abnormality of subjects with (inv)16 was $75.0 \pm 21.7\%$ and subject with t(8;21) was $36.0 \pm 16.1\%$.⁵

Our study was the first study in Indonesia to examine the karyotype in children with AML. The data obtained from this study could be very useful for further management to treat AML in children in Indonesia. The limited number of study subject may explain the insignificance statistical results. In addition, our study encountered obstacles in fulfilling the sample size due to the limited numbers of new diagnosed AML patients who came to the study hospitals (Dr. Cipto Mangunkusumo Hospital/CMH, Gatot Soebroto Army Hospital, Dharmas Cancer Hospital). The COVID-19 pandemic has also affected the diagnosis confirmation in new AML patients and former patients who discontinued the treatment. The other difficulty faced in this study was technical examination of karyotypes and mutations which was complex and required several trials. The examination were carried out collectively hence re-sampling could not be done because patient had already received chemotherapy or did not survive. Another study limitation was that the laboratory could not examine samples due to the damage of the incubator and data processing computer, or the supply of reagents taking longer duration. The time discrepancy between the schedule of receiving samples and taking specimens from the subjects was also another obstacle in this study so that some patients failed to become study subjects due to the damaged samples. These factors hinder the achievement of having more study subjects.

In conclusion, this study fails to show that cytogenetic profiles of t(8;21) and inv(16) as favorable prognostic factors in pediatric AML. Nevertheless, this study finds the tendency of subjects with these chromosomal aberrancies to have better prognosis.

Conflict of Interest

None declared.

Funding Acknowledgement

The authors received no specific grant from any funding agency

in the public, commercial, or not-for-profit sectors.

References

1. Jaime-Pérez JC, Padilla-Medina JR, Fernández LT, Herrera-Garza JL, Gutiérrez-Aguirre CH, Tarín-Arzaga L, et al. Outcomes of adolescents and young adults with acute myeloid leukemia treated in a single Latin American Center. *Clin Lymphoma, Myeloma Leuk.* 2018;18:286-92. DOI: 10.1016/j.clml.2018.02.002.
2. JE R. Current management of childhood acute myeloid leukemia. *Paediatr Drugs.* 2017;19:1-10. DOI: 10.1007/s40272-016-0200-6
3. Mansoor N, Jabbar N, Arshad U, Maqsood S, Habib MA, Raza MR. Outcome of core binding factor acute myeloid leukemia in children: A single-center experience. *J Pediatr Hematol Oncol.* 2020;42:E423-7. DOI: 10.1097/mp.0000000000001853.
4. Jastaniah, Alsultan A, Daama S Al, Ballourah W, Bayoumy M, Al-Anzi F, et al. Is the outcome of childhood acute myeloid leukemia with t(8;21) inferior in Saudi Arabia? A multicenter SAPHOS leukemia group study. *J Appl Hematol.* 2017;8:41. DOI: 10.4103/joah.joah_16_17.
5. Jastaniah W, Bayoumy M, Alsultan A, Al Daama S, Ballourah W, Al-Anzi F, et al. Identifying prognostic factors that influence outcome of childhood acute myeloid leukemia in first relapse in Saudi Arabia: results of the multicenter SAPHOS Study. *Clin Lymphoma Myeloma Leuk.* 2018;18:773–80. DOI: 10.1016/j.clml.2018.09.001.
6. So CC, Wan TS, Chow JL, Hui KC, Choi WW, Lam CC, et al. A single-center cytogenetic study of 629 Chinese patients with de novo acute myeloid leukemia - evidence of major ethnic differences and a high prevalence of acute promyelocytic leukemia in Chinese patients. *Cancer Genet.* 2011;204:430-8. DOI: 10.1016/j.cancergen.2011.06.003.
7. Horibe K, Tsukimoto I, Ohno R. Clinicopathologic characteristics of leukemia in Japanese children and young adults. *Leukemia.* 2001;15:1256-61. DOI: 10.1038/sj.leu.2402194.
8. Amare PSK, Jain H, Kabre S, Deshpande Y, Pawar P, Banavali S, et al. Cytogenetic profile in 7209 Indian patients with de novo acute leukemia: a single centre study from India. *J Cancer Ther.* 2016;7:530-44. DOI: 10.4236/jct.2016.77056.
9. Tyagi A, Pramanik R, Chaudhary S, Chopra A, Bakhshi S. Cytogenetic profiles of 472 indian children with acute myeloid leukemia. *Indian Pediatr.* 2018;55:469-73. PMID: 29978812.
10. Pession A, Masetti R, Rizzari C, Putti MC, Casale F, Fagioli F, Luciani M, Lo Nigro L, Menna G, Micalizzi C, Santoro N. Results of the AIEOP AML 2002/01 multicenter prospective trial for the treatment of children with acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology.* 2013;122:170-8. DOI: 10.1182/blood-2013-03-491621.
11. Kiem Hao T, Van Ha C, Huu Son N, Nhu Hiep P. Long-term outcome of childhood acute myeloid leukemia: a 10-year retrospective cohort study. *Pediatric reports.* 2020;12:22-5. DOI: 10.4081/pr.2020.8486.
12. Ghafoor T, Ahmed S, Khalil S, Farah T. Optimum induction chemotherapy for pediatric acute myeloid leukemia: experience from a developing country. *The Journal of Pediatric Pharmacology and Therapeutics.* 2020;25:288-94. DOI: 10.5863/1551-6776-25.4.288
13. Sjakti HA, Gatot D, Windiastuti E. Hasil pengobatan leukemia mieloblastik akut pada anak. *Sari Pediatri.* 2012;14:40-5. DOI: 10.14238/sp14.1.2012.40-5.
14. Zhai XW, Cheng FW, Lee V, Leung WK, Ng MH, Tsang KS, Shing MK, Li CK. Improved survival outcome of childhood acute myeloid leukemia with intensified chemotherapy in Chinese children. *Pediatr Hematol Oncol.* 2011;28:269-78. DOI: 10.3109/08880018.2010.533249.
15. Jastaniah W, Al Ghemlas I, Al Daama S, Ballourah W, Bayoumy M, Al-Anzi F, et al. Clinical characteristics and outcome of childhood de novo acute myeloid leukemia in Saudi Arabia: A multicenter SAPHOS leukemia group study. *Leuk Res.* 2016;49:66-72. DOI: 10.1016/j.leukres.2016.08.009.