IJBC 2017; 9(2): 44-47

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

The Difference in Initial Leukocyte Count, Bone Marrow Blast Cell Count and CD 34 Expression in Patients with Acute Myeloid Leukemia with and without NPM1 gene Mutation

Notopuro Paulus Budiono[1*], Notopuro Harianto[2], Budiwijono Imam[3], Adhipireno Purwanto[3]

[1]Departement of Clinical Pathology, Faculty of Medicine Airlangga University, Indonesia
[2]Departement of Biochemistry, Faculty of Medicine Airlangga University, Indonesia
[3]Departement of Clinical Pathology, Faculty of Medicine Diponegoro University, Indonesia

ARTICLE INFO

Article History:
Received: 28.11.2016
Accepted: 20.01.2017

Keywords:
Acute myeloid leukemia
Initial leukocyte count
Bone marrow blast cell count
CD34 expression
NPM1 gene mutation

**Corresponding author:*
Notopuro Paulus Budiono, MD
Address: Jalan Klampis Aji 1 No 12
Surabaya 60117, Indonesia
Tel: +62 81 23579714
Email: paulusbudiono@yahoo.com

ABSTRACT

Background: Mutation in NPM1 gene has been reported to be the most common genetic mutation in de novo acute myeloid leukemia (AML). AML with NPM1 gene mutation usually presents with higher initial leukocyte and blast cell counts and negative CD34 expression. We aimed to investigate the difference of initial leukocyte counts, bone marrow blast cell counts and expression of CD34 among patients with AML with and without NPM1 mutation.
Methods: In this study, 25 de novo patients with AML were investigated for NPM1 exon 12 gene mutation using ASO-RT-PCR. Initial leukocyte counts, bone marrow blast cell counts and expression of CD34 on blasts were examined in all patients.
Results: 13 of 25 de novo patients with AML (52%) had NPM1 gene mutation. Initial leukocyte counts in AML patients with NPM1 gene mutation was not significantly higher than patients without this mutation (23.400 /µL versus 16.000 /µL, P=0.53). Blast cell counts were not significantly higher in AML patients with NPM1 gene mutation than patients without mutation. (41% versus 19%, P=0,18). Expression of CD34 was not significantly different between AML patients with and without NPM1 gene mutation (P=0.48).
Conclusion: There were no difference in initial leukocyte count, blast cell count and CD34 expression among patients with AML with and without NPM1 exon 12 type A gene mutation.

Please cite this article as: Budiono NP, Harianto N, Imam B, Purwanto A. The Difference in Initial Leukocyte Count, Bone Marrow Blast Cell Count and CD 34 Expression in Patients with Acute Myeloid Leukemia with and without NPM1 gene Mutation. IJBC 2017; 9(2): 44-47.

Introduction

Acute myeloid leukemia (AML) is a heterogenous disease with various clinical features and several genetic abnormalities. The incidence of AML is reported to be 3.7 per 100.000 persons in the world.[1] It has proposed that age of the patients, leukocyte count at diagnosis, bone marrow blast cell count and expression of CD34 are important prognostic factors in AML patients.[2,3] Based on cytogenetic and molecular abnormalities, AML can be classified into low, intermediate and high risk groups. Low risk AML is defined as patients with normal karyotype with mutated NPM1 and no FLT3 gene mutation. Intermediate risk group is defined as abnormal karyotype, such as +8 and all other combinations of NPM1 and FLT3 gene mutation. High risk group of AML patients have high risk cytogenetic features such as inv (3)(q21q26), t(3;3) (q21;q26), monosomy 7, monosomy 5, 5q-, 7q-, 11q23, t(9;11) and complex karyotype with >3 abnormalities.[4] In 40-50% of patients with AML with normal karyotype, various clinical and prognostic factors have been reported. It can be related to some genetic mutations such as NPM1, FLT3, and CEBPA

gene mutations that also contribute to the pathogenesis of AML. The exon 12 NPM1 gene mutation is a common genetic mutation in AML (35% in de novo AML and 45% in the case of AML with normal cytogenetic).[5] There is evidence that mutation in exon 12 NPM1 gene in AML is correlated with leukocyte count at diagnosis, bone marrow blast cell count and CD34 expression on blast cells. Patients with NPM1 gene mutation have reported to have higher leukocyte counts and bone marrow blast cell counts than patients without this mutation. Patients with NPM1 gene mutation have also down regulated CD34 expression.[2,3,6] In this study, we investigated the difference of leukocyte count, bone marrow blast cell count and CD34 expression among AML patients with and without exon 12 NPM1 gene mutation.

Materials and Methods

This study was approved with ethical clearance by the Ethical Committee, Faculty of Medicine, Airlangga University. Inform consent was taken from the patients. Bone marrow aspirates of 25 newly diagnosed patients with AML were collected from January 2015 to August 2015 from hospitals in Surabaya and Indonesia. The study was done in the department of biochemistry and clinical pathology, faculty of medicine, Airlangga University, Dr Soetomo general hospital Surabaya. Diagnosis of AML was made on the bone marrow aspirate cytology, considering cut off point of 20% blast cell count to establish the diagnosis. AML subtypes were defined based on FAB (French American British) criteria. Immunophenotyping by flowcytometry was used to confirm the diagnosis and determine the subtype of AML. The specimen was also studied for detection of exon 12 NPM1 gene mutation.

We extracted mRNA with RNA extraction kit (Trizol® LS reagent Invitrogen Cat: 10296-010) according to manufacturer's instruction. Exon 12 NPM1 gene mutation was detected by allele specific (ASO) RT-PCR (Reverse Transcriptase Allele Specific Polymerase Chain Reaction). Extract of mRNA was incubated in 55°C for 30 minutes with reverse transcriptase enzyme (Superscript III One Step RT-PCR with Platinum Taq Polymerase "Invitrogen" Cat No: 12574-026) to produce cDNA. We amplified cDNA of NPM1 mutant with RT-AS PCR technique. Amplification (resulting 319 bp amplicon) was achieved after 40 cycles of the following steps: hot start (95°C for 3 minutes), denaturation (94°C for 50 seconds), annealing (60°C for 50 seconds) and extension (68°C for 1 minute). We used forward primer NPM1-AN: 5'CAA-GAG-GCT-ATT-CAA-GAT-CTC-TGT-CTG-3' and reverse primer NPM-Rev6: 5'-ACC-ATT-TCC-ATG-TCT-GAG-CAC-C-3' to detect exon 12 NPM1 gene mutation.[7] Normal primer set was not used because all NPM1 mutations in patients with AML were heterozygote and homozygote states for NPM1 mutation were lethal.

Analysis of leukocyte count at diagnosis was done by ADVIA 2120i hematology analyzer system (Siemens®) as a routine procedure. Bone marrow aspirate blast cell counts were counted among 500 nucleated cells.

CD34 expression was examined in BD (Becton Dickinson®) Facs Calibur Flowcytometry, using anti CD34 monoclonal antibody. We examined the expression of CD34 in population of cells in blast gate with blastic gating strategy (low side scatter, moderate CD45 expression). Isotypic control was used to determine positive marker expression. Statistical analysis was done with SPSS version 22. Mann-whitney U-test was used to determine the difference in leukocyte count and bone marrow blast cell count and Fisher exact X2-test to indicate the difference between expression of CD34 in AML patients with and without exon 12 NPM1 gene mutation.

Results

Among 25 de novo patients with AML, 13 (52%) patients had exon 12 NPM1 gene mutation based on the positive 319 bp fragment in RT-AS PCR result; whereas 12 patients did not show any mutation in this exon. Figures 1 and 2 depict the positive and negative results of RT-AS PCR from de novo AML patients. Positive result was determined based on the positivity of 319bp fragment.

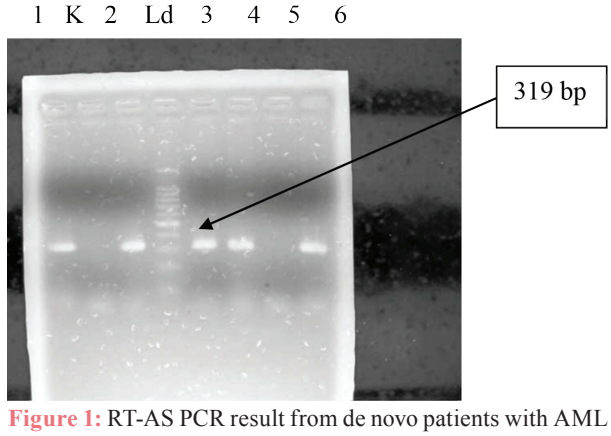

Figure 1: RT-AS PCR result from de novo patients with AML with positive exon 12 NPM1 gene mutation (positive 319 bp fragment in patients number 1,2,3,4,6), negative result in patient number 5, K=Negative control, Ld=DNA Ladder 100bp.

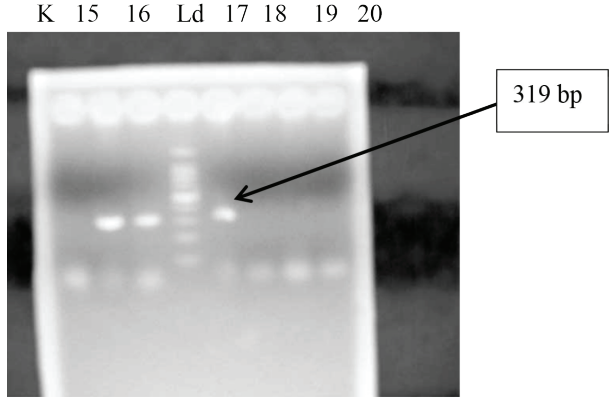

Figure 2: RT-AS PCR result from de novo AML patients with positive exon 12 NPM1 gene mutation (positive 319 bp fragment in patients number 15,16,17), negative result in patient number 18,19,20, K=Negative control, Ld=DNA Ladder 100bp.

The patients' characteristic (sex, age and FAB classification for AML subtype) is shown in table 1.

The initial leukocyte count and bone marrow blast cell

Table 1: Characteristic of AML patients

		Mutated NPM1	Non Mutated NPM1
Sex	Male	9	9
	Female	4	3
Age	Mean	50.6 years	46 years
	Range	17–78 yearrs	36–72 yearrs
FAB classification	M0	0	0
	M1	2	0
	M2	2	2
	M3	3	2
	M4	0	3
	M5	6	4
	M6	0	1
	M7	0	0

count is described in table 2. The median initial leukocyte count in AML patients with mutated NPM1 is 23,400 /µL, and in non mutated NPM1 group is 16,000 /µL (P=0.53). The median bone marrow blast cell count is 41% in AML patients with mutated NPM1 and 19% in non mutated NPM1 group (P=0.18).

The distribution of CD34 expression is shown in table 3. In AML patients with mutated NPM1, the CD34 expression can be seen in 6 patients (from total 8 mutated NPM1 patients) and in non mutated NPM1 group, CD34 expression can be found in 5 patients (from total 5 patients) (P=0.48) .

Discussion

The median initial leukocyte count in AML patients with exon 12 NPM1 mutation was 23,400 /µL which was higher than median leukocyte count (16,000/µL) in patients without this mutation, but not statistically significant. Previous studies reported that leukocyte counts were significantly higher in AML patients with mutated NPM1 gene than non-mutated group. This difference might be explained by other genetic mutations such as mutation in the FLT3-ITD and CEBPA mutation that could have some correlation with the leukocyte count at diagnosis. The other explanations for such dissimilarities were infectious complications of the patients and different frequency of mutations in geographic areas where the study was performed.[2,3,6,8]

The median bone marrow blast cell count in AML patients with exon 12 NPM1 mutation was 41% which was higher than in patients without this mutation (19%), but statistically was not significant. This result was different from previous studies reporting bone marrow blast cell counts were significantly higher in patients with AML with mutated NPM1 gene. Again, this difference might be interpreted by existence of other genetic mutations or difference in geographic area. Studies have reported that AML patients with higher bone marrow blast cell counts had a worse prognosis.[8,9] Based on French-American-British classification for AML, the majority of AML patients with exon 12 NPM1 gene mutation in this study were AML-M5 (46,2%). Other studies reported the same results they stated that AML patients with mutated NPM1 had a trend of differentiation to myelomonocytic series.[2,3]

The CD34 expression was not statistically different in AML patients with and without exon 12 NPM1 gene mutation. This result was different from the previous studies. Cauhan et al. reported that NPM1 gene mutation was significantly correlated with negative expression of CD34 and HLA-DR. Falini et. al described that 90-95% of their AML patients with mutated NPM1 gene were negative for CD34 expression. The possible causes proposed were other genetic mutations. Another possibility for lack of correlation between CD34 expression and NPM1 gene

Table 2: Leukocyte count and bone marrow blast cell count in patients with and without exon 12 NPM1 gene mutation

Parameter	Mutated NPM1	Non-mutated NPM1	P value
Leukocyte count ($\times 10^3/\mu L$)			
mean±SD	28.1±19.6	23.2±17.2	0.53
median	23.4	16	
range	6.3–65	5.9–42	
Bone marrow blast cell count (%)			
mean±SD	43.8±25.1	30.2±26.6	0.18
median	41	19	
range	9-75	4-72	

Table 3: The CD34 expression in patients with and without mutated NPM1 gene

	Mutated NPM1	Non mutated NPM1	P value
$CD34^+$	6 patients	5 patients	0.48
$CD34^-$	2 patients	0 patients	

mutational status in our study was the number of patients with acute promyelocytic leukemia that comprised almost 25% of the studied patients. In normal myeloid progenitor cell development, CD34 is mainly expressed on myeloblasts. This expression will be decreased in later maturation stages such as promyelocytes. In acute promyelocytic leukemia, most cells in bone marrow consist of promyelocytes that do not express CD34.[2,6] Martelli et. al reported that lack of expression of CD34 in AML with mutated NPM1 is caused by down regulation of CD34 gene expression. It is also related with the increment of HOX gene expression. In AML patients with mutated NPM1 gene, nucleolus and ribosomal stress cause down regulation of CD34 expression. Meanwhile, the positivity of CD34 expression has inverse correlation with the rate of complete remission. AML patients with CD34 expression show longer time to achieve complete remission than patients who lack its expression.[10]

Limitations of this study were small sample size and lack of data of other genetic mutations and cytogenetic abnormalities that could affect initial leukocyte counts, bone marrow blast cell counts and CD34 expression. In addition, infection status, ethnicity and genetic polymorphism of the patients are also required in order to determine all contributing factors.

Conclusion

Initial leukocyte counts, bone marrow blast cell counts and CD34 expression were not statistically different in the patients with and without exon 12 NPM1 gene mutation.

Conflict of Interest: None declared.

References

1. Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. Cancer. 2006;107(9):2099-108. doi: 10.1002/cncr.22233. PubMed PMID: 17019734.
2. Falini B, Martelli MP, Bolli N, Sportoletti P, Liso A, Tiacci E, et al. Acute myeloid leukemia with mutated nucleophosmin (NPM1): is it a distinct entity? Blood. 2011;117(4):1109-21. doi: 10.1182/blood-2010-08-299990. PubMed PMID: 21030560.
3. Verhaak RG, Goudswaard CS, Putten Wv, Biji MA, Sanders MA, Hugens W, et al. Mutations in nucleophosmin NPM1 in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood. 2005;106(12):3747-55. doi: 10.1182/blood-2005-05-2168. PubMed PMID: 16109776.
4. Sekeres MA, Kalaycio ME. Definition of Remission, Prognosis and Follow Up. In: Sekeres MA, Kalaycio MA, Bolwell BJ, editors. Clinical Malignant Hematology. 1st ed: The Mc-Graw Hill; 2007. p. 83-90.
5. Owen CJ, Fitzgibbon J. The genetics of acute myeloid leukemias. In: Provan D, Gribben J, editors. Molecular Hematology. 1st ed: Wiley-Blackwell; 2010. p. 42-50.
6. Chauhan PS, Ihsan R, Singh L, Dipta DK, Mittal V, Kapur S. Mutaiton of NPM1 and FLT3 Genes in Acute Myeloid Leukemia and Their Association with CLinical and Immunophenotypic Features. Dis Markers. 2013;35(5):581-8. doi: 10.1155/2013/582569
7. Ottone T, Ammatuna E, Lavorgna S, Noguera NI, Buccisano F, Venditti A, et al. An allele specific RT-PCR assay to detect type A mutation of the nucleophosmin-1 gene in acute myeloid leukemia. J Mol Diagn. 2008;10(3):212-6. doi: 10.2353/jmoldx.2008.070166. PubMed PMID: 18403613.
8. Thiede C, Koch S, Creutzig E, Steudel C, Lilmer T, Scaich M, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006;107(10):4011-22. doi: 10.1182/blood-2005-08-3167. PubMed PMID: 16455956.
9. Tong WG, Sandhu VK, Wood BL, Hendrie PC, Becker PS, PAgel JM, et al. Correlation between peripheral blood and bone marrow regarding FLT-3 and NPm1 mutational status in patients with acute myeloid leukemia. Haematologica. 2015;100(3):97-8. doi: 10.3324/haematol.2014.118422. PubMed Central PMCID: PMC4349287.
10. Martelli MP, Pettirossi V, Thiede C, Bonifacio E, Mezzasoma F, Ceccini D, et al. CD34+ cells from AML with mutated NPM1 harbor cytoplasmic mutated nucleophosmin and generate leukemia in immunocompromised mice. Blood. 2010;116(19):3907-24. doi: 10.1182/blood-2009-08-238899. PubMed PMID: 20634376.