Research Article

Mutations of *NPM1* and *FLT3* and deletion of chromosome 5 del(5q) as predictors of myelodysplastic syndrome becoming acute myelocytic leukemia

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ABSTRACT

Aim: This study aimed to determine the predictors of myelodysplastic syndrome (MDS)-related acute myelocytic leukemia (AML). **Materials and method:** A total of 36 patients, 31 were diagnosed as AML and five others as clinical MDS. Diagnoses were done using peripheral blood smears and bone marrow aspiration. Several tests were done to characterize the patient sample. **Results:** Immunophenotyping showed that 92% of the overall patient group had a myeloid lineage. A polymerase chain reaction (PCR)-based test found bands characteristic of mutations in *FLT3* and *NPM1*, and the chromogenic *in situ* hybridization test found a deletion in chromosome 5 del(5q). A logistic regression found the mutations of *FLT3* and *NPM1* with deletion of 5 del(5q) to be a predictor of MDS-related AML, at $P = 0.036$. The low and high predictor are <0.782 and >0.782, respectively. Receiving operating curve is a predictor of 76.5% area under curve. The sensitivity, specificity, and cutoff for each were 70.8%, 72.4%, and 0.782. The highest cutoff was in mutations of *FLT3* and *NPM1* with deletion of 5 del(5q), which stands in 0.969. **Conclusion:** Thus, mutations in *NPM1* and *FLT3* and deletion of 5 del(5q) were a predictor toward the changes of MDS into AML in the patients studied.

KEY WORDS: Chromosome deletion, Immunophenotyping, Leukemia, Myelodysplastic syndrome

INTRODUCTION

Acute myelocytic leukemia (AML) is a heterogenic disease that consists of many kinds of clinical descriptions and genetic disorders including cytogenetic mutation, genetic mutation, and changes in genetic expression (Bain *et al*., 2012). Research shows that different genetic changes work together with leukemogenesis. Research in animal models notes that single mutations (e.g., in *RUNX1, FLT3,* and *NPM1*) are not enough to cause phenotypic AML changes; for example, a fuse mutation of *RUNX-RUNX1T1* and *CBFB-MYH11*, each of which are caused by t(8;21) and inv(16)/t(16;16), succeeded in influencing cell differentiation but did not cause a leukemic phenotype (Ceesay *et al*., 2010). This study focuses on myelodysplastic syndrome (MDS)-related AML, which fits into the multiple mutation paradigm; Owen and Fitzgibbon (2010) found the molecular pathomechanism in MDS-related AML involves an *NPM1* and *FLT3* mutation and chromosome 5 deletion del(5q) or normal chromosome 5 that impacts MDS-related AML progress.[1]

MDS is a hematopoiesis that first has a regular organization (apoptosis), then becoming dysplastic and ineffective (antiapoptosis) in the spinal cord. Next, the numbers of immature cell (blasts) increase, then the transformation of MDS into AML can occur. The frequency of MDS cases that develop into AML, i.e., MDS-related AML, is about 20–30% (Falini *et al*., 2009). In the previous research conducted in the Department of Clinical Pathology, Airlangga University School of Medicine, Dr. Soetomo Hospital, Surabaya, East Java, Indonesia, from January 2 to October 30, 2014, 181 admitted patients between 14 and 77 years old suffering from leukemia

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were included in a study on the disease. Leukemia was characterized using the morphology-based French–American–British (FAB) classification system, 70 people (38.66%) had AML and 9 people (4.98%) had MDS (Gilliland and Gribben, 2010).[2]

The transformation of MDS into AML may be caused by a combination of abnormal genetics, epigenetic factor, and receptor signals, as well as microenvironmental factors and treatments (MDS secondary incidence 80%) (Greenberg *et al*., 1997; Hoffbrand and Moss, 2011; Quinitas-Cadarma *et al*., 2013). Genetic mutations such as mutations to *NPM1, FLT3,* and cytogenetic abnormalities such as deletion of chromosome 5 del(5q) are predictors of MDS, which will develop into AML for some patients. Genetic mutation can cause hematopoietic problems such as hampered differentiation caused by an *NPM1* mutation. In MDS, chromosome abnormalities such as a deletion of chromosome 5 del(5q) occur in 65–100% of cases.[3,4]

Berghe found that cytogenetic and genetic abnormalities occur in a different percentage in MDS-related AML cases than other AML cases, that is, all the cytogenetic and genetic abnormalities that are found in MDS are also found in AML even though they have a different incidence (Osato, 2004). The *NPM1* mutation is predominant in AML subgroups with a normal karyotype about 50% have this mutation. The *NPM1* mutation is accompanied by an internal tandem duplication in *FLT3* (*FLT3*-ITD) in between 40 and 60% of cases (Bain *et al*., 2012; Owen and Fitzgibbon, 2010). Other genetic abnormalities can also accompany the *NPM1* mutation, occurring in AML with normal karyotypes in about 45–55% of cases (Bain *et al*., 2012; Papaemmanuil *et al*., 2016). In adult patients with AML, an estimated 27–35% have an *NPM1* mutation and 11% an *FLT3*-ITD mutation (Patel *et al*., 2018; Simanjorang *et al*., 2010). *FLT3*-ITD is found in 30% of AML cases with normal karyotype.[5-7]

MDS needs to be accurately diagnosed because it can develop into AML during the course of the disease. The objective of this study is to determine the predictors of MDS-related AML.

MATERIALS AND METHODS

The research study was a cross-sectional study to understand the influence of *FLT3* and *NPM1* mutations and the deletion of chromosome 5 del(5q) as predictors of the change of MDS into AML. The research was conducted over 11 months from May 2017 to April 2018. Patients were diagnosed through samples (taken as described below) processed either in the laboratory or at the Hematology and Medical Oncology Department of Internal Medicine, Airlangga University School of Medical, Dr. Soetomo Hospital, Surabaya, Indonesia. Ethical clearance was obtained (No. 573/Panke.KKE/X/2016). Gene and chromosome investigations were done in Biomedical Sciences of Medical Faculty of Brawijaya University, Malang, East Java, Indonesia. There were 36 patients sampled in this research, 31 samples were diagnosed as AML; meanwhile, five were diagnosed as having clinical MDS.

Peripheral blood (blood smear) and bone marrow aspiration (bone marrow smear) samples were taken in Hematology and Medical Oncology Department of Internal Medicine, Airlangga University School of Medical, Dr. Soetomo Hospital, Surabaya, Indonesia. From a clinical check of the patient, AML is preliminarily diagnosed and cell morphology read by two clinical pathologists and a hematology-oncology consultant (i.e., FAB classification). Samples were also subjected to PCR (*FLT3* and *NPM1*) (Roche Diagnostic, USA), chromogenic *in situ* hybridization detection of chromosome 5 del(5q) with cDNA probe del(5q) biotin, and microscope Olympus CX-41 and immunophenotyping (BD Facs Calibur, Becton Dickinson, USA). Logistic regression and receiver operating characteristics (ROC) were used to determine high-risk or low-risk prediction after cutoff is determined.

RESULTS

Research Subject

The baseline data of the research subjects were as follows: 47–68 years old, 39%; 14–35 and 36–46 years old, both 25%; and ages 69–90 years old, 11%. Males were found higher (67%) than females (33%) in the sample. Immunophenotyping found

Table 1: The result of logistic regression relationship of gene predictor and MDS-related AML

Variables	*β* (Coeff.)	Wald	Df
FLT3	0.147	0.012	1
NPM1	1.618	1.688	1
Constant del (5q)	−0.761	0.160	1

Variable factors taken into account were FLTS3 and *NPM1*, affecting MDS turning into AML: MDS-related AML (chromosome del (5q)).

Table 2: *FLT3* and *NPM1* variation toward the probability MDS-related AML

FLT3	*NPM1*	Prob. MDS-related AML	MDS-related AML
−	−	0.079	−
+	−	0.090	−
−	+	0.302	−
+	+	0.334	−
−	+	0.833	+
−	−	0.844	+
+	−	0.862	+
+	+	0.969	+

92% myeloid lineage and 8% non-myeloid lineage. Patient diagnoses, based on the clinical checkup, immunophenotyping, and bone marrow aspiration smear, found that 86% of the subjects had AML (86%) and 14% non-AML.

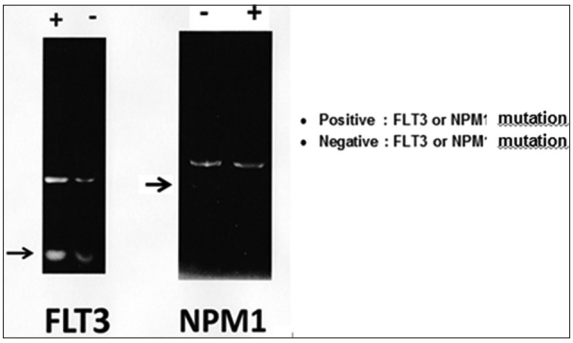

Figure 1: Mutation of *FLT3* and *NPM1* based on PCR test

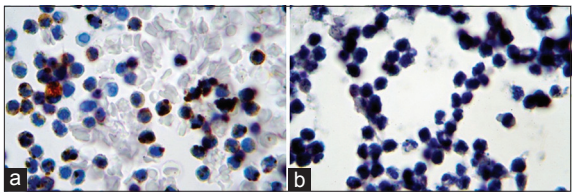

Figure 2: Cytogenetic examination, (a) chromosome 5 del(5q) deletion; (b) normal chromosome 5. (Chromosome 5 del(5q) deletion is brownish color and indicates positive [+]. Normal chromosome shown as blue cell indicates negative [−])

Significance of *FLT3* and *NPM1* affecting MDS-related AML

According to Table 1, ROC analysis showed 76.5% area under curve (AUC) with a confidence interval of 95%, and significance (P = 0.036) showing that factors *FLT3* and *NPM1* affecting MDS-related AML were valid and able to obtain cutoff value. The presence of a chromosome 5 deletion del(5q) was positively correlated with various mutations of *FLT3* and *NPM1*. The presence of normal chromosome 5q was negatively correlated with various mutations of *FLT3* and *NPM1* [Table 2].

Mutation of *FLT3* and *NPM1*

Figure 1 shows PCR examination of *FLT3* and *NPM1*. *FLT3* right side shows positive, *FLT3* left side shows negative, *NPM1* left side shows negative, and *NPM1* right side shows positive. As shown from the analysis, mutation of *FLT3* and *NPM1* with deletion of chromosome 5 del(5q) [Figure 2] was predictors of MDS-related AML (P = 0.036).

Immunophenotyping Examination of CD13, CD33, MPO, and CD34

ROC had a predictor of 76.5% AUC, with a probability under 0.782 (low predictor), above 0.782 (high predictor), sensitivity 70.8%, specificity 71.4%, and a cutoff of 0.782 [Table 1]. This result showed that CD13, CD 33, MPO, and CD34 were positive

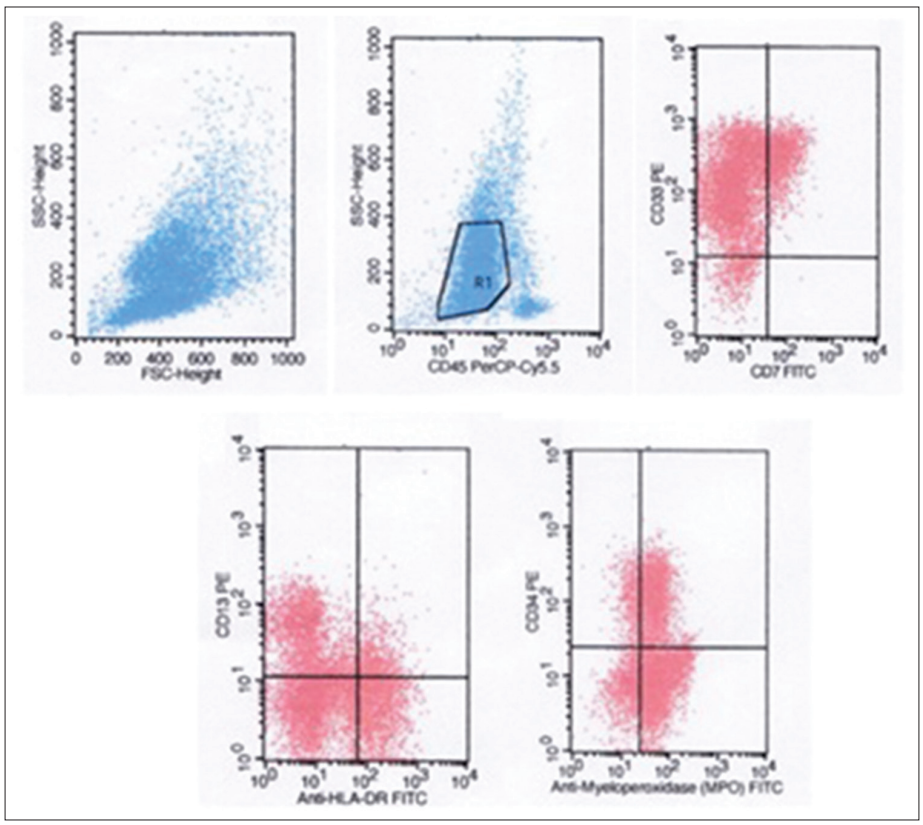

Figure 3: Immunophenotyping examination of myeloid lineage (CD10, CD34, CD79a, HLA-DR, and CD20)

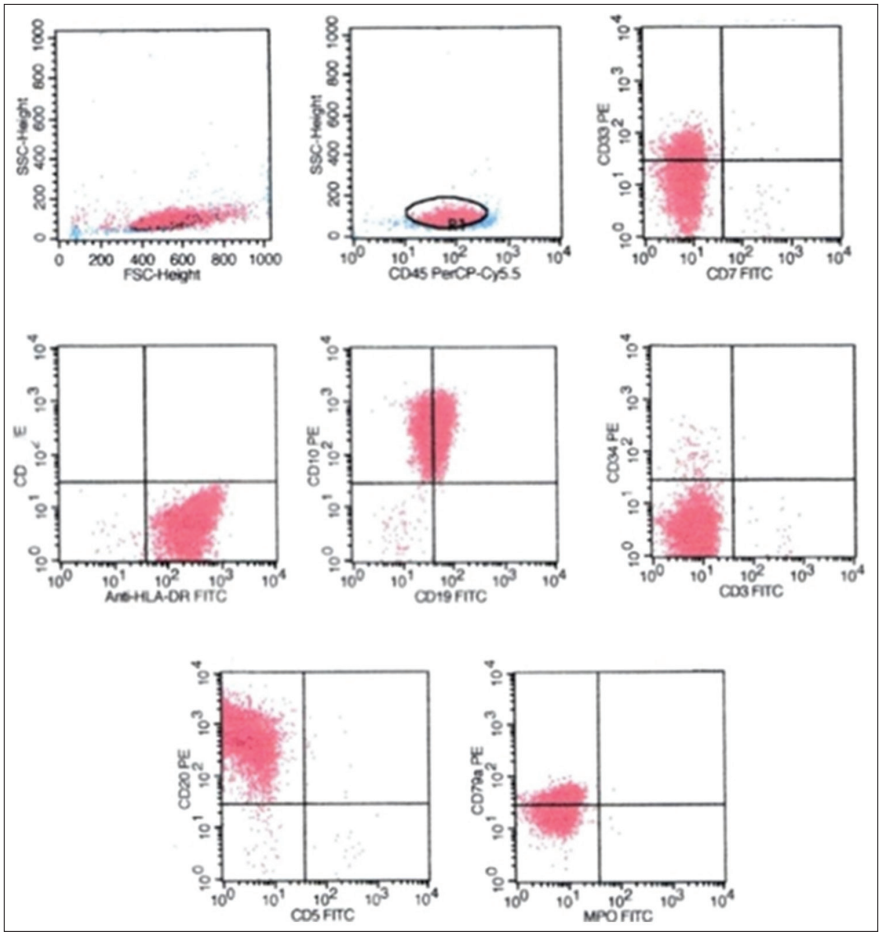

Figure 4: Immunophenotyping examination of lymphoid lineage

[Figures 3 and 4] was a result of immunophenotyping examination of lymphoid lineage.

DISCUSSION

Clinical checkup, immunophenotyping, and bone marrow aspiration smear allowed diagnoses of AML (86%) and non-AML (14%). Some potential risk factors for AML are the use of pesticide, electric field, the history of miscarriage from mother, chemical (benzene), virus, and genetic disorder, a mother has late age when she delivered a baby, magnetic field, parents' job, and diet (Vardiman *et al*., 2002). No definitive causes of the diagnosed leukemia were found for sure, however.[8,9]

MDS diagnosis may become a challenge because clinical and pathological description and diagnosis sometimes differ at the age of 70 years old when the highest number of illnesses is found. Hence, the diagnosis must be correct because there are many medications that can be used to repair the hematopoietic function and restore the quality of life of patients (Quinitas-Cadarma *et al*., 2013; Walter *et al*., 2012; Wetzler *et al*., 2012).[10-14]

The result of the immunophenotyping check was 92% myeloid lineage and 8% non-myeloid lineage. Using AML diagnosis, it can be seen that the series of myeloid (myeloblastic) is <20%.

The cutoff value sensitive is 70.8% and the specific is 71.4%, indicating that the studied mutations and deletions influence 0.7825584010 of MDS-related AML change. Other researchers have stated that in MDS-related AML, all the cytogenetic and genetic abnormalities that are found in MDS are also found in AML even though they have a different incidence. Cytogenetic abnormalities in MDS case with deletion of chromosome 5 del(5q) incidence had a lower limit of 57.4% and an upper limit of 95.6%. Translocations that are found in AML, for example, *t*(15;17), inv(16) and *t*(8;21), those abnormalities above are rarely found in MDS (Greenberg *et al*., 1997; Young, 2012).[15,16]

CONCLUSION

FLT3 mutation and 5 del(5q) chromosome deletion are as a predictor of MDS that changes into AML in some patients. *NPM1* as a predictor to determine MDS-related AML, using MDS-related AML, used a cutoff 0.782. The highest cutoff was found in *FLT3* and *NPM1* mutation with deletion of chromosome 5 del(5q) which was 0.969.

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REFERENCES

1. Bain B, Bates I, Laffan M, Lewis S. Dacie and Lewis Practical Haematology. 11th ed. London: Churchill livingstone; 2012. p. 139-74.
2. Ceesay MM, Wendy I, Mufti G. Myelodysplastic syndrome. In: Provan D, Gribben JG, editors. Molecular Hematology. 3th ed. London: Wiley-Blackwell; 2010. p. 89-103.
3. Falini B, Bolli N, Liso A, Martelli MP, Mannucci R, Pileri S, *et al.* Altered nucleophosmin transport in acute myeloid leukaemia with mutated NPM1: Molecular basis and clinical implications. Leukemia 2009;23:1731-43.
4. Gilliland DG, Gribben JG. Secondary myelodysplasia/acute myelogenous leukemia: Assessment of risk. In: Provan D, Gribben JG. editors. Molecular Hematology. 3th ed. London: Wiley-Blackwell; 2002. p. 51-6.
5. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, *et al.* International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079-88.
6. Hoffbrand AV, Moss PA. Essential Haematology. 6th ed. London: Wiley-Blackwell; 2011. p. 1150-222.
7. Quinitas-Cadarma A, Cortes JE, Kantarjianv H. Biology of chronic and acute myeloid leukemia. In: Hoffman R, Benz EJ Jr., Silberstein LE, editros. Hematology Basic Principles and Practice. 6th ed. New York: Elservier Saunders; 2013. p. 853-62.
8. Osato M. Point mutations in the RUNX1/AML1 gene: Another actor in RUNX leukemia. Oncogene 2004;23:4284-96.
9. Owen CJ, Fitzgibbon J. Myelodysplastic syndrome. In: Provan D, Gribben JG, editors. Molecular Hematology. 3th eds. London: Wiley-Blackwell; 2010. p. 42-9.
10. Papaemmanuil E, Gerstung M, Bullinger L, Verena I, Gaidzik MD. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2010;374:2209-21.
11. Patel SS, Kuo FC, Gibson CJ, Steensma DP, Soiffer RJ, Alyea EP 3rd, *et al.* High NPM1-mutant allele burden at diagnosis predicts unfavorable outcomes in *de novo* AML. Blood 2018;131:2816-25.
12. Simanjorang C, Adisasmita AC, Tehuteru ES. Gambaran epidemiologi kasus leukemia anak di rumah sakit Kanker "dharmais", 2004-2008. Indones J Cancer 2010;4:15-21.
13. Vardiman JW, Harris NL, Brunning RD. The world health organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292-302.
14. Walter MJ, Dong S, Li D, Jin S. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med 2012;366:1090-3.
15. Wetzler M, Marcucci G, Bloomfield CD. Acute and chronic myeloid leukemia. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo L, editors. Harrison's Principles of Internal Medicine. 18th ed. New York: McGraw-Hill Education; 2012. p. 905-14.
16. Young NS. 'Harrison's principles of internal medicine. In: Longo DL, Kasper DL, Jameson J, editors. Apalstic Anemia Myelodysplasia, and Releted Bone Marrow Failure Syndrome. 18th ed. New York: McGraw-Hill; 2012. p. 887-97.

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