Original Article

Incidence of Nucleophosmin (NPM1) and FMS-like Tyrosine Kinase (FLT3) Mutation in Adult Patients with Acute Myeloid Leukaemia (AML) and Its Influence on Survival; A Single Centre Study

Akut Myeloid Lösemi (AML) Tanılı Erişkin Hastalarda Nükleofosmin (NPM1) ve FMS Like Tirozin Kinaz (FLT3) Mutasyonun İnsidansı ve Sağ Kalıma Etkisi; Tek Merkez Deneyimi

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ABSTRACT

Introduction: Acute Myeloid Leukaemia (AML) is a disease characterized by bone marrow failure due to increased proliferation resulting from the protection of myeloid precursor cells from differentiation and apoptosis control in the bone marrow. Nucleophosmin (NPM1) and FMS-like tyrosine kinase (FLT3) are mutations frequently seen in AML, which has heterogeneous genetics. The present study aimed to evaluate the clinical characteristics and lifespan of the patients with NPM1 and FLT3 in AML.
Materials and Methods: The study retrospectively investigated the clinical, immunophenotypical, and genetic parameters of the patients diagnosed with AML between 1 January 2012 and 31 June 2019 in the haematology clinic of Dicle University following WHO 2016 criteria. The study primarily focused on the patients' response to the disease, genetic characteristics, and the relationship of NPM1 and FLT3 mutations with their lifespan
Results: The study was performed 107 cytogenetically normal patients were investigated using RT-PCR (Real-Time Polymerase Chain Reaction) in NMP1 and FLT3 mutations in 269 AML patients. While the median survival time in the NPM1-positive patient group was 12.3 months (95% confidence interval (C.I.): 0.1-32 months), it was 10.6 months (%95 C.I: 4,9-16,3 months) in the NPM1-negative group. On the other hand, the median survival time in the FLT3-positive patient group was 8,4 months (95% C.I: 1,2-15,6 months), and it was 12,3 months (95% C.I: 3,8-20,8 months) in the FLT3-negative group. While the number of NPM1-positive patients was 31 (29%), one of the FLT3-positive patients was 19 (17.8%)
Discussion: The study found that while the NPM1 positivity rate in AML cytogenetically normal patients was 29%, the FLT3 positivity rate was 17.8%, representing the first-ever data in this respect in our country. The results indicate that overall survival was better in cases with NPM1 and worse in those with FLT3.

Keywords: AML, NPM1, FLT3, survival

ÖZET

Giriş: Akut Myeloid Lösemi (AML) kemik iliğinde myeloid öncü hücrelerin diferansiyasyon ve apoptozis kontrolünden korunup proliferasyon hızının artması sonucu kemik iliği yetmezliği ile giden bir hastalıktır. Nükleofosmin (NPM1) ve FMS like tirozin kinaz (FLT3), heterojen bir genetiğe sahip olan AML'de sık görülen mutasyonlardır. Bu çalışmamızda AML'de NPM1 ve FLT3 tespit edilen hastaların klinik özelikleri ve yaşam sürelerini değerlendirmeyi amaçladık.
Gereç ve yöntemler: Çalışmaya 01 Ocak 2012-31 Haziran 2019 tarihleri arasında Dicle Üniversitesi hematoloji kliniğinde WHO 2016 kriterlerine göre AML teşhisi konan hastaların klinik, immünofenotipik ve genetik parametreleri retrospektif olarak incelendi. Hastaların tedaviye cevapları,

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yaşam süreleri, genetik özelikleri ve NPM1 ve FLT3 mutasyonlarının yaşam süresi ile ilişkisi incelenmiştir.
Bulgular: Allojenik hematopoietik hücre naklinden sonra akut GVHH'li 59 hasta ve akut GVHH'si olmayan Çalışmamızda 269 AML tanısı alan hastalardan 'sitogenetik olarak normal 107 hasta RT-PCR (Gerçek Zamanlı Polimeraz Zincir Reaksiyonu) kullanılarak NMP1 ve FLT3 mutasyonlarında araştırıldı. NPM1 pozitif hasta grubunda ortanca sağkalım 12,3 ay (% 95 confidence interval(C.I.): 0,1-32 ay) tespit edildi, NPM1 negatif hasta grubunda ise 10,6 ay (% 95 C.I: 4,9-16,3 ay) olarak tespit edildi. FLT3 pozitif hasta grubunda ortanca sağkalım süresi 8,4 ay (%95 C.I: 1,2-15,6 ay) olup FLT3 negatif grupta ise 12,3 ay (%95 C.I: 3,8-20,8 ay) olarak tespit edildi. NPM1 pozitif hasta sayısı 31 (%29), FLT3 pozitif hasta sayısı 19(%17,8) izlendi
Tartışma: Ülkemizin ilk verisi olarak AML sitogenetik normal hastalarda NPM1 pozitiflik oranı %29, FLT3 pozitiflik oranı %17,8 bulundu. NPM1 ile genel sağkalım daha iyi, FLT3 ile genel sağkalım daha kötü olarak izlendi.

Anahtar kelimeler: AML, NPM1, FLT3, sağkalım

Introduction

AML is a malignant haematological disease that causes bone marrow failure and the related symptoms and signs resulting from the abnormal accumulation of myeloblasts from hematopoietic precursor cells in the bone marrow. It is a disease with heterogeneous genetic characteristics. In its 2016 AML criteria, the World Health Organization (WHO) defines it as a condition where the myeloblast ratio detected in the bone marrow or peripheral blood is at least 20% by multi-parameter flow cytometry. An exception to this is that AML diagnosis can be made even if the myeloblast rate is below 20% in the presence of cytogenetic or molecular AML-specific mutations. [1]

It is essential to detect genetic mutations before treatment in AML patients. Genetic mutations determine the prognosis and play a role in defining the treatment process to be performed. The detected mutation also provides information about the minimal residual disease, which is essential in monitoring it [2].

Previous research demonstrates that molecular translocations and genetic mutations in AML can differ between societies. Some translocations seen in AML are RUNX1 / RUNX1T1 t (8; 21), CBFB / MYH11 inv (16), KMT2A / MLLT3 t (9; 11), PML-RARA t (15; 17) and BCR-ABL t (9 ; 22). PML-RARA mutation is a cause of acute promyelocytic leukaemia and is associated with a good prognosis. RUNX1 / RUNX1T1 t (8; 21), CBFB / MYH11 inv (16) mutations are also frequent translocations associated with a good prognosis. Apart from these, other chromosomal anomalies are associated with poor prognosis, and the absence of any chromosomal anomalies constitutes the intermediate-risk group. [3]

While nucleophosmin-1 (NPM-1) presence is associated with a good prognosis, the presence of FMS-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD) causes a poor prognosis. Establishing these mutations in AML patients provides direction for treatment decisions and predicts the response to induction and consolidation chemotherapy and the risk of relapse and overall survival. [2]

Nucleophosmin (NPM1) is a ubiquitously expressed phosphoprotein generally located between the nucleus and the cytoplasm. It plays a role in the ribosomal protein assembly and transport and regulation of tumour suppressor ARF (cyclin-dependent kinase inhibitor 2A). When an NPM1 mutation exists in AML, transportation of such proteins and enzymes from the cytoplasm to the nucleus is reduced [4, 5].

FMS-like tyrosine kinase 3 (FLT-3) regulates the differentiation, proliferation, and survival

of hematopoietic progenitor cells and is also required for normal haematopoiesis. Mutations such as those of the Juxta-membrane domain of FLT3 receptor tyrosine kinase and those of internal tandem duplication lead to uncontrolled proliferation of myeloblasts [6,7].

The present study aimed to determine the frequency of NPM1 and FLT3 in cytogenetic negative AML patients and determine the prognostic difference between the NPM1 positive and NPM1 negative patient groups and the one between the FLT3 positive and FLT3 negative patient groups.

Material and Method

The sample of the present study consisted of the patients presented to the clinic of our university between 1 January 2012 and 31 June 2019 and were diagnosed with peripheral smear, flow cytometry, bone marrow smear, and AML according to 2008 -2016 WHO classification. Translocation analyses of the patients were performed on RT-PCR on LightCycler 480 (Roche, Germany) device with t (15; 17), t (8; 21), inv 16, and t (9; 22). Of this patient group, the patients with negative translocation and analyzed using EZ1 Blood mini kit (Qiagen, Germany) and FLT3 ITD Toolset (Teknigen Biyoteknoloji, Istanbul, Turkey) and Lightmix FLT D835 kit (TıbmolBiol, Berlin, Germany) kit NPM1 ve FLT3-ITD and FLT3 D895 RT- PCR were included in the study. The study compared the overall survival of the NPM1 positive and the NPM1 negative patient groups. In the same patient group, survival was compared in the FLT3-ITD and FLT3-D895 positive patient group and the FLT3-ITD and FLT3-D895 negative patient group.

Characteristics of the Patients: Cytogenetic and molecular mutations were screened in 269 patients diagnosed with AML, and the lifespan of the patients and its relationship with the genetic mutation were examined.

Treatment: The standard "7 + 3" treatment was administered to the patients. Cytosine arabinoside 100mg / m² was given for seven days (24 hours continuous infusion), and idarubicin treatment of 12 mg/m² was administered for three days. Bone marrow evaluation was performed on day 28 of the treatment. In patients in remission, high-dose cytosine arabinoside was administered 3 or 4 times, once in 28 days in a dosage of 3 g/m² twice a day on days 1, 3, and 5. Allogeneic stem cell transplantation was planned for patients with a poor prognosis or those who did not complete remission. Patients who were not suitable for standard treatment received 5 + 2 treatment or the patients whose performance score was low and could not tolerate these two treatments received 5-azacitidine, decitabine, and supportive treatment.

NPM1 Screening: Genomic DNA was obtained from blood samples using an EZ1 Blood mini kit (Qiagen, Germany). The screening was initiated using the ipsogen NPM MutaScreen (Qiagen, Hilden) Kit in patient samples whose DNA concentrations were adjusted. Final reaction volume: Using 12.5µl TaqMan Universal PZR Master mix, 1.0 µl Primer and probe mix separately (PPM-Total NPM1, PPM-Mut NPM1, PPM-NPM1 MutA, PPM-NPM1 MutB or PPM-NPM1 MutD), 6.5µlNuclease-free PZR-grade water was calculated to yield 5.0µlDNA, 2x 25 µl.

FLT3 Screening: DNA isolation was performed on the MagnaPure LC 2.0 device (Roche Diagnostics GmBH, Mannheim, Germany) in line with the instructions of the manufacturer company using the Magna Pure LC DNA isolation kit from blood tubes with EDTA taken from the patients. To specify the FLT3-ITD mutations in the DNAs thus obtained, an analysis was performed on the device LightCycler 480 in line with the manufacturer's instructions using the toolset (Teknigen Biyoteknoloji, Istanbul, Turkey) and Lightmix FLT-D835 kit (TıbmolBiol,

Table 1: General characteristics of the patients diagnosed with AML

	Disease Variables	Number – Rate
Cytogenetic Distribution of the Patients with AML	Number of Patients	269
	t(15;17)	61(%22,7)
	t(8;21)	17(%6,3)
	inv 16	12(%4,5)
	Patients with cytogenetics normal	169
	Patients who could not undergo cytogenetic evaluation	10
	NPM1 and FLT3 evaluated patient group	107
General Characteristics of the Patients with Cytogenetic Normal AML Examined for NPM1, FLT3-ITD, and FLT3-D895 mutations	Age	51,5 (17-91)
	Haemoglobin	8,9(3,5-16) gr/dl
	White Blood Cells	27 900(1 600-388 000) /mm³
	platelet	53 000(7 000-452 000) /mm³
	CD34 +	41(38,3%)
	NPM1+	31(29%)
	FLT3 +	17(17, 8%)
Treatments Administered to Patients with Cytogenetic Normal:	7+3 [a]	79(73,8%)
	5+2 [b]	6(5,6%)
	5- azacitidine	15(14%)
	decitabine	3(2,8%)
	Supportive Treatment	4(3,7%)

a: 7+3 ARA-C 100 mg/m^2 1 to 7 day, İdarabucin 12 mg/m^2 1 to 3 day
b: 5+2 ARA-C 100 mg/m^2 1 to 5 day, İdarabucin 12 mg/m^2 1 to 2 day
nucleophosmin-1 (NPM-1), FMS-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD)

Table 2. Median survival time and the survival rates over five years concerning cyto-negative AML patients

	NPM1(+)	NPM1(-)	FLT3 (+)	FLT(-)
Median Survival Time (months)	12,3	10,6	8,4	12,3
Survival Rates over a 5-Year Period	37%	20%	19%	29%

Berlin, Germany). The results emerging from the FLT3-ITD detection were analyzed using the high-resolution melting method, and the Tm calling analysis was used to analyze the results obtained in the one for FLT-D835, both following the instructions of the manufacturing company.

Statistics: The data were analyzed using IBM SPSS v. 24 (IBM Corp., Armonk, USA). The descriptive analysis-explore test was used to define the normal distribution of the continuous variables. The continuous variables with normal and non-normal distribution were presented using mean, standard variation, and median values. P value of <0.05 was considered statistically significant. On the other hand, numbers and percentages were used for categorical variables. Kaplan-Meier survival curve was performed using the log-rank test.

Results

The study sample consisted of 269 patients diagnosed with AML, of whom 146 (54.3%) were men and 123 (45.7%) were women. The cases were examined with the RT-PCR method in terms of three cytogenetic parameters frequently detected through this method, as a result of which 61 patients were detected as t(15; 17) positive (22.7%), 17 as

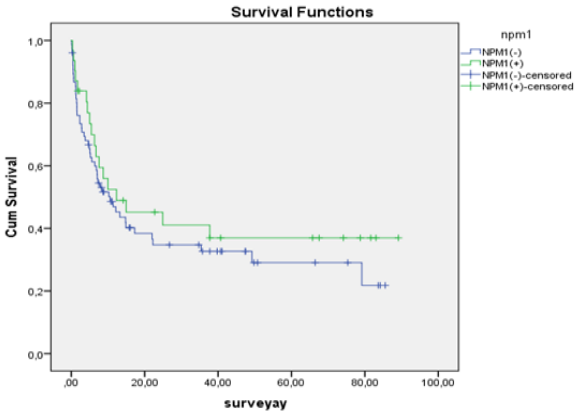

Figure 1: The overall survival (months) in NPM1 (+) and NPM1 (-) patients

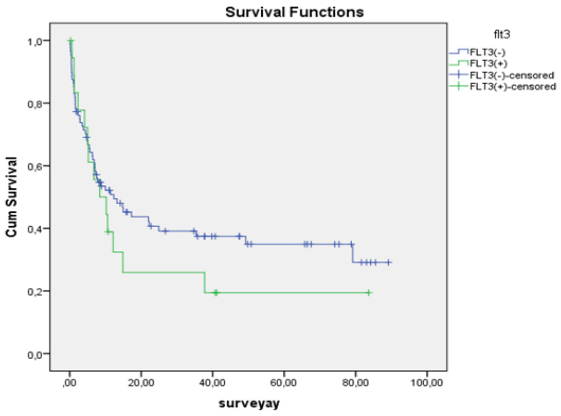

Figure 2: The overall survival (months) in FLT3 (+) and FLT3 (-) patients

t(8;21) positive (6.3%) and 12 as inv16 positive (4.5%). The patients diagnosed with AML who had had positive translocation were not included in the study. 107 patients examined for NPM1, FLT3-ITD, and FLT3-D895 mutations were evaluated.

Of the patients with normal cytogenetic, 60 (56.1%) were men, and 47 (43.9%) were women. The mean age of the patients was 51.5 (17-91) years. The haemogram values of the patients at the time they presented to the clinic were as follows: WBC mean value was 27 900/mm³ (1 600-388 000), haemoglobin mean value 8.9 gr/dl (3.5-16gr/dl), platelet mean value 53 000 (7 000-452000/mm³). AML molecular mutations were NPM1 31(29%), FLT3-ITD 19 (17.8%) FLT3-D895 2 (1.9%). NPM1 mutation and FLT3 mutation positive only 1(0.9%). (Table 1)

While the standard 7+3 treatment was administered to 79 patients (73.8%), 15 (14%) patients received 5- azacitidine treatment, six patients (5.6%) 5 + 2 treatment, three patients (2.8) decitabine treatment and four patients (3.7%) supportive treatment. While 40 (37.8%) patients moved on with their lives during the monitoring period, 67 (62.6%) patients died. While 42 (39.3%) of the patients were refractory to treatment, 34 (31.8%) patients had a recurrence in the later periods.

Based on the analysis of the cases with normal cytogenetic, the present study found that the rate of NPM1 positive incidence was 29% and FLT 3 positivity rate of 17.9%.

The median survival time of the patients was 10.6 months, which was found to be 12.3 months in NPM1 positive patients and 10.6 months in NPM1 negative patients. Even though the patients with NPM1-positive AML seemed advantageous in terms of a longer survival time, the result was not statistically significant ($p>0.05$). (Table 2, Figure 1))

The median survival time in patients with FLT3-ITD-positive AML was 8.4 months, 12.3 months in the FLT3-ITD negative patients. Another statistically non-significant result was that the patients with FLT3-positive AML had a shorter survival time ($p>0.05$). The survival rate over five years in NPM1 positive patients was 37%, 19% in FLT3 positive patients. (Table 2., Figure 2.)

Discussion

The FLT3-ITD and NPM1 mutations in patients with AML play an essential role in prognostic risk classification. Defining the correct mutation can therefore help optimize the therapeutic approaches in AML. Past research suggests that the presence of an

NPM1 mutation might indicate a good prognosis [2]. In the present study, we investigated the overall survival time of 107 cytogenetically normal patients with those with positive NPM1 mutation and compared the results with the NPM1 negative patient group. The group with NPM1 positive AML had a median survival time of 12.3 months; the rate of the median overall survival time over five years was 37%. The overall survival in patients with NPM1 negative AML, on the other hand, was 10.6 months. The analysis of the NPM1 mutation regardless of FLT3 mutation indicated a relationship between NPM1 positivity and a longer survival time. Though observed as an advantage in clinical practice in terms of a longer survival time, it was not found to be statistically significant p (0,374). The lack of a statistically significant difference may be attributed to the retrospective structure of the study, the study sample consisting of a small number of patients, and the non-inclusion of other prognostic parameters in the evaluation.

Patients with FLT3-positive AML have a higher risk of prognosis and a possibility of recurrence after achieving remission. The patient group with FLT3 positive AML had a mean survival time of 8.4 months, 12.3 months in the group with FLT3 negative AML. Even though the mean survival time was longer in patients in FLT3-negative patients, the results indicate no statistically significant difference in this respect p (0.34). Since FLT3 TKD is in 2(1.9) patients, survival analysis is not performed. Although there have been no survey studies investigating FLT3 cases in our society, the results should be interpreted with caution due to the retrospective structure and the small sample size of the study, and the difference between consolidated treatment options. The survival rate over five years was 19% in FLT3-positive patients and 29% in patients with FLT3-negative AML. Much of the previous research reports that FLT3 positivity is related to a shorter survival time. Similarly, our study results also indicate that the FLT 3 positivity is related to a shorter survival time in our society [8, 9].

Many parameters influence the prognosis in patients with AML, one of the most critical genetic mutations. Previous research on the frequency of genetic mutations suggests that it might vary from one society to another. Based on the analysis of the cases with normal cytogenetic, the present study found that the rate of NPM1 positive incidence was 29%. While WHO reports an NPM1 positivity rate between 45 to 64% in the patient group with adverse cytogenetic in its statistics for 2016, research reports that it is 28.3% in Egypt, 24.9% in Japan, and lower than 10% in South Africa [10-13].

The FLT3-ITD mutation is frequently observed in AML, and the FLT3-D895 mutation can be observed in some patients. Its incidence, like the NPM1 mutation, may differ from one society to another. According to WHO, its incidence has been reported to be between 20 and 40% [14]. Research reports a lower positivity rate between 10 and 20% in India and China [15, 16]. The present study, on the other hand, found an FLT 3 positivity rate of 17.9%.

The frequency of NPM1 and FLT3 mutations in our patients, which was observed not to be very high in the present study, is lower than the one observed in western countries (such as the USA, England, Germany, etc.) but similar to the one observed in eastern countries (such as India, China, Japan, etc.).

In conclusion, some limitations that partially limited the present study need to be considered, such as the retrospective structure and the small sample size of the study, as a study on a heterogeneous disease like AML, and differences in treatment options. Previous studies report that frequency of mutation may differ between societies. As there has been no

research investigating the frequency of genetic and molecular mutations and their effect on survival time in our country, we believe that the present study will provide essential insights into the current literature. Further research should be performed in more centres and larger samples.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127: 2391-2405
2. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017; 129: 424-447.
3. Swerdlow SH, Harris CE, Jaffe NL, Pileri ES, Stein SA, Thiele HJ (2017) WHO classification of tumors of hematopoietic and lymphoid tissues, 4th edn, vol 2IARC WHO Classification of Tumours, No 2, 2017
4. Ivey A, Hills RK, Simpson MA, Grech GA, Patel GY, Bhudia N, et al. UK National Cancer Research Institute AML Group. Assessment of minimal residual disease in standard-risk AML. N Engl J Med. 2016; 374(5): 422-433.
5. Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients acute myeloid leukemia (AML). Blood. 2006; 107(10): 4011-4020.
6. El Fakih R, Rasheed W, Hawsawi Y, Alsermani M, Hassanein M. Targeting FLT3 Mutations in Acute Myeloid Leukemia. Cells. 2018; 7: 4-8.
7. Richard F. Schlenk Differential impact of allelic ratio and insertion site in FLT3-ITD–positive AML with respect to allogeneic transplantation Blood 2014; 124 (23): 3441-3449.
8. Yanada M, Matsuo K, Suzuki T, Kiyoi H, Naoe T. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. Leukemia. 2005;19(8):1345–1349.
9. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002; 99(12): 4326–4335.
10. Swerdlow SH, Harris CE, Jaffe NL, Pileri ES, Stein SA, Thiele HJ (2017) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn, vol Blood. 2016; 127(20):2375-90.
11. Sofan MA, Elmasry S, Salem DA, Bazid MM. NPM1 gene mutation in Egyptian patients with cytogenetically normal acute myeloid leukemia. Clin Lab 2014; 60(11):1813–1822
12. Suzuki T, Kiyoi H, Ozeki K, et al Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood 2005; 106(8):2854–2861
13. Marshall RC, Tlagadi A, Bronze M, et al. Lower frequency of NPM1 and FLT3-ITD mutations in a South African adult de novo AML cohort. Int J Lab Hematol 2014; 36(6):656–664
14. Swerdlow SH, ed (2008) World Health Organization classification of tumours, Lyon, 4th edn Blood. 2011; 117(19): 5019-32.
15. Sazawal S, Singh N, Jain S, et al NPM1 and FLT3 mutations in acute myeloid leukemia with normal karyotype: an Indian perspective. Indian J Pathol Microbiol 2017; 60(3):355–359.
16. Gou H, Zhou J, Ye Y, et al. The prevalence and clinical profiles of FLT3- ITD, FLT3-TKD, NPM1, C-KIT, DNMT3A, and CEBPA mutations in a cohort of patients with de novo acute myeloid leukemia from southwest China. Tumour Biol 2016; 37(6): 7357–7370.

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