Open Access Original Article

Outcomes of Patients with NPM1 Positive Acute Myeloid Leukemia, An Experience from a Tertiary Care Hospital in Karachi, Pakistan

***Maria Zulfiqar*[1]**
***Natasha Ali*[2]**
***Usman Shaikh*[2]**
***Hamzah Jehanzeb*[3]**
***Salman Arif*[1]**
***Nabiha Saeed*[1]**
***Zurrya Fasih Khan*[1]**
***Zeeshan Ansar*[4]**

[1]Department of Oncology, Aga Khan University Karachi, Pakistan
[2]Department of Pathology and Laboratory Medicine/ Oncology, Aga Khan University Karachi, Pakistan
[3]Medical College, Aga Khan University Karachi, Pakistan
[4]Department of Pathology and Laboratory Medicine, Aga Khan University Karachi, Pakistan

Address for Correspondence
Dr. Maria Zulfiqar
Department of Oncology
Aga Khan University Karachi, Pakistan.
dr.mariaali@yahoo.com

Abstract

Objectives: To determine the response to induction chemotherapy, overall survival, and relapse rate in patients with NPM1-positive acute myeloid leukaemia.
Methodology Patients diagnosed with AML from January 2015 to July 2022 at Aga Khan University Hospital Karachi were included in the study. Patient demographics, clinico-haematological parameters, and molecular analysis for the NPM1 mutation were performed. Response to standard induction chemotherapy, overall survival, and relapse rate were assessed.
Results: A total of 76 cases of AML were analysed. The mean age of the sample was 33.7 years, of which 63.2% were males and 36.8% were females. The patients were stratified into two groups: those who were positive for NPM1 while negative for FLT3 (NPM+/FLT3-), representing 18.4%, and those who were negative for both NPM1 and FLT3 (FLT3-/NPM-), representing 81.6% of cases. On day 28 post-induction, the complete remission rate was 78.6% in the NPM1 positive group and 77.4% in the NPM1 negative group. In the NPM1+/FLT3-group, 54.5% of cases who were in remission at day 28 subsequently relapsed, compared to 50.0% of NPM1-/FLT3-cases. The overall survival could not be assessed between both the groups due to the low number of deaths.
Conclusion: AML patients harbouring only the NPM1 mutation do not show significant differences in outcome as compared to those negative for both mutations.
Keywords: Acute myeloid leukemia (AML), Complete remission (CR), Relapse rate (RR), Overall survival (OS), FLT-3 ITD, FLT-3TKD and NPM-1.

Introduction

Molecular genetic abnormalities in acute myeloid leukaemia play an important role not only in diagnosis but also in determining the clinical course and prognosis. Mutations in the NPM1 gene confer a good prognosis, while internal tandem duplication of the fms-like tyrosine kinase-3 gene (FLT3-ITD) is associated with a bad prognosis. Knowledge of these mutations not only aids in treatment decisions but also in predicting response to induction chemotherapy, overall survival, and relapse rates. Acute myeloid leukaemia is a heterogeneous group of disorders characterised by abnormal proliferation of hemopoietic progenitor cells and is the most common leukaemia in adults.[1] There are various cytogenetic aberrations and molecular genetic alterations identified over the past years that have a role not only in pathogenesis but also in disease progression.[2] Cytogenetic analysis provides the most important information regarding prognosis. However, by conventional chromosomal banding analysis, 50% of AML patients have a normal karyotype.[3] In this intermediate risk group, molecular genetic markers are useful to stratify patients into favourable and adverse groups.[4] For example, FMS-like tyrosine kinase-3 gene (FLT3) mutations of internal tandem duplication (ITD) type[5] and partial tandem duplication of the mixed lineage leukaemia gene[6] are poor prognostic factors; likewise, mutations in NPM genes in the absence of mutations in FLT3 ITD carry a good prognosis.[7] These molecular markers aid not only in risk stratification but also in treatment decisions and can be a useful tool for the assessment of minimal residual disease.

In adults, 50–60% of AML patients with a normal karyotype have NPM gene mutations; thus, it is one of the most frequent genetic abnormalities in AML.[8] Nucleophosmin, or nucleolar phosphoprotein B23, located within the nucleolus, is a nucleocytoplasmic shuttling protein

Authorship Contribution: [1,3]Conceived and planned the idea of the study, [7]final approval of the version to be published, [1,2,4]drafting the work or revising it critically for important intellectual content, [3,4]Collecting the data, Active participation in active methodology

Funding Source: none
Conflict of Interest: none
Received: June 13, 2023
Accepted: Nov 08, 2023

involved in chaperoning ribosomal proteins and core histones from the nucleus to the cytoplasm as well as regulating the ARF-p53 tumour suppressor pathway.[9] As per the 2022 World Health Organisation (WHO) classification of myeloid neoplasms, NPM1*mut*-AML is recognised as a distinct entity, irrespective of the blast counts.[10] Clinically, patients with the NPM mutation often present with high blasts, white cells, and platelet counts and with increased extramedullary involvement.[11] Also, it has been observed that patients with this mutation respond better to chemotherapy and have improved outcomes.[12].

The present study was done to determine the clinical relevance and prognostic significance of patients with NPM mutations in terms of rate of complete remission followed by induction, relapse rate, and overall survival rate.

Methodology

A total of 76 adult patients were included in this study who were registered at Aga Khan University (AKU) Karachi during the time period of January 2015 until July 2022. They belonged to different ethnic groups. The diagnosis of AML was made after bone marrow and peripheral blood film examinations based on WHO criteria. Patients with AML-M3 were excluded from the study due to different induction protocols. Baseline characteristics, including age, gender, haemoglobin, white blood cell, and platelet counts at presentation, were noted. All the data collection was done via the patient's medical records, and the patient information was recorded in a predesigned Performa. Approval was obtained from AKU's ethical review committee (ERC # 2022-8203-23491) before starting the study.

A 3-ml whole blood or bone marrow sample was used for molecular studies. PCR for the NPM1 mutation was performed using complimentary DNA using the Amplification Refractory Mutation System Methodology. NPM1 was amplified by nested PCR using specific primers. Amplification was performed in a thermal cycler (S-1000). The samples were then run on gel electrophoresis using 3% polyacrylamide gel for NPM1. For gene amplification, denaturation at 94°C for five minutes, annealing at 94°C for 45 seconds, extension at 62°C for 60 seconds, final extension at 72°C for one minute, and holding at 72°C for three minutes were done. Positive and negative controls were also run.

All the patients received induction chemotherapy 3+7 (45–60 mg/m2 of daunorubicin or idarubicin 12 mg/m2 for 3 days plus 100 mg/m2/day of cytarabine for 7 days) followed by consolidation with either an allogenic stem cell transplant or four cycles of cytarabine 1-3 g/m2/day for a total of 3 days on days 1, 3, and 5 based on their risk stratification. A bone marrow examination was done on day 14 of induction to see clearance of blast cells and at day 28 to see morphological remission. The patients were followed for physical examination and haematological assessment at the outpatient services of Aga Khan University Karachi. Complete remission, relapse, and overall survival rates were evaluated. Complete remission was defined as bone marrow blasts <5%, absence of circulating blasts and blasts with Auer rods, absence of extramedullary disease, ANC ≥1.0 × 10^9/L and platelet count ≥100 × 10^9/L. Relapse was defined as the reappearance of leukaemia cells in the bone marrow, peripheral blood, or elsewhere (extra medullary disease) after the attainment of a CR. Overall survival was defined as the duration in days from the diagnosis till death regardless of disease recurrence, or the date of the last follow-up visit.

The rate of remission at day 28 and relapse after remission were compared between the NPM1+/FLT3- and NPM1-/FLT3- groups through a Chi-square test. Kaplan-Meier models for survival and remission were used to calculate the median survival time and median time to remission for both groups as well as for the overall cohort. These times were then tested for any significant differences through a log-rank test. Additionally, a univariate Cox proportional hazards model was used to calculate hazard ratios for survival and remission for both the groups. Moreover, two multivariable logistic regression models considering FLT3 positivity, gender, age, haemoglobin, platelet, and WBC counts were constructed with remission at day 28 and relapse after day 28 remission as the outcomes of interest. The analysis was carried out using R version 4.2.2.

Results

Patients with known NPM1 and FLT3 status were divided into two groups: NPM+/FLT3- and NPM-/FLT3-. Our sample (n = 76) consisted mostly of NPM1- and FLT3- cases (n = 62, 81.6%), with NPM1+/FLT3- cases

representing only 18.4% of the sample (n = 14). The sample had a greater representation of males as opposed to females (63.2% vs. 36.8%). The mean age was 33.7 years. The means for the haematological parameters were as follows: Hb 8.71 g/dl, WBCs 28.3 x 10^9/L and platelets 71 x 10^9/L (Table 1). The overall median time to remission from the time of diagnosis was 36 days (95% CI: 35–39), while that of the NPM1+ and NPM1– groups was 35.5 days (95% CI: 31-41) and 36.5 days (95% CI: 36-41). The difference between the medians of these two groups was found to be statistically insignificant under a log-rank test (*p-value* 0.3). A hazard ratio calculated for this comparison using a univariate Cox proportional hazard model was also found to be statistically insignificant (HR: 1.367, 95% CI: 0.7541–2.479). On day 28 post-diagnosis, 78.6% of NPM1+ cases were in remission, compared to 77.4% of NPM1-cases, a statistically insignificant difference (*p-value* 1.0). Additionally, a multivariable logistic regression model found the odds of remission at day 28 post-diagnosis to be unrelated to NPM1 positivity (*p-value* 0.7), gender (*p-value* 0.07), age (*p-value* 0.7), haemoglobin (*p-value* 0.6), WBCs (*p-value* 0.4), and platelet count (*p-value* 0.5).

Table I: Patient demographics and hematological parameters.	
Variable	N (%)
Male	48 (63.2)
Female	28 (36.8)
NPM1+/ FLT3-	14 (18.4)
NPM1-/ FLT3-	62 (81.6)
	Mean ± SD
Age (years)	33.6 ± 11.0
Hb gm/dl	8.7± 1.8
WBC x 10^9/L	28.3 ± 36.6
Platelets x 10^9/L	71.5 ± 96.6

The median survival times could not be estimated, neither for the overall sample nor for the NPM1+ and NPM1- groups, due to the low number of deaths in the sample. A log-rank test found the median survival to not be significantly different between the NPM1+ and NPM1- groups (*p-value* 0.8). Moreover, a univariate Cox proportional hazard analysis found no statistical difference in the hazard of death between the two groups (HR: 0.84, 95% CI: 0.28–2.5, *p-value* 0.7).

At day 28, 54.5% of NPM1+ cases subsequently relapsed, compared to 50.0% of NPM1-cases. This difference was not statistically significant (*p-value 1.0*). Similarly, on logistic regression, none of NPM1 positivity (*p-value* 0.4), gender (*p-value* 0.07), age (*p-value* 0.11), haemoglobin (*p-value* 0.08), WBCs (*p-value* 0.48), or platelet count (*p-value* 0.58) were found to be associated with increased or decreased odds of relapse.

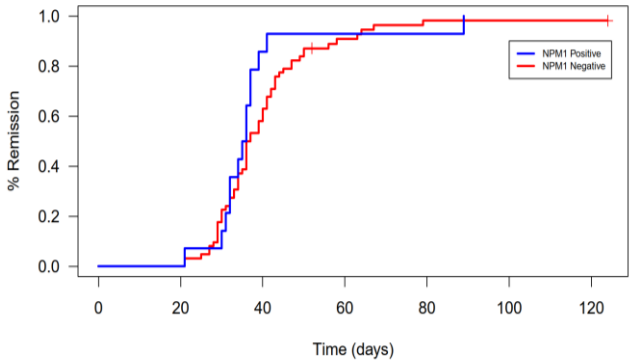


Figure I. Kaplan-Meier Curve for Remission.

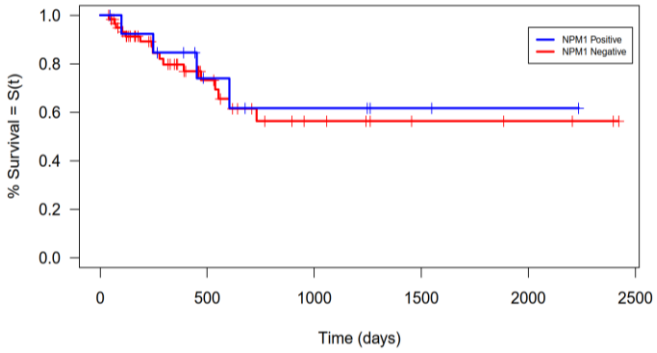


Figure II. Kaplan-Meier Curve for Survival.

Discussion

In a recent WHO classification of myeloid neoplasms (2022), AML with the NPM1 mutation is classified as a separate entity, thus, the genetic molecular markers have a role not only in disease pathogenesis but also in predicting the likelihood of attaining complete remission and subsequent disease-free survival in patients with acute myeloid leukaemia.[13] In our study, we focused on 76 AML patients fulfilling the inclusion criteria, out of which 81.6% were negative for both FLT3 and NPM mutations. This is in comparison with a study done by Mahmood et al.[14] who reported FLT3/NPM negative cases of about 54.6% in a Pakistani population. In our study, the frequency of NPM mutations was 18.4%. These results were comparable with a study done by Pazhakh et al. [15] who estimated it at 17.5%. Other studies reported a much higher frequency of NPM mutation in their populations, as reported by Becker H et al.[16] and Nafea et al.[17] (56% and 47.9%, respectively).

Following standard induction chemotherapy, complete remission was observed in 78.6% of NPM positive cases and 77.4% of NPM negative cases, suggesting a good overall response rate at day 28, but no significant difference in attaining remission in both groups. A similar study done by Mahmood et al.[14] has shown a small difference in remission rates of 72% and 67% in NPM-positive and NPM negative patients, respectively. Another study by Wang et al.[18] demonstrated no significant differences in CR rates between NPM positive and NPM-negative groups. However, there are some studies that show significant differences in outcome between both groups. In a large study done by Dohner K et al.[19] the highest remission rate was reported in the NPM1-mutated/FLT3 ITD-negative group (86%), followed by the NPM1-unmutated/FLT3 ITD-positive group (76%), and then the group without mutations (68.5%). In another study by Schneider et al. (20), CR rates were 77% for the NPM+/FLT3-group and significantly lower at 56% in the NPM-/FLT3-group. The patients who achieved complete remission on day 28 post induction were followed to see the overall survival and relapse rate. In our study, the relapse rate was 54.5% in the NPM positive group and 50% in the NPM negative group, suggesting that nearly half of the patients have relapsed after achieving CR. There is no significant difference in the relapse rate in both groups, but on the other side, a slightly higher relapse rate is observed in the NPM positive group. This is contrary to the study done by Dohner K et al.[19], who reported a better relapse-free survival in the NPM+/FLT3- group, and Thiede C et al. [11] who also showed a significantly lower cumulative incidence of relapse in patients with a normal karyotype who are NPM positive only. (CIR at 4 years: 25% in the NPM+/FLT3 negative group). However, similar to our study, Suzuki et al.[21] identified the NPM mutation as an independent adverse factor for relapse.

However, overall survival could not be assessed in our study due to the low proportion of deaths that occurred over the period of observation, which might have been a result of the short period of observation. Additionally, the hospital records used only reported deaths that took place within the hospital where the study was conducted and did not account for patients who might have died out-of-centre, thereby leading to an underreporting of deaths.

However, Thiede C et al.[11] and Dohner K et al.[19] have shown good overall survival in patients who are NPM positive only as compared to those who are NPM negative.

Additionally, in this study, the parameters including age, gender, haemoglobin levels, WBC, and platelet counts did not influence complete remission and relapse rates in terms of NPM positivity.

As a result, there were no differences in outcomes between the NPM+ and NPM-groups in this study. This finding can be attributed to the smaller sample size, and we also conducted the study without taking into account the patients' cytogenetic profiles and Next Generation Sequencing (NGS) panel because they may have had an impact on the overall findings.

Conclusion

This study did not reveal any association between the presence of the NPM mutation and patient outcomes, as no significant differences were found between the two groups. Hence, further studies with a larger sample size are required to confirm the prognosis of the NPM1 mutation in AML.

References

1. Hoffbrand AV, Higgs DR, Keeling D, Mehta AB, editors. Acute Myeloid Leukaemia. In: Postgraduate haematology. Seventh edition. Chichester, West Sussex ; Hoboken, NJ: John Wiley and Sons, Inc; 2016. https://doi.org/10.1002/9781118853771

2. Mrózek K, Marcucci G, Nicolet D, Maharry KS, Becker H, Whitman SP, et al. Prognostic Significance of the European LeukemiaNet Standardized System for Reporting Cytogenetic and Molecular Alterations in Adults With Acute Myeloid Leukemia. J Clin Oncol. 201220;30(36):4515-23. https://doi.org/10.1200/JCO.2012.43.4738

3. Byrd JC. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 2002;100(13):4325-36. https://doi.org/10.1182/blood-2002-03-0772

4. Fröhling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood. 2002;100(13):4372-80. https://doi.org/10.1182/blood-2002-05-1440

5. Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and

prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood. 2002 Jul 1;100(1):59-66. https://doi.org/10.1182/blood.V100.1.59

6. Döhner K, Tobis K, Ulrich R, Fröhling S, Benner A, Schlenk RF, et al. Prognostic Significance of Partial Tandem Duplications of the MLL Gene in Adult Patients 16 to 60 Years Old With Acute Myeloid Leukemia and Normal Cytogenetics: A Study of the Acute Myeloid Leukemia Study Group Ulm. J Clin Oncol. 2002 Aug 1;20(15):3254-61. https://doi.org/10.1200/JCO.2002.09.088
7. Verhaak RGW, Goudswaard CS, van Putten W, Bijl 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 Dec 1;106(12):3747-54. https://doi.org/10.1182/blood-2005-05-2168
8. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic Nucleophosmin in Acute Myelogenous Leukemia with a Normal Karyotype. N Engl J Med. 2005 Jan 20;352(3):254-66. https://doi.org/10.1056/NEJMoa041974
9. 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):1109-20. https://doi.org/10.1182/blood-2010-08-299990
10. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-19. https://doi.org/10.1038/s41375-022-01613-1
11. 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 with acute myeloid leukemia (AML). Blood. 2006 ;107(10):4011-20. https://doi.org/10.1182/blood-2005-08-3167
12. Liu Y, He P, Liu F, Shi L, Zhu H, Zhao J, et al. Prognostic significance of NPM1 mutations in acute myeloid leukemia: A meta-analysis. Mol Clin Oncol. 2014;2(2):275-81. https://doi.org/10.3892/mco.2013.222
13. Ivey A, Hills RK, Simpson MA, Jovanovic JV, Gilkes A, Grech A, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. N Engl J Med. 2016;374(5):422-33. https://doi.org/10.1056/NEJMoa1507471
14. Mahmood R, Altaf C, Malik HS, Khan SA. Clinico-Haematologic association and prognostic relevance of NPM1 and FLT3-ITD mutations in acute Myeloid Leukaemia: Clinico-Haematologic association with AML. Pak J Med Sci [Internet]. 2019 Jan 22 [cited 2023 Apr 19];35(1). Available from: http://www.pjms.org.pk/index.php/pjms/article/view/285 https://doi.org/10.12669/pjms.35.1.285
15. Pazhakh V, Zaker F, Alimoghaddam K, Atashrazm F. Detection of Nudeophosmin and FMS-Like Tyrosine Kinase-3 Gene Mutations in Acute Myeloid Leukemia. Ann Saudi Med. 2011 ;31(1):45-50. https://doi.org/10.5144/0256-4947.2011.45
16. Becker H, Marcucci G, Maharry K, Radmacher MD, Mrózek K, Margeson D, et al. Favorable Prognostic Impact of NPM1 Mutations in Older Patients With Cytogenetically Normal De Novo Acute Myeloid Leukemia and Associated Gene- and MicroRNA-Expression Signatures: A Cancer and Leukemia Group B Study. J Clin Oncol. 2010 ;28(4):596-604. https://doi.org/10.1200/JCO.2009.25.1496
17. Nafea D. Incidence and Prognostic Value of NPM1 and FLT3 Gene Mutations in AML with Normal Karyotype. Open Hematol J. 2011 ;5(1):14-20. https://doi.org/10.2174/1874276901105010014
18. Wang L, Xu W lai, Meng H tao, Qian W bin, Mai W yuan, Tong H yan, et al. FLT3 and NPM1 mutations in Chinese patients with acute myeloid leukemia and normal cytogenetics. J Zhejiang Univ Sci B. 2010 Oct;11(10):762-70. https://doi.org/10.1631/jzus.B1000052
19. Döhner K, Schlenk RF, Habdank M, Scholl C, Rücker FG, Corbacioglu A, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood. 2005 Dec 1;106(12):3740-6. https://doi.org/10.1182/blood-2005-05-2164
20. Schneider F, Hoster E, Unterhalt M, Schneider S, Dufour A, Benthaus T, et al. NPM1 but not FLT3-ITD mutations predict early blast cell clearance and CR rate in patients with normal karyotype AML (NK-AML) or high-risk myelodysplastic syndrome (MDS). Blood. 2009 May 21;113(21):5250-3. https://doi.org/10.1182/blood-2008-09-172668
21. Suzuki T. Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood. 2005;106(8):2854-61. https://doi.org/10.1182/blood-2005-04-1733