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Original Article

Outcomes of Patients with FLT3 Positive Acute Myeloid Leukaemia; an Experience from a Tertiary Care Hospital in Karachi, Pakistan

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Abstract

Introduction: Molecular genetic abnormalities in acute myeloid leukaemia (AML) are essential for disease diagnosis and determining prognosis and clinical course. Mutations in FLT3 and nucleophosmin (NPM) genes are the most frequent genetic abnormalities, which are also known to impact disease outcomes. FLT3 mutations have been identified in approximately 30% of *de novo* AML patients and are associated with poor prognoses. This study aimed to determine the response to induction chemotherapy, overall survival (OS) and relapse rate (RR) in patients with FLT3-positive AML. **Materials and Methods:** In this study, a retrospective analysis was performed of 75 newly diagnosed patients with AML registered between January 2015 and July 2022. Patient demographics and clinical-haematological parameters were noted and molecular analysis for FLT3 ITD/TKD and NPM mutations was performed. All the patients received standard induction chemotherapy and their response to treatment, OS and RR were assessed. **Results:** A total of 75 cases of AML were analysed. The mean age of the sample was 34.9 years, of which 65.3% were males and 34.7% were females. The patients were stratified into two groups: Those who were positive for FLT3 while negative for NPM (FLT3+/NPM-), representing 17.3% and those who were negative for both FLT3 and NPM (FLT3-/NPM-), representing 82.7% of cases. On day 28 post-induction, the complete remission rate was 69.2% in the FLT3 positive group and 77.4% in the FLT3 negative group. In the FLT3+/NPM- group, 55.6% of cases who were in remission at day 28 subsequently relapsed, compared to 50.0% of FLT3-/NPM- cases. The overall median survival time for the cohort and FLT3+ group was 1467 days, while that of the FLT3-group could not be estimated due to the very high survival rate. **Conclusion:** No significant differences in outcomes were observed in patients who were FLT3 positive compared to those who were FLT3 negative.

Key words: Acute myeloid leukaemia, Complete remission, FLT-3 ITD, FLT-3TKD, NPM-1, Overall survival Relapse rate

Introduction

Acute myeloid leukaemia (AML) is a malignant transformation of immature hematopoietic cells that develops through a complex, multistep process requiring different genetic alterations.[1] It is the most common form of leukaemia in adults and accounts for 80% of cases in this age group. The median age at diagnosis is 65 years and the incidence increases with age. AML has been associated with environmental factors (e.g., chemical exposure, radiation, tobacco and chemotherapy) and genetic abnormalities. These mutations occur in a group of genes that are responsible for the proliferation and survival of hematopoietic progenitors, such as receptor tyrosine kinases (FLT3, KIT and RAS), oncogenic transcription factors (CEBP/alpha) and nucleophosmin (NPM1) mutations. These genetic mutations play an important role in the revised fifth edition of the World Health Organization (WHO) classification of haematolymphoid tumours: Myeloid and histiocytic/dendritic neoplasms.[2]

At present, the prognostic effect of FLT3 internal tandem duplication (ITD) and NPM1 mutations in AML has become well-established.[3] In approximately 30% of patients with AML, mutations in the FLT3 receptor are found; these mutations can occur either as ITDs (approximately 25%) or point mutations in the tyrosine kinase domain (TKD) (7–10%).[4] Although both mutations promote ligand-independent auto-phosphorylation and constitutive receptor activation, the FLT3-ITD mutation has more clinical impact than the FLT3-TKD mutation.[5,6] It has been proven in previous studies that FLT3-ITD is strongly associated with high blast counts, leucocytosis, increased risk of relapse, shorter overall survival (OS), normal cytogenetics, t(15; 17), t(6; 9), NPM1 and DNMT3A mutations.[7]

The allelic ratio of FLT3 mutation also has prognostic significance in that a higher ratio correlates with higher relapse risk. Similarly, the coassociation of FLT3 and NPM1 mutations has a modulating effect on the negative impact of the FLT3 mutation. For example, if a patient has FLT3 and NPM1 mutations, it is considered an intermediate risk unless the allelic ratio is high. On the contrary, if a patient has an FLT3 mutation without NPM, it is characterised as a poor risk, whereas the presence of only NPM1 without FLT3 has a favourable risk.[8]

This retrospective analysis was performed to examine the complete remission (CR), relapse and survival rates of newly diagnosed patients with FLT3-mutated AML.

Materials and Methods

Study participants and setting

The study was conducted in the Department of Oncology at Aga Khan University (AKU), a tertiary care hospital in Karachi, Pakistan, from January 2015 to July 2022. The sample consisted of 75 newly diagnosed AML patients aged 18–65 years. Diagnosis of AML was made after reviewing routinely stained peripheral blood smears and bone marrow biopsies/aspirations according to the WHO criteria. Patients with APML (AML-M3) and those positive for both FLT3 and NPM1 mutations were excluded from the study.

All the data collection was done through the patient's medical records. All follow-ups with patient information were recorded in a predesigned proforma and the confidentiality of patients was maintained. Approval was obtained from AKU's ethical review committee (ERC) (ERC #: 2022-8203-23491) before starting the study.

Clinicohaematological parameters

A detailed history and physical examination were done. The diagnostic workup included bone marrow examination, complete blood count with peripheral blood film examination, renal function, liver function test and molecular studies, including FLT3 and NPM mutation analysis by polymerase chain reaction (PCR). Baseline characteristics, including age, gender, haemoglobin, white blood cell and platelet counts at presentation, were noted.

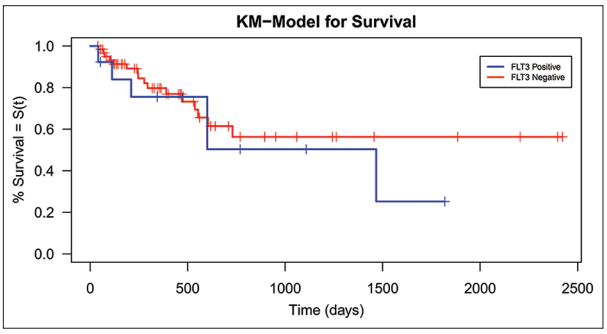

Figure 1: Kaplan–Meier curve for survival

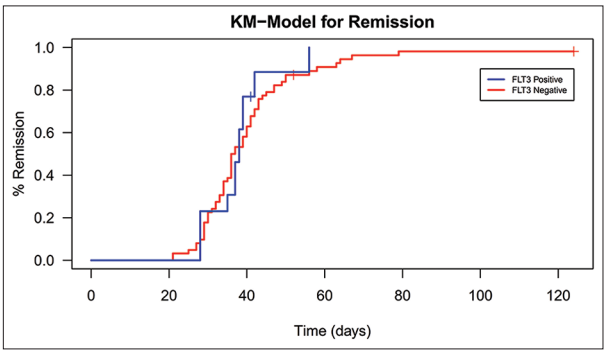

Figure 2: Kaplan–Meier curve for remission

Molecular analysis for FLT3 ITD and TKD

A 3-mL whole blood or bone marrow sample was used for molecular studies. The sample was run on gel electrophoresis using 3% polyacrylamide gel for D835/TKD mutation and for FLT3 ITD, 2% was used along with positive and negative controls. For FLT3-ITD mutation analysis, conventional PCR using allele-specific primers was used to amplify the ITD mutant region and for D835/TKD RFLP (restriction fragment length polymorphism). For gene amplification, denaturation at 95°C for 9 min, annealing at 94°C for 30 s, extension at 60°C for 60 s, final extension at 72°C for 2 min and holding at 72°C for 10 min were done. Samples showing additional longer PCR products were considered FLT3-ITD positive. Positive and negative controls were also run.

Treatment and response to induction chemotherapy

All the patients were given induction chemotherapy 3 + 7 (45–60 mg/m^2/day Daunorubicin or 12 mg/m^2/day Idarubicin for 3 days and 100 mg/m^2/day Cytarabine for 7 days), followed by consolidation with either an allogeneic stem cell transplant or four cycles of Cytarabine 1-3 g/m^2/day for 3 days on day 1, 3 and 5 based on their risk stratification. A bone marrow biopsy was done on day 14 of induction to see clearance of blast cells and on day 28 to see morphological remission. The patients were followed as outpatients for a detailed physical examination and haematological assessment to document the response. CR 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; platelet count ≥100 10^9/L. Relapse was defined as the reappearance of leukaemia cells in the bone marrow, peripheral blood or elsewhere (extramedullary disease) after the attainment of a CR and OS was defined as the time from diagnosis to death, regardless of disease recurrence or the date of the last follow-up visit.

Table 1: Summary of demographic and diagnostic characteristics of the study population

Variable	N (%) or mean±standard deviation
Sex	
Male	49 (65)
Female	26 (35)
Age (years)	34.9±11.7
FLT3+/NPM-	13 (17.3)
FLT3-/NPM-	62 (82.7)
Hb (g/dL)	8.6±1.8
WBC×10^9/L	30.9±46.5
Platelets×10^9/L	73±97
Cytarabine consolidation	
FLT3+/NPM-	09 (12)
FLT3-/NPM-	57 (76)
Allogenic Stem cell transplant	
FLT3+/NPM-	04 (5)
FLT3-/NPM-	05 (7)

Statistical analysis

Chi-square tests were run to compare the frequencies of remission at day 28 and relapse after day 28 remission between the FLT3+/

NPM1- and FLT3-/NPM1- groups. In addition, a Kaplan–Meier model was used to calculate the median survival time and time to remission for these two groups as well as for the overall cohort. A log-rank test was then used to compare the differences in these median values for the two groups. In addition, hazard ratios for survival and remission for the two groups were calculated using a Cox proportional hazards model. A multivariable logistic regression model for remission at day 28 was also made, considering FLT3 positivity, gender, age, haemoglobin, platelet and white blood cell (WBC) counts. All analysis was carried out in R version 4.2.2.

Results

Our cohort consisted of 75 AML patients with a mean age of 34.9 years (standard deviation = 11.7 years). Most patients were both FLT3 and NPM1 negative (82.7%, *n* = 62), with only 17.3% (*n* = 13) of patients being positive for FLT3 but negative for NPM1. In addition, the sample was unevenly split between males (65.3%, *n* = 49) and females (34.7%, *n* = 26). As for the haematological parameters at baseline, the mean haemoglobin levels were 8.67 g/dL, while those for WBCs and platelets stood at 31 × 10^9/L and 73.3 × 10^9/L, respectively.

All the patients received 3 + 7 induction chemotherapy. In FLT3+/NPM- group, four patients underwent allogenic stem cell transplant as consolidation and nine patients received cytarabine. In FLT3-/NPM- group, a total of 57 patients received cytarabine consolidation, while five patients had allogeneic stem cell transplants. None of the FLT3-positive patients received FLT3 inhibitors during their treatment (Table 1).

The overall median survival time for the cohort (from the time of diagnosis) was 1467 days (95% CI = 6000–N/A days), while that of the FLT3+ group was also 1467 days (95% CI = 6000–N/A days). However, the median survival time for the FLT3-negative group could not be ascertained due to a very high survival rate (Figure 1). The median survival time for the FLT3 positive and negative groups was not found to be significantly different under a log-rank test (*P* = 0.5). Moreover, a statistically insignificant hazard ratio of 1.426 (95% CI = 0.5–3.6) was obtained through a univariate Cox proportional hazards model. Fewer deaths have occurred in both groups as the study included only hospital death records while the cause of death was primarily due to primary disease or sepsis.

The median time to remission after diagnosis stood at 37 days (95% CI: 36–40) for the overall sample, 38 days (95% CI: 35–N/A) for the FLT3 positive group and 36.5 days (95% CI: 36–41) for the negative group. A log-rank test found no statistically significant differences between the two groups (*P* = 0.5). A univariate Cox proportional hazard model additionally found a statistically insignificant hazard ratio between the two groups (HR: 1.2, 95% CI: 0.65–2.32, reference: FLT3 negative group). The day 28 remission rate for the FLT3+/NPM1- and FLT3-/NPM1- was 69.2% and 77.4%, a statistically non-significant result (*P* = 0.7) (Figure 2). In addition, on logistic regression, none of the factors included in the model (FLT3 positivity, gender, age, haemoglobin, platelets and WBCs) were associated with day 28 remission, including FLT3 positivity (*P* = 0.4).

After day 28 of remission, the relapse rate (RR) was not significantly different in the FLT3+/NPM1- and FLT3-/NPM1- groups (55.6% vs. 50.0%, *P* = 1.0). In addition, a multivariable logistic regression model found no association between the odds of relapse after remission and FLT3 positivity (*P* = 0.5), gender (*P* = 0.3), age (*P* = 0.2), platelet count (*P* = 0.4) or WBC count (*P* = 0.2). However, it uncovered significantly lower odds of relapse with higher haemoglobin levels, representing a nearly 35% decrease (OR: 0.64, 95% CI: 0.4–0.9, *P* = 0.02).

Discussion

In approximately one-third of patients with AML, activating mutations in FLT3 genes are found; thus, they are the most widely recognised genetic changes. These mutations play an important role

in the diagnosis, risk assessment and therapy guidance. It has been widely accepted that the presence of FLT3 mutation confers a poor prognosis; therefore, in this study, we examined the outcome of AML patients with FLT3 mutations who were treated with standard chemotherapy protocols.

In the present study, the prevalence of FLT3 mutation was 17.3%, while in a study done by Mahmood *et al*.[9], the frequency of FLT3 ITD was 18.5% in a Pakistani adult population and 19.2% in patients in North-east Thailand, according to a study done by Kumsaen *et al*.[10] Elyamany *et al*.[11] reported a frequency of 14.4% in the Saudi AML patients in their study. Likewise, Elyamany *et al*.[11] and Kumsaen *et al*.[10] have included paediatric and adult populations, while we have included only the adult population. Furthermore, all the studies have focused only on FLT3-ITD, but in our research, we have included both FLT3-ITD and TKD mutations.

The mean age of our patients was 34.9 years. In our study, males were more common than females (65.3% vs. 34.7%, respectively). Similar to our study, Zaidi *et al*.[12] reported 62.1% of males and 37.9% of females in their study. Mahmood *et al*.[9] and Mohammed *et al*.[13] have observed a similar male predominance among AML patients. As for the haematological parameters at baseline, Zaidi *et al*.[12] reported mean haemoglobin levels of 8.2 g/dL, a white blood cell count of 26 × 10^9/L and a platelet count of 76 × 10^9/L. These results were comparable to the blood counts of our patients. The patients in the FLT3 negative group achieved a better CR rate (77.4%) than those in the FLT3 positive group (69.2%). However, the results were statistically non-significant. Similarly, Tao *et al*.[14] reported no significant differences in the CR rates of FLT3 positive and FLT3 negative groups (65.22 vs. 62.5%; P = 0.812). A study by Mahmood *et al*.[9] reported a CR of 60% and 67% in FLT3-ITD positive and FLT3-ITD negative groups, respectively. However, Thiede *et al*.[15] reported a much lower CR of 49.3% in the FLT3-ITD positive group and 41% in the FLT3-ITD negative group. Similar to the above observation, a study was performed by Meshinchi *et al*.[16] on paediatric AML patients and it showed that the remission induction rate was 40% in the FLT3 ITD+ group and 74% in the FLT3 ITD- group (P = 0.005).

All the patients who achieved remission were followed to determine OS and RRs. The RR of the FLT3+ group was 55.6%, while 50.0% of patients in the FLT3-group relapsed at the time of the data analysis. These results were not statistically significant. In comparison, a study by Tao *et al*.[14] showed that the RR was significantly higher in the FLT3-ITD-positive group compared with the FLT3-ITD-negative group (34.78 vs. 15.0%, P = 0.03). Another study by Thiede *et al*.[15] showed similar results, in which the RR was 51.3% in FLT3 positive groups and 32.7% in FLT3 negative groups. In addition, in our study, there was no association between relapse and the age of the patient, gender, white blood cell count or platelet count. However, it showed that fewer patients relapsed who had higher haemoglobin levels.

The median survival time of FLT3-negative patients could not be estimated due to the low number of deaths in our cohort over the short observation period. The low number of deaths might have been due to a substantial proportion of deaths occurring out-of-centre, as the records used in the study only reported patient deaths that had occurred at the hospital where the study was conducted.

The study conducted had some limitations, including a small sample size and a lack of information about the patient's infection status during presentation. In addition, there were missing data on the cytogenetic profile and the NGS panel at the time of data collection. To validate the findings on AML patients, a larger study that considers NGS and cytogenetics results should be conducted.

In conclusion, this study did not show any significant differences in outcomes between patients harbouring the FLT3 mutation and those without the mutation.

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Authorship Contributions

Conceived and designed the analysis: NA and US; Collected the data: NA, SA, ZFK and NS; Contributed data or analysis tools: US, HJ, SA, ZFK and NS; Performed the analysis: MZ, HJ and ZA; Wrote the paper: MZ, US and ZA.