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ORIGINAL ARTICLE

Frequency and prognostic impact of FLT3/ITD mutation in patients with acute myeloid leukaemia

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Summary

Purpose: *This study was designed to evaluate the prevalence and the prognostic significance of fms-like tyrosine kinase-3 internal tandem duplication (FLT3/ITD), in acute myeloid leukaemia (AML).*

Methods: *We reviewed 123 newly diagnosed AML patients who have been treated at the Clinic of Hematology, Clinical Center of Nis, Serbia, during a 5-year period. The correlation between presence of the FLT3/ITD mutation and the subtype of disease according to FAB classification, white blood cell count, incidence of early relapse (<12 months) and overall survival was studied.*

Results: *Among 103 patients for whom molecular analyses had been done, FLT3/ITD mutation was present in 46 (44.7%) cases; the highest frequency was seen in the M0 subtype (63.6%), and the lowest in the M1 subtype (16.7%). There were no statistically significant differences in the FLT3/ITD presence for the 3 groups of patients having different leucocyte counts. The FLT3/ITD mutation was associated with a higher incidence of early relapse compared with no mutation cases (78.7 vs. 21.4%; $p < 0.001$), and with a shorter survival time (<40 vs. >60 months; $p < 0.001$).*

Conclusion: *The FLT3/ITD mutation is a poor prognostic factor, which occurs frequently in AML, and is associated with higher incidence of early disease relapse and shorter overall survival.*

Key words: acute leukaemia, FLT3, mutation, prognosis

Introduction

The term "acute myeloid leukaemia" refers to a heterogeneous group of malignant hematologic conditions, characterized by clonal proliferation of myeloid progenitors and accumulation of immature blasts in bone marrow. AML is a rare disease (3% of all malignancies) and the most frequent cause of death from neoplasia between the 3rd and 4th decade of life. The mean age at the time of diagnosis is around 60 years [1].

Biology of FLT3

Fms-like tyrosine kinase-3 receptor (FLT3) is a transmembranic protein encoded by a gene located on chromosome 13q, and is expressed in early hematopoietic progenitors. Binding of tyrosine kinase-3 to FLT3 leads to its autophosphorylation and activation of cytoplasmic effector molecules involved in apoptosis, proliferation and differentiation of hematopoietic cells in bone marrow [2,3].

FLT3 receptor mutations have been detected in about 40% of patients with AML [4]. Three types of activating FLT3 mutations were described: a) FLT3 internal tandem duplication (FLT3/ITD) with 30% prevalence [5]; b) point mutation in tyrosine kinase domain (FLT3/TKD) with prevalence of 7% [6]; and c) point mutation in juxtamembrane domain (FLT3/JMD), which is observed in 2% of the patients [7,8].

The study by Abu-Duhier et al. established FLT/ITD as the sole poor prognostic factor, also in conjunction with frequent disease deterioration and shorter survival, independent of conventional cytogenetic findings. In that study, patients with no FLT3/ITD mutation had considerably longer survival (29.1 months) than those with FLT3/ITD mutation (12.8 months) [9].

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In another study, correlation between the FLT3/ITD mutation and hyperleukocytosis (>100.000×10^9/L), as well as shorter survival has been observed [10]. Albeit the FLT3/ITD mutation could be found in any subtype of AML according to French-American-British (FAB) classification, the results of one research showed a statistically higher incidence of this mutation in AML M5 than in either AML M2 or M6 [11].

Our survey was conducted in order to evaluate the prevalence and the prognostic significance of the FLT3/ITD mutation in our group of patients with AML.

Methods

All patients underwent physical examination, full blood count, serum biochemistry, and bone marrow aspiration in order to establish the diagnosis of AML. Then, standard cytochemical staining of bone marrow smears, conventional cytogenetic analyses, as well as immunophenotyping were performed to establish the FAB subtype of AML. Simultaneously, one non-stained bone marrow smear for each patient was used for PCR analysis of the FLT3/ITD mutation.

All patients were treated with standard combination of anthracycline and cytarabine.

During the 60 months of the study, all patients were followed and monitored. During the follow up period, data over their age, AML subtype, leucocyte count, relapse rate and overall survival were registered and analyzed.

Statistical analysis

Comparison of data was performed for the whole group of patients, for patients within each AML subtype, and finally between these subtypes.

Continuous variables were expressed as mean ± SD. Categorical variables were expressed as percentages. After testing for normality of distribution, continuous variables were compared using Student's t-test. Categorical variables were compared using the x^2 test (Yates correction as appropriate). Survival times were evaluated using the Kaplan-Meier's method. A p-value < 0.05 was considered significant. Statistical analysis was performed with SPSS 15.0 statistical software (SPSS, Chicago, IL).

Results

Patients with newly diagnosed AML (n=123) and treated at the Clinic of Hematology, Clinical Center of Nis, Serbia, between 2005-2009 were studied. Their characteristics are presented in Table 1.

This group included 59 (48%) males and 64 (52%) females with mean age of 56.33±17.12 years; the youngest patient was 17 and the oldest 88 years old. There were no significant differences in the mean patient age regarding gender.

Table 2 shows the distribution of patients concerning FAB subtypes in relation to age.

M5 patients (n=36; 29%) prevailed, whereas the lowest number was observed in M1 subtype patients (n=7; 6%). No patient with FAB M6 or M7 subtype was registered.

The youngest patients belonged to M3 subtype (47 years approx.), and the oldest ones to the M1 subtype (66 years approx.); this difference was statistically significant ($p < 0.05$). Statistically significant differences concerning age were observed between subtypes M3/M2 ($p < 0.05$), and M3/M5 ($p < 0.05$).

FLT3/ITD frequency

Of the total 123 patients, FLT3/ITD mutation analysis was carried out in 103 (84%), whereas in the remaining 20 (16%) patients this analysis was not performed due to technical reasons. Table 3 gives the frequency of FLT3/ITD mutation for each observed AML subtype.

The FLT3/ITD mutation was most frequent in the M0 subtype (63.6%), and least frequent in the M1 subtype (16.7%). No statistically significant differences between AML subtypes were observed regarding the FLT3/ITD mutation.

Table 1. Distribution of the patients with AML regarding gender and age

Gender	*Patients n (%)*	*Age, years mean±SD (range)*
Males	59 (47.97)	55.10±18.42 (17-88)
Females	64 (52.03)	57.45±15.89 (19-83)
Total	123 (100)	56.33±17.12 (17-88)

SD: standard deviation

Table 2. Frequency of patients regarding FAB subtype in relation to age

FAB subtype	*Patients n (%)*	*Age, years mean±SD*
M0	14 (11.38)	56.93±21.04
M1	7 (5.69)	66.14±12.98
M2	33 (26.83)	58.12±15.45
M3	21 (17.07)	46.67±18.15
M4	12 (9.76)	57.67±20.06
M5	36 (29.29)	57.72±14.64
Total	123 (100)	56.33±17.12

SD: standard deviation

Table 3. Frequency of FLT3/ITD mutation in relation to AML subtypes

	FLT3/ITD negative		*FLT3/ITD positive*	
FAB subtype	*n*	*%*	*n*	*%*
M0	4	36.36	7	63.64
M1	5	83.33	1	16.67
M2	15	53.57	13	46.43
M3	12	70.59	5	29.41
M4	5	62.50	3	37.50
M5	16	48.48	17	51.52
Total	57	55.34	46	44.66

x^2=5.93, df=5 (nonsignificant), p=0.3128

FLT3/ITD and leucocyte count

Concerning leucocyte counts, all patients were divided into 3 groups: patients with a decreased total number of white blood cells (WBC < 4×10^9/l), patients with leukocytosis (WBC up to 100×10^9/l), and those with hyperleukocytosis (WBC >100×10^9/l). The presence/absence of the FLT3/ITD mutation in these groups is shown in Table 4.

The highest incidence of FLT3/ITD mutation was observed in the group with leucopenia, and the lowest in the group of patients with moderate leukocytosis, but x^2 test did not reveal statistically significant differences between FLT3/ITD incidence regarding the leucocyte count.

FLT3/ITD and occurrence of early disease relapse

Table 5 depicts the incidence of early relapse (< 12 months) for the groups of FLT3/ITD positive and negative patients.

In FLT3/ITD positive patients the incidence of early relapse differed significantly from the patients without FLT3/ITD mutation (78.6 vs. 21.4%, p < 0.001).

FLT3/ITD and overall survival

Figure 1 shows the Kaplan-Meier survival analysis of the patients with or without the FLT3/ITD mutation.

Table 5. Incidence of FLT3/ITD mutation in relation to early relapse (<12 months)

	Patients without relapse		*Patients with relapse*		*All patients*	
FLT3/ITD	*n*	*%*	*n*	*%*	*n*	*%*
Negative	51	68.92	6	21.43	57	51
Positive	23	31.08	22	78.57	45	23
Total	74	100.00	28	100.00	102	74

x^2=16.71, df=1, p<0.001

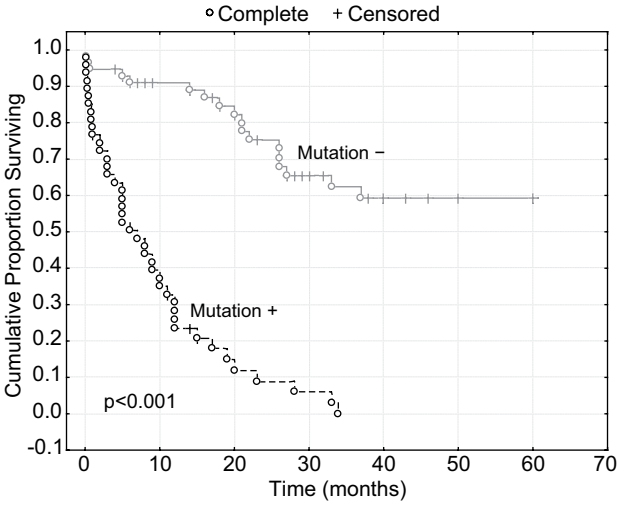

Figure 1. Kaplan-Meier overall survival in patients with or without FLT3/ITD mutation.

Patients with FLT3/ITD mutation had shorter overall survival compared to patients without mutation (35 vs. 60^+ months; log-rank p < 0.001).

Discussion

Our examined group of patients had mean age of 56.81±17.05 years; this is in accordance with epidemiological data, that AML is a disease which most frequently affects older people aged approximately 60 years [1].

In a study conducted by Fröhling et al. FLT3/ITD

Table 4. Incidence of FLT3/ITD mutation in relation to the leucocyte count in peripheral blood

	Leukocytes <4×10⁹/l		*Leukocytes 4-100×10⁹/l*		*Leukocytes >100×10⁹/l*		*All patients*	
FLT3/ITD	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Negative	11	42.31	40	63.49	6	42.86	57	55.34
Positive	15	57.69	23	36.51	8	57.14	46	44.66
Total	26	100.00	63	100.00	14	100.00	103	100.00

x^2=4.36, df=2 (nonsignificant), p=0.1128

mutation was observed in 30% of the patients [4]. In our group of patients we observed FLT3/ITD mutation more frequently, in approximately 45% of the patients.

The study of Abu-Duhier et al. [9] established FLT3/ITD as an independent poor prognostic factor, correlated with a higher incidence of early relapse and shorter overall survival. Our findings coincide since patients with FLT3/ITD mutation most frequently had early relapses than patients without this mutation (78.6 vs. 21.4%), and with shorter survival time (11 vs. 29 months).

In contrast to some previous studies, we did not observe statistically significant differences in the frequency of FLT3/ITD mutation regarding AML subtype [11], as well as leucocyte count [10].

Finally, overall survival analysis revealed a significant difference in survival times between patients with or without FLT3/ITD mutation (35 vs. 60 months), which is in accordance with previous studies that FLT3/ITD acts as an independent poor prognostic factors [9,10].

Conclusion

The FLT3/ ITD mutation is a poor prognostic factor which occurs frequently in AML, and is associated with a higher incidence of early disease relapse and shorter overall survival.

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