FLT3 internal tandem duplication and JAK2 V617F mutations in *de novo* acute myelogenous leukemia: relation with induction chemotherapy and overall survival

Magda M Assem[1], Magda M Noshy[2], Ghada M Elsayed[1], Hanan R Nassar[3] Gamal Thabet[1], Ghada M Sherif [4] and Aida K Ahmad[2]

[1]Clinical Pathology and Oncologic Laboratory Medicine, National Cancer institute, Cairo University, Egypt
[2]Zoology Department, Faculty of Science, Cairo University, Egypt
[3]Medical oncology, National Cancer Institute, Cairo University, Egypt
[4] Biostatistics and Epidemiology Department, National Cancer institute, Cairo University, Egypt
elsayed276@yahoo.com; magda_assem123@yahoo.com

Abstract: Molecular characterization of acute myeloid leukemia (AML) allows prognostic stratification and assessment of the chances of durable treatment response. The presence of FLT3 internal tandem duplication (ITD) mutation as well as the allelic ratio (ITD-AR) and JAK2 V617F mutation may be associated with clinical outcome in patients with AML. FLT3-ITD and JAK2 V617F mutation status was determined for 194 patients with de novo AML. ITD-AR was calculated for patients with FLT3- ITD. Clinical characteristics and outcomes for patients with different FLT3 genotypes were compared. In the total group of 194 patients, FLT3- ITD mutation was detected in 34 (17.5%) patients, 30 (18.8%) adults and 4 (11.8%) pediatric. JAK2-V617F mutation was detected in one patient (0.5%). Among the adult group, patients with FLT3/ITD had a significantly elevated diagnostic white blood cell count (WBC) compared to patients with FLT3 WT/WT genotype (p=0.02).Sixty three (61.2%) achieved complete remission (CR), 52 (82.5 %) were of the FLT3 WT/WT genotype and 11(17.5%) of the FLT3 WT/ITD genotype (p=0.75). Overall survival (OS) of patients with FLT3 WT/ITD group was shorter (28.5%) when compared with for the FLT3 WT/WT group (40.8%) although no significant difference was detected(p=0.2). The disease free survival (DFS) for patients with FLT3 WT/ITD genotype was (100%) compared to (86%) for patients with FLT3 WT/WT genotype, with no significant difference (p=0.3) between the two groups. In conclusion we found that FLT3-ITD mutation is a frequent finding in adult patients with *de novo* AML. There is a significant association between FLT3-ITD mutation with high WBC count and a tendency towards a worse prognosis. The ratio of mutant to wild allele level may have a strong relation to the patient outcome. JAK2-V617F mutation is infrequent finding in *de novo* AML.

[Magda M Assem, Magda M Noshy, Ghada M Elsayed, Hanan R Nassar, Gamal Thabet, Ghada M Sherif and Aida K Ahmad. **FLT3 internal tandem duplication and JAK2 V617F mutations in *de novo* acute myelogenous leukemia: relation with induction chemotherapy and overall survival.** *Life Sci J* 2012;9(4):1053-1060]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 160

Key words: AML, FLT3-ITD, JAK2 V617F

1. Introduction

Acute myeloid leukemia is being revealed as an increasingly heterogeneous entity as the molecular aberrations underlying it are defined. Such information is fundamental in assessment of the chances of durable treatment response. Morphological complete remission is now achieved in the majority of patients with current chemotherapeutic regimens, so the main determinants of prognosis are therefore those variables that influence treatment-related death or relapse risk [(1)].

The Fms-like tyrosine kinase 3 (FLT3) genes encodes a class III tyrosine kinas that plays important roles in cellular proliferation and differentiation. To date, 2 types of FLT3 mutations have been found to induce autophosphorylation through ligand-independent FLT3 dimerization, leading to uncontrolled hematologic progenitor cell proliferation and malignancy. One such mutation is an internal tandem duplication of the region between exon 14 and 15 encoding the juxtamembrane domain; the other, in exon 20, is a point mutation in aspartic acid residue 835 (D835) within the activation loop of the second tyrosine kinase domain [(2)]. FLT3-ITD mutation is a common finding in approximately 25% of younger adult AML patients. Many studies have found that FLT3-ITD mutation is associated with adverse prognosis [(3)].

An acquired mutation in the JAK2 gene has recently been described in human myeloproliferative disorders (MPD). JAK2 is a cytoplasmic tyrosine kinase that plays an essential role in the signaling pathways of cytokines and growth factors. The mutation 1849 G>T, which leads to amino acid substitution of phenylalanine for a highly conserved valine (V617F), renders JAK2 kinase constitutively active and leads to cell proliferation in the absence of the growth factors [(4)]. Now there is evidence in the

literature that patients with AML with an antecedent MPD often have JAK2 V617F mutations [5].

Only recently, a few patients with AML without previous hematologic disorders were found to have the JAK2-V617F mutation. It can be assumed that mutations of JAK2-V617F lead to a more aggressive subtype of leukemia because of the activation of the JAK2-STAT5 cascade which substantially alters apoptotic response, self-renewal and proliferative capacity of myeloid cells [6]. It is of note that a high prevalence of co-operating mutations of FLT3, KIT, or N-RAS in AML patients with the JAK2 mutation has been reported.

The aim of this study was to assess the frequency and the prognostic impact of FLT3-ITD and JAK2- V617F gene mutations on the outcome of patients with *de novo* AML.

2. Patients and methods

The study population

DNA was available from bone marrow (BM) or peripheral blood (PB) samples at diagnosis from 194 patients with *de novo* AML. All patients presented to outpatient clinic of the National Cancer Institute (NCI), Cairo University, in a six month period from May 2010 to December 2010. Thirty four patients were children (≤ 16 years) with a median age of 7.5 (0.5-16) years and 160 were adults (> 16 years) with a median age of 40 (18-80) years. This study was approved by the local ethics committee at the university. No potential conflicts exist.

All adult patients received the 3 and 7 protocols which consisted of adriamycin 30mg/m2 for 3 days and ARAC 100mg/m2 by continuous infusion for 7 days. Further treatment of AML patients was according to their risk group. Patients with favorable risk group were treated with high dose ARAC containing usually HAM protocol(ARAC 1 gm/m2 over 3 hours infusion every 12 hours day 1 to 3 and mitoxantrone 12mg/m2 short infusion days 3 to 5. If relapse occurred to these patients a second induction by HAM or AVVV protocol was given. Then if donor was available, an allogenic BM transplantation was carried out. For unfavorable risk group, allogenic BMT was carried out if a suitable donor was available, but if not HD-ARAC containing regimen was given for 3 cycles then autologous BMT was done. If relapse occurred for this group of patients they were treated with palliative care only.

Pediatrics protocol consisted of two induction courses of ADE protocol (doxorubicin 50mg/m2 D 1, 3, 5, ARAC 3.3 MG/KG D 1 to 10 and etoposide 100mg/m2 D 1 to D 5). Intensification course was done by 4 cycles of MIDAC (ARAC 1gm/m2 every 12 hours for 6 doses and mitoxantrone 8mg/m2 D 1to D3). For special subgroups of AML, special treatment was used. Patients with AML M5 have high risk of central nervous system disease, so they were given triple intrathecal prophylaxis (methotrexate 15mg, ARAC 40mg and dexamethasone 4mg) every 8 weeks for a total 6 injections with induction treatment. If CNS disease was diagnosed at presentation, triple intrathecal injections was given until CSF is free then craniospinal irradiation was given to be followed by intrathecal injection of ARAC and dexamethasone every 8 weeks for 7 doses. Patients with AML M3, induction treatment was consisted of ATRA 45mg/m2 oral daily in divided doses every 12 hours till complete remission or for maximum 90 days, and adriamycin 30mg/m2 for 3 days for every month for 3 courses. These patients received maintenance treatment after complete remission with ATRA (45mg/m2 oral daily for two weeks every 2 years) and 6 mercaptopurine and methotrexate for 2 years.

Morphologic analysis

Peripheral blood and BM smears were stained by standard techniques for diagnosis of AML and assignment of French- American- British (FAB) subtypes [7].

FLT3-ITD gene mutation analysis

High molecular weight DNA was extracted from EDTA anticoagulated BM samples using of QIAamp DNA blood Mini Kit (QIAGEN).Earlier studies have showed that the location of ITD of FLT3 gene is at exon 14 and exon 15 [8]. PCR amplification was performed on the 194 samples using the (11F): 5'-GCAATTTAGGTATGAAAGCCAGC -3' and (12R): 5'- CTTTCAGCATTTTGACGGCAACC -3'. In brief, 1ul DNA was amplified in a volume of 25 µl containing 50 mM KCl, 10 mM Tris-HCl, PH 8.3, 1.5 Mm MgCl2, 200mM dNTPs, 0.5uM of each primer and 1U Taq DNA polymerase (QIAGEN). The PCR consisted of an initial incubation step at 94 °C for 150 seconds followed by 35 cycles at 94 °C for 30 seconds, 57 °C for 60 seconds, and 72 °C for 120 seconds, and a final elongation step at 94 °C for 30 seconds and 60 °C for 10 minutes. The PCR product was analyzed on standard 3% agarose gel. A fragment of 328 base pair (bp) was produced from wild- type (WT) alleles. All patient with an additional higher molecular weight band was considered to be FLT3/ITD+.

Semiquantitative analysis of FLT3-ITD gene mutation

To calculate the allelic ratio (mutant/wild), densitometric estimations of ethidium bromide–stained agarose gels with (BioDocAnalyze (BDA) software (Biometra), Germany) which is included in BioDocAnalyze gel documentation system was performed. The intensity of the mutant and the wild fragments were measured and then the ratio of the mutant to the wild allele was calculated [9]. ITD-

allelic ratio threshold (>0.4) was used to separate FLT3-ITD population into risk groups [10].

V617F genotyping by amplification refractory mutation system (ARMS)

Genomic DNA was analyzed for V617F JAK-2 mutation as previously described by Jones et al [11]. PCR primers were: forward outer (FO), 5`-TCCTCAGAACGTTGATGGCAG-3`; reverse outer (RO), 5`ATTGCTTTCCTTTTTCACAAGAT-3`; forwardwild-type–specific(Fwt),5`-GCATTTGGTTTTAAATTATGGAGTATaTG-3`; reverse-mutant–specific (Rmt), 5`GTTTTACTTACTCTCGTCTCCACAaAA-3`. Amplifications were performed for 30 cycles with HotStar Taq polymerase (QIAGEN), an annealing temperature of 60°C, 25 ng genomic DNA, and standard amplification conditions, except that the final concentrations of the outer primers and the Mutant/wild-type–specific inner primers were 1μM and 0.5 μM, respectively. Products were resolved on 3% agarose gels and visualized after staining with ethidium bromide.

Statistical methods

SPSS package version 17.0 was used for data management. Non-parametric ANOVA and t- test compared means of independent groups. Chi-square tested proportion independence. Kaplan-Meier method was used to estimate survival and log rank compared curves. *P* value ≤ 0.05 is significance.

Endpoints

Complete remission required a normocellular BM containing fewer than 5% blasts with evidence of normal maturation of other marrow elements, a neutrophil count of 10^9/L and a platelet count of 100 > 10^9/L. Remission failures were classified by the clinicians as either partial remission (PR, defined as 5%-15% blasts or fewer than 5% blasts but a hypocellular BM), resistant disease (RD, more than 15% blasts in the BM), or induction death (ID; i.e., related to treatment or hypoplasia). Where the clinician's evaluation was not available, deaths within 30 days of entry were classified as ID and deaths later than 30 days after entry as RD. DFS was defined only for patients who achieved CR and was measured from the documented date of CR until date of relapse or death regardless of cause (death in first CR or relapse), censoring for patients alive in continuous CR. Overall survival was measured from the protocol on-study date until date of death, regardless of cause of death, censoring for patients alive [12].

3. Results

Frequencies, laboratory and clinical characteristics

The study included 194 *de novo* AML patients, 160 adults and 34 children. In the total AML group, FLT3- ITD mutation was detected in 34 (17.5%) patients. In the adult AML group, 30 (18.8%) were found to be FLT3-ITD positive, while in pediatric AML group; the mutation was detected in 4 (11.8%) patients. Details of clinical characteristics for the adult and pediatric groups are shown in table 1 and 2. In two of the 34 cases with FLT3-ITD more than one mutant allele was detected, and in one case no DNA PCR evidence of FLT3 (WT) allele was detected. This indicates that the mutant allele was predominant in three cases, figure1. Laboratory and clinical characteristics of adult patients in the FLT3 WT/WT and FLT3 WT/ITD genotype groups were compared in table 3. Patients with FLT3/ITD had a significantly elevated diagnostic white blood cell count (WBC) with a median of $36.5x10^9$/L compared with $19.4x10^9$/L for patients with FLT3 WT/WT genotype (*p*=0.02). No significant difference was found when we compared other clinical and laboratory parameters.

The mean value for the ITD-allelic ratio (ITD-AR) was 1.2± 0.75 and median was 1.14(0.13 -3.7). When we used the threshold (0.4) for risk stratification of FLT3-ITD positive patients, only three patients were found to have ITD- AR the (≤ 0.4). Statistical analysis of FLT3-ITD patients using ITD-AR threshold could not be done because of the small number of patients in the (≤ 0.4) group. Among the pediatric group FLT3- ITD was detected in 4 out of 34 patients (11.8%) which was a small number to compare among FLT3 genotypes.

JAK2-V617F mutation was detected in one out of 194 AML patients (0.5%). This patient was fifteen years old male, with AML M1 with no hepatomegaly or splenomegaly. WBC count was 30x 10^9/L, hemoglobin level was 7.9 g/dl, and platelet count was 13x 10^9/L, blast percentage was 80%. The patient was FLT-3 ITD negative.

Clinical outcome

Of the 103 adult patients, 63 (61.2%) achieved CR within 28 days of chemotherapy. Among these, 52 (82.5%) were of the FLT3 WT/WT genotype and 11(17.5%) of the FLT3 WT/ITD genotype. Although there was an apparent difference between the two groups, it was not statistically significant (*p*=0.75).Thirty two (24.8%) patients had treatment failure caused by an early death (death before evaluation of response) and 21 (16.2%) died within 7 days after completion of therapy. The main causes of deaths were uncontrolled infection, febrile neutropenia and cerebral hemorrhage. Median follow up period for all adult patients was relatively short, 2 months ranging from (0.03-15) months. Overall survival of adult patients with FLT3 WT/ITD group was shorter (28.5%) when compared with the FLT3 WT/WT group (40.8%) although no significant difference was detected(p=0.2), figure 2. The disease

free survival for patients with FLT3 WT/ITD genotype was (100%) compared to (86%) to patients with FLT3 WT/WT genotype, with no significant difference (p=0.3) between the two groups, figure 3.Overall survival for the pediatric group at 6 months period was 65.3±8.8 and DFS was 71.9±10.

Table 1 Clinical and laboratory characteristics for the adult group at presentation

	N=160
Age(years), median, range	40 (18-80)
Gender, (male) n. (%)	77 (48.1)
Hemoglobin (g/dl), median, range	7.3 (2.5-13.2)
Platelets (10^9/L), median, range	36(3-359)
TLC (10^9/L), median, range	23(0.8-312)
Percentage of bone marrow blasts, median, range	55(1-94)
FAB classification, n. (%)	
Mo	1 (0.7)
M1	19(12.8)
M2	64(43.2)
M3	23(15.5)
M4	34(23)
M5	6(4)
M7	1(0.7)
Hepatomegaly, n. (%)	39(30.7)
Splenomegaly, n. (%)	33(26)
DFS	
Mean (months) ±SE	10.6±5.7
Percentage at 6 months, (95% confidence interval)	88.2 (9.6-11.6)
Survival	
Mean (months)±SE	2 ±4.3
Percentage alive at 6 months, (95% confidence interval)	38.5 (1.6-2.4)
Clinical outcome	
Percentage of complete remission, n. (%)	63(61.2)

We had only one patient with JAK2-V617F mutation who had achieved complete remission after induction chemotherapy, DFS was three months and OS was seven months.

Table 2 Clinical and laboratory characteristic of the pediatric group at presentation

	N=34
Age(years), median, range	7.5 (0.5-16)
Gender, percentage (male)	21(61.8)
Hemoglobin (g/dl), median, range	7.1(2.6-10)
Platelets(10^9/L), median, range	35.5(7-164)
TLC (10^9/L), median, range	30(4.4-257)
Percentage of bone marrow blasts, median, range	70(1-96)
FAB classification, n. (%)	
Mo	1 (3.2)
M1	9(29)
M2	7(22.6)
M3	5(16.1)
M4	6(19.4)
M7	3(9.7)
Hepatomegaly, n. (%)	13(46.4)
Splenomegaly, n. (%)	12(42.9)
DFS	
Mean (months)±SE	7.7±0.9
Percentage at 6 months, (95% confidence interval)	71.9 (6.1-9.2)
Survival	
Mean (months)±SE	7.5±8.8
Percentage at 6 months, (95% confidence interval)	65.3 (5.8-9.2)
Clinical outcome	
Percentage of complete remission	23 (85.2)

Table 3 Clinical characteristics at presentation for adult patients in the FLT3 WT/WT and FLT3 WT/ITD genotype groups

	FLT3 WT/WT N=130	FLT3 W/ITD N= 30	*P*-value
Age(years)			
Median, range	40.5(18-80)	38.5(18-65)	0.29
Gender, n. (%)			
Male	61(46.9)	16(53.3)	0.53
Female	69(53.1)	14(46.7)	
FAB subtypes, n. (%)			
M1+M2	68(56.7)	15(57.7)	
M3	19(15.8)	4(15.4)	0.99
M4+M5	33(27.5)	7(26.9)	
Hemoglobin (g/dl), median, range	7.3(3.4-9.4)	6.9(2.5-11)	0.26
Platelets (10^9/L), median, range	37(3-359)	29(4-193)	0.35
TLC (10^9/L), median, range	19.4(0.8-257)	36.6(5.6-312)	0.02
Splenomegaly, n. (%)	29(28.4)	4(16)	0.20
Hepatomegaly, n. (%)	34(33.3)	5(20)	0.22
Percentage of BM blasts, n. (%)	50(1-94)	65(6-90)	0.42

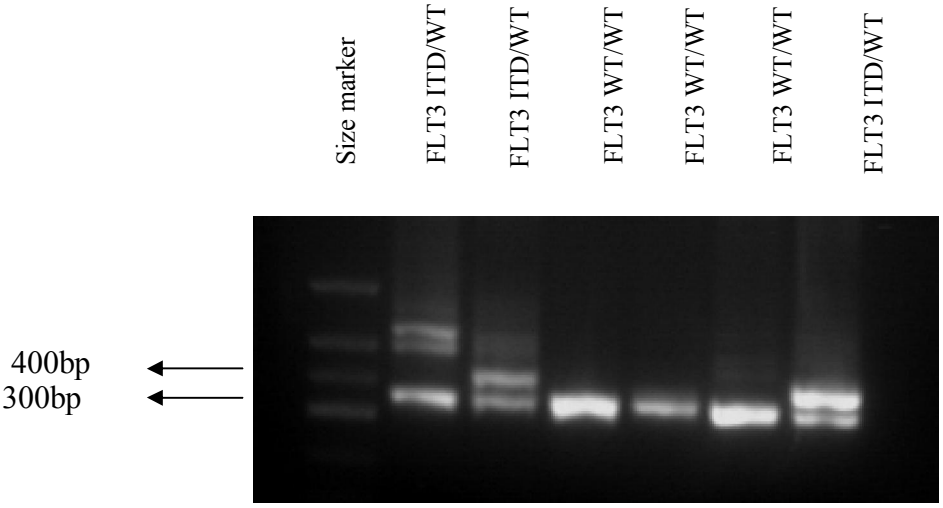

Figure 1: PCR detection of FLT3-ITD. The WT FLT3 genomic PCR product is indicated by the arrow at 330bp. Lane 1 is showing 100bp size marker, lane 2 is showing patient with FLT3 WT/ITD with two mutant alleles, lane 3 and 7 is showing FLT3 WT/ITD with one mutant allele, lanes 4,5 and 6 is showing FLT3 WT/WT.

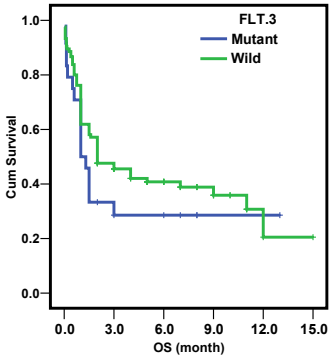

Figure 2: Comparison of OS between adult patients with FLT3 WT/WT and FLT3WT/ ITD mutation.

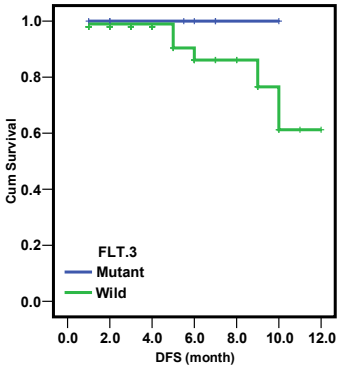

Figure 3: Comparison of DFS between adult patients with FLT3 WT/WT and FLT3WT/ ITD mutation.

4. Discussion

We examined the frequency and prognostic impact of FLT3-ITD and JAK-2 V617F mutations in 194 patients with *de novo* AML. The overall frequency of FLT3 mutation was (17.5%) and JAK-2 V617F mutation was (0.5%). The frequency of FLT3-ITD in the adult group of patients was (18.8%) which was lower than reported for other ethnic population, in Germans (31%, 53%) [13-15] and in Japanese (28.0%, 47.4%) [16-17], but approximated to those reported by Colovic *et al.,* [18] (17.7%). It is possible that studies using DNA banks slightly overestimate the true incidence of FLT3/ITD mutations because there may be bias toward availability of DNA from patients with higher peripheral WBC counts [19-20].

In this study, FLT3-ITD mutation was detected in 6/25 (24%) of elderly adult (≥60years) patients and 24/105 (22.8%) young adults (p>0.05). A higher frequency of FLT3-ITD mutation has been reported in elderly patients, although without these mutations having a prognostic impact [21]. Whereas, Schnittger *et al.,* [9] found that the median age of FLT3-ITD mutation was significantly lower than FLT3 negative patients.

In agreement with previous studies [22], the frequency of FLT3-ITD was higher (18.8%) in adult

compared to pediatric AML patients (11.8%). This observation may partially explain why adult AML has a poorer clinical outcome than pediatric AML

We found a significantly increased leucocyte count in adult patients with FLT3-ITD (p=0.02). This finding was consistent with some previous reports [17-18, 23-24]. Although the effect of FLT3-ITD on inducing leukomogenesis was not directly proved, the ligand – independent constitutive activation of FLT3 induced by ITD mutation could activate some downstream signal molecules including mitogen activated protein (MAP) kinase, signal transducer and activator of transcription 5 (STAT5), and serine-threonine kinases Akt, which contribute to cell proliferation and survival advantages [25-29]. These findings might partially explain the close relationship between FLT3/ITD and higher WBC count.

Furthermore, we found AML FLT3-ITD in all FAB subtypes but we could not reach a significant association with any FAB subtype. Previous studies demonstrated high percentage of FLT3-ITD in certain FAB subtypes, M3 especially the M3v group both with t (15; 17) [9, 30], M5 [15 -31] and M1 [32]. In this work, we had a small number of patients in each FAB subgroup of the FLT3-ITD AML patients, with which we could not reach a significant association.

In line with previous studies, there was no significant difference with respect to age, sex, or other clinical characteristics such as, splenomegaly or hepatomegaly [33].

Previous studies indicated that FLT3 mutation had no influence on complete remission rates and overall survival especially young adults with normal karyotype [9,15,32,33]. However, other studies have found that the presence of FLT3-ITD mutation and the relative level of FLT3-ITD allele have a major impact on long term outcome in predicting relapse from complete remission [12, 19, 34].

When we analyzed the clinical outcome of adult patients with and without FLT3-ITD mutation, patients with FLT3WT/ITD had tendency toward lower CR rate and shorter OS when was compared with the FLT3 WT/WT group although, no significant difference between the two groups could be reached. Moreover, we found no difference in DFS between FLT3 genotypes because of the small number of patients who achieved CR with FLT3WT/ITD genotype.

The prognostic impact of FLT3-ITD in this study was less pronounced than in other reports. The mutation status at diagnosis could not predict the clinical outcome and we could not evaluate the prognostic impact of the mutant allele. However, we cannot ignore that high level of mutant allele (> 0.4) in most of our ITD positive patients might explain the poor outcome of this group of patients.

One possible explanation for the discordant results is that , a large group of our patients (41%) died during induction therapy (ID) and with that short median follow up period (2, 0.03-15) months, CR rate and the long term outcome (OS and DFS) was hard to predict. Our analysis suffered from low statistical power due to limited number of patients and short follow up period.

Another explanation could be related to differences in the cohorts and treatment regimens of various intensities. Our adult group of patients was heterogeneous with respect to cytogenetic which was not available, and age (above and below 60 years) and these two factors are very important for risk stratification. Moreover, recent studies have found FLT3-ITD positive patients are heterogeneous with respect to mutant level, number and size of mutant and with the potential interaction of FLT3-ITD with other molecular abnormality such as nucleophosmin 1 gene (NPM1) [12].

In this study, JAK2-V617F mutation was found in one (0.5%) patient who had AML M1 subtype, high WBC and BM blast count, anemia and thrombocytopenia. The frequency in our patients was similar to that of Levine *et al.,* [35]. Previous studies from the literature have shown that V617F mutation to be distributed mainly among the more immature AML M1 and M2 subtype suggesting a correlation with less differentiated AML [36]. Although the mutation has been found in a small number of AML patients, a relatively high incidence of JAK2-V617F mutation was often seen in *de novo* and therapy-related t(8;21) AML patients and was associated with high WBC count in AML [37]. The small number of mutated cases in our study did not permit correlation with clinical or laboratory parameters.

In conclusion, we found that FLT3-ITD mutation is a frequent finding in adult patients with *de novo* AML. There is a significant association between FLT3-ITD mutation with high WBC count and a tendency towards a worse prognosis. The ratio of mutant to wild allele level may have a strong relation to the patient outcome. JAK2-V617F mutation is infrequent finding in *de novo* AML.

Corresponding author
Ghada M Elsayed
Clinical Pathology and Oncologic Laboratory
Medicine, National Cancer institute, Cairo
University, Egypt
elsayed276@yahoo.com

References

1- Smith ML, Hills RK, Grimwade D. (2011): Independent prognostic variables in acute myeloid leukaemia. Blood Rev., 25:39-51.

2- Park SH, Chi HS, Min SK, Cho YU, Jang S, Park CJ, Lee JH, Lee JH, Lee KH, Im HJ, Seo JJ.(2011): Prognostic significance of the FLT3 ITD mutation in patients with normal-karyotype acute myeloid leukemia in relapse. Korean J Hematol., 46:88-95.
3- Bienz M, Ludwig M, Leibundgut EO, Mueller BU, Ratschiller D, Solenthaler M, Fey MF, Pabst T. (2005): Risk assessment in patients with acute myeloid leukemia and a normal karyotype. Clin Cancer Res 11:1416-1424.
4- Chen Q, Lu P, Jones AV, Cross NC, Silver RT, Wang YL. (2007): Amplification refractory mutation system, a highly sensitive and simple polymerase chain reaction assay, for the detection of JAK2 V617F mutation in chronic myeloproliferative disorders. J Mol Diagn., 9:272-276.
5- Frohling S, Lipka DB, Kayser S, Scholl C, Schlenk RF, Dohner H, Gilliland DG, Levine RL, Döhner K. (2006): Rare occurrence of the JAK2 V617F mutation in AML subtypes M5, M6, and M7. Blood, 107:1242-3.
6- Illmer T, Schaich M, Ehninger G, Thiede C; DSIL2003 AML study group. (2007): Tyrosine kinase mutations of JAK2 are rare events in AML but influence prognosis of patients with CBF-leukemias. Haematologica, 92:137-138.
7- Muñoz L, Aventín A, Villamor N, Juncà J, Acebedo G, Domingo A, Rozman M, Torres JP, Tormo M, Nomdedéu JF. (2003): Immunophenotypic findings in acute myeloid leukemia with FLT3 internal tandem duplication. Haematologica, 88:637-640
8- Kiyoi H, Towatari M, Yokota S, Hamaguchi M, Ohna R, Saito H, and Naoe T. (1998): Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product**.** Leukemia 12: 1333– 1337.
9- Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, Löffler H, Sauerland CM, Serve H, Büchner T, Haferlach T, Hiddemann W. (2002): 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 100:59-66.
10- Meshinchi S, Alonzo TA, Stirewalt DL, Zwaan M, Zimmerman M, Reinhardt D, Kaspers GJ, Heerema NA, Gerbing R, Lange BJ, Radich JP. (2006): Clinical implications of FLT3 mutations in pediatric AML. Blood 108:3654-61.
11- Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, Score J, Seear R, Chase AJ, Grand FH,*et al.* (2005): Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 106:2162-8.
12- Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, Linch DC. (2008): Medical Research Council Adult Leukaemia Working Party. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood 111:2776-84
13- Döhner K, Schlenk RF, Habdank M, Scholl C, Rücker FG, Corbacioglu A, Bullinger L, Fröhling S, Döhner H. (2005): Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood 106:3740-3746.
14- Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF, Haferlach T, Hiddemann W, Falini B. (2005): Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood 106:3733-9.
15- Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G. (2006): Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 107:4011-20.
16- Suzuki T, Kiyoi H, Ozeki K, Tomita A, Yamaji S, Suzuki R, Kodera Y, Miyawaki S, Asou N, Kuriyama K. (2005): Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood 106:2854-2861
17- Schlenk RF, Döhner K, Krauter J, Fröhling S, Corbacioglu A, Bullinger L, Habdank M, Späth D, Morgan M, Benner A. (2008): Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N. Engl. J. Med 358:1909-1918.
18- Colovic N, Tosic N, Aveic S, Djuric M, Milic N, Bumbasirevic V, Colovic M, Pavlovic S .(2007): Importance of early detection and follow-up of FLT3 mutations in patients with acute myeloid leukemia. Ann. Hematol 86:741-747.
19- Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, Walker H, Wheatley K, Bowen DT, Burnett AK, Goldstone AH, Linch DC. (2001): The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 98:1752-9.
20- Murphy KM, Levis M, Hafez MJ, Geiger T, Cooper LC, Smith BD, Small D, Berg KD. (2003): Detection of FLT3 internal tandem duplication and D835 mutations by a multiplex polymerase chain reaction and capillary electrophoresis assay. J Mol Diagn 5:96-102.
21- Stirewalt DL, Radich JP. (2003): The role of FLT3 in haematopoietic malignancies. *Nat* Rev Cancer 3:650- 65.
22- Gregory TK, Wald D, Chen Y, Vermaat JM, Xiong Y, Tse W. (2009):Molecular prognostic markers for adult acute myeloid leukemia with normal cytogenetics. J Hematol Oncol 2:23.

23- Fröhling, S, Schlenk, RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, Döhner H, DöhnerK. (2002): 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 100:4372-4380.

24- Huang Q, Chen W, Gaal KK, Slovak ML, Stein A, Weiss LM. (2008): A rapid, one step assay for simultaneous detection of FLT3/ITD and NPM1 mutations in AML with normal cytogenetics. Br. J. Haematol 142: 489-492

25- Hayakawa F, Towatari M, Kiyoi H, Tanimoto M, Kitamura T, Saito H, Naoe T. (2000):Tandem-duplicated *FLT3* constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3- dependent cell lines. Oncogene 19:624-631.

26- Kiyoi H, Naoe T. (2002): FLT3 in human hematologic malignancies. Leuk Lymphoma 43:1541-7.

27- Tse KF, Allebach J, Levis M, Smith BD, Bohmer FD, Small D. (2002): Inhibition of the transforming activity of FLT3 internal tandem duplication mutants from AML patients by a tyrosine kinase inhibitor. Leukemia 16: 2027-2036.

28- Grundler R, Miething C, Thiede C, Peschel C, Duyster J. (2005): FLT3-ITD and tyrosine kinase domain mutants induce 2 distinct phenotypes in a murine bone marrow transplantation model. Blood 105:4792-4799.

29- Rocnik JL, Okabe R, Yu JC, Lee BH, Giese N, Schenkein DP, Gililand DG. (2006) : Roles of tyrosine 589 and 591 in STAT5 activation and transformation mediated by FLT3-ITD. Blood 108:1339-1345

30- Kuchenbauer F, Kern W, Schoch C, Kohlmann A, Hiddemann W, Haferlach T, Schnittger S .(2005): Detailed analysis of FLT3 expression levels in acute myeloid leukemia. Haematologica 90:1617-25

31- Koh Y, Park J, Ahn KS, Kim I, Bang SM, Lee JH, Yoon SS, Soon Lee D, Yiul Lee Y.(2009): Different clinical importance of FLT3 internal tandem duplications in AML according to FAB classification: possible existence of distinct leukemogenesis involving monocyte differentiation pathway. Ann Hematol., 88:1089- 97

32- Wang L, Xu WL, Meng HT, Qian WB, Mai WY, Tong HY, Mao LP, Tong Y, Qian JJ, Lou YJ, Chen ZM, Wang YG, Jin J. (2010): FLT3 and NPM1 mutations in Chinese patients with acute myeloid leukemia and normal cytogenetics. J Zhejiang Univ Sci B11:762-70.

33- Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, Carroll AJ, Mrózek K, Vardiman JW, George SL, Kolitz JE, Larson RA, Bloomfield CD, Caligiuri MA. (2001): Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res., 61:7233-9.

34- Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U, Wermke M, Bornhäuser M, Ritter M, Neubauer A, Ehninger G, Illmer T. (2002): Analysis of FLT3-activating mutations in 979 patients with acute myeloid leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 99: 4326-4335

35- Levine RL, Loriaux M, Huntly BJ, Loh ML, Beran M, Stoffregen E, Berger R, Clark JJ, Willis SG, Nguyen KT, Flores NJ, Estey E, Gattermann N, Armstrong S, Look AT, Griffin JD, Bernard OA, Heinrich MC, Gilliland DG, Druker B, Deininger MW. (2005): The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. Blood 106:3377-9.

36- Vicente C, Vázquez I, Marcotegui N, Conchillo A, Carranza C, Rivell G, Bandrés E, Cristobal I, Lahortiga I, Calasanz MJ, Odero MD. (2007): JAK2-V617F activating mutation in acute myeloid leukemia: prognostic impact and association with other molecular markers. Leukemia 21:2386-90.

37- Iwanaga E, Nanri T, Matsuno N, Kawakita T, Mitsuya H, Asou N. (2009): A JAK2-V617F activating mutation in addition to KIT and FLT3 mutations is associated with clinical outcome in patients with t(8;21)(q22;q22) acute myeloid leukemia. Haematologica 94:433-5.

9/22/2012