Leukemia Research Reports 10 (2018) 20–25

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Detection of KIT mutations in core binding factor acute myeloid leukemia

Passant Badr[a], Ghada M Elsayed[b,*], Dalia Negm Eldin[c], Bahia Y Riad[d], Nayera Hamdy[e]

[a] BSc Biotechnology, Faculty of Science, Cairo University, Cairo, Egypt
[b] Professor of Clinical Pathology and Oncologic Laboratory Medicine, National Cancer institute, Cairo University, Cairo, Egypt
[c] Lecturer of biostatistics, Department of Biostatistics and Cancer Epidemiology, National Cancer Institute, Cairo University, Cairo, Egypt
[d] Professor of Organic Chemistry, Faculty of Science, Cairo University, Cairo, Egypt
[e] Professor of Clinical Pathology and Oncologic Laboratory Medicine, National Cancer institute, Cairo University, Cairo, Egypt

ARTICLE INFO

Keywords:
CBF-AML
KIT mutations
FLT-3 mutations
HRM analysis

ABSTRACT

We have investigated the frequency and the effect of *KIT* mutations on the outcome of patients with CBF-AML. 69 patients (34 pediatrics and 35 adults) with CBF-AML were enrolled in the study. The frequency of *KIT* mutations was higher in adults compared to pediatrics (22.9% and 14.7%, $p = 0.38$) respectively. Leukocytosis $\geq 20 \times 10^9$ /L was significantly associated with pediatrics compared to adults. t(8;21)(q22;22) was significantly associated with thrombocytopenia in adults. We conclude that no significant difference is found between *KIT* mutated and unmutated CBF-AML in adults and pediatrics. Children with CBF-AML present with leukocytosis. t(8;21) is associated with thrombocytopenia.

1. Introduction

Core binding factor acute myeloid leukemia (CBF-AML) represents 4–12% of all AML, 15% of adults and 25–30% of pediatrics. Patients with CBF-AML are characterized with high complete remission (CR) rates (86–88%), however, 30–50% of patients relapse, and the 5-year survival is only 50% [1].

Mutations in the *KIT* gene are the most common (15–45%) in CBF-AML. The *KIT* gene is located on, chromosome band 4q11-12 and encodes a 145-kDa transmembrane glycoprotein that is a member of the type III tyrosine kinase family. Binding of stem cell factor (KIT ligand) to the KIT receptor activates downstream signaling pathways important for cell proliferation, differentiation, and survival [2]. *KIT* mutations result in ligand- independent activation and most commonly affects the extracellular portion of the receptor (exon 8), and the tyrosine kinase domain (exon 17). Mutations affecting the juxta-membrane domain (exon 10 and 11) are less common KIT mutations have been associated with poor outcome in CBF-AML [3].

The national comprehensive cancer network (NCCN) guidelines have included *KIT* mutations as a prognostic marker that can change CBF-AML from favorable to intermediate risk group [4]. In contrast, the European Leukemia Net did not add KIT mutations in the routine workup for patients with CBF-AML Unlike the cytogenetic classification, the outcome of CBF-AML is heterogeneous [5].

The aims of this work were to analyze the different clinical and prognostic characteristics of CBF-AML and to investigate the prevalence and prognostic effect of *KIT* mutations (exon 8 and exon 17) on the outcome of this group of AML patients.

2. Materials and methods

2.1. Patients

Patients were recruited in a period of two years, retrospectively and prospectively from June 2014 to June 2016. 765 patients were diagnosed with AML, 234 pediatrics and 531 adults. A total of 69 patients (34 pediatrics and 35 adults) were diagnosed with CBF AML and included in this study. Sub classification of AML according to French-American-British (FAB) subtypes was based on morphology, cytochemistry (chloroacetate esterase and myeloperoxidase) and immunophenotyping. The diagnosis CBF-AML was confirmed by the detection of inv (16)(p13q22) or t(8,21) (q22;q22)/RUNX1-RUNX1T1 fusion genes using reverse transcriptase–polymerase chain reaction (RT-PCR). All patients gave informed consent and the study was approved by the Institutional Review Board (IRB), (201516028.4) of the National Cancer Institute (NCI), Egypt.

All patients received the 3 and 7 induction chemotherapy at the NCI, induction chemotherapy consisted of Adriamycin 30 mg/m2 for 3

Abbreviations: CBF-AML, core binding factor AML; PCR-RFLP, Polymerase chain reaction restriction fragment length polymorphism; HRM, High resolution melting curve analysis; NCCN, National comprehensive cancer network; ELN, European Leukemia Network; FLT-3-ITD, FLT3 internal tandem duplication

* Corresponding author.
E-mail addresses: passant.badr@nci.cu.edu.eg (P. Badr), ghada.elsayed@nci.cu.edu.eg (G.M. Elsayed), Nayera.e-shakankiry@nci.cu.edu.eg (N. Hamdy).

https://doi.org/10.1016/j.lrr.2018.06.004
Received 1 May 2018; Received in revised form 5 June 2018; Accepted 29 June 2018
Available online 21 July 2018
2213-0489/ © 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

days and ARAC 100 mg/m2 by continuous infusion for 7 days, after complete remission high-dose cytarabine 3 g/m2 IV over 3 h every 12 h on days 1, 3, and 5 for four cycles was given for consolidation (**5**). Achievement of CR was defined by the detection of less than 5% blasts in normocellular BM. Overall survival (OS) was measured for all living patients from the date of entry to the date of death or last time follow up. Disease free survival (DFS) was calculated from the date of CR to the date of relapse in the first CR.

2.2. Detection of C-KIT mutations

DNA extraction: Genomic DNA was extracted from BM samples using GeneJET Whole Blood Genomic DNA Purification Mini Kit (#K0781- Thermo Scientific). DNA quantity and quality were checked using Thermo Scientific NanoDrop™ 1000 Spectrophotometer.

2.3. HRM analysis for KIT exon 8 mutations

HRM analysis was used for the analysis of *KIT* exon 8 mutations. All samples were tested in triplicates on 7500 fast real-time pcr-Applied Biosystems. Positive and negative controls were included in each run. Twenty nanograms of DNA were amplified in 20 µl reaction volume containing 0.5 µM forward and reverse primers designed by [6] and 10 µl of MeltDoctor™ HRM (Applied Biosystems) master Mix with its thermal profile for 45 cycles with a ramp of 0.02 ˚C/ S. The expected PCR product was 219 bp.

Upon completion of the run, data were analyzed as fluorescence versus temperature graphs (temperature shifting, difference plots, and derivative melting curves) using High Resolution Melting (HRM) Software version 2.0 (#4,397,808).

2.4. PCR amplification and cycle sequencing for KIT exon 8 mutations

Samples positive for exon 8 mutations with same primers were confirmed by sequencing. PCR products were purified using QIAquick PCR Purification Kit (#28,104). Cycle sequencing was performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit and the sequencing product was purified using Centri-Sep™ Spin Columns (#401,762) according to manufacturer instructions. Sequencing products were then resuspended with 10 µl of Hi-Di™ Formamide (#4,311,320), incubated at 95C for 5 minutes and then chilled on ice for 5 min. Bidirectional sequencing was performed on the Applied Biosystems™ 3500 Genetic Analyzer. Sequencing traces were analyzed by Applied Biosystems SeqScape Software v2.5. The analysis of data was done according to Gene Bank accession number (U63834.1).

2.5. Fragment analysis for KIT exon 8 mutations

Fragment analysis was used to help in the analysis of indel mutations of *KIT* exon 8. PCR reaction was performed as previously described by [7] using a Fluorescently labeled forward primer (FAM). Next, these PCR products were added to 7 µL of Hi-Di™ Formamide (#4,311,320) and 2 µL of GeneScan™ 500 LIZ™ dye Size Standard (#4,322,682).The mixture was then injected to Applied Biosystems™ 3500 Genetic Analyzer, analysis and verification of fragments size done using GeneMapper® Software Version 4.1 Microsatellite Analysis.

2.6. PCR-RFLP for KIT exon 17 mutations (D816)

KIT exon 17 was amplified as previously described by [8]. PCR products were digested by AatII (10 U/µL) (#ER0991) at 37 ˚C overnight. Heterozygous samples create 106, 85 and 21 bp fragments, while wild samples create 85 and 21-bp fragments.

2.7. Statistical analysis

Descriptive statistics were calculated for all variables. Patient follow-up was updated on April 1, 2017. Disease-free survival (DFS) and relapse-free survival (RFS) were estimated from the date of complete remission. Differences in proportions were assessed using the v2 or Fisher exact statistic. Survival was plotted with Kaplan–Meier curves and the data for the various groups were compared with independent T-test, [9]. All survival estimates were reported 1 standard error (SE). All P values were 2-sided, *P* value less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 24.0 software statistical package (SPSS, Chicago, IL, USA).

3. Results

3.1. Comparison of initial clinical and laboratory characteristics of CBF-AML between pediatrics and adults patient's groups

Mean age was (7.1 ± 4.7 vs 32.1 ± 10.2) years for pediatrics and adults respectively, and median was (6.0 (1.0–16) vs 30.0 (19–59)) years for pediatrics and adults respectively. The prevalence of CBF-AML was 69/765 (9%), (14.9%, 6.36%) for pediatrics and adults respectively. t(8;21)(q22;22) was detected in (64.7% vs 62.9%, $p = 0.87$) for pediatrics and adults respectively. Inv(16) (p13q22) was detected in (35.3% vs 37.1%, $p = 0.87$) for pediatrics and adults respectively. Leukocytosis $\geq 20 \times 10^9$ /L was significantly associated with pediatrics compared to adults, (64.7% vs 40%, $p = 0.04$). The frequency of FLT3-ITD and FLT-3 TKD mutations was (2.9% vs 5.7%, $p = 0.57$, 2.9% vs 8.6%, $p = 0.3$) for pediatrics and adults respectively. 26 pediatrics and 26 adults were followed till the end of induction chemotherapy, 61.5% of pediatrics and 69.2% of adults achieved complete remission with no significant difference, $p = 0.771$. The total death rate was 31(44.9%). Four (12.9%) patients died before starting chemotherapy from disease progression and 12 (38.7%) patients died during induction chemotherapy. Death was contributed mainly to infections and febrile neutropenia. OS at 6 months was significantly higher in adults compared to pediatrics (63.3% vs 35.7%, $p = 0.043$).

3.2. Comparison of initial clinical and laboratory characteristics between inv (16) and t(8;21) in CBF AML

Inv (16) (p13q22) was significantly associated with FAB subtypes M4 + M5 and t(8;21))(q22;22) was significantly associated with M1 + M2 in pediatrics and adults ($p < 0.001$, $p = 0.004$) respectively.

In pediatrics, inv (16) (p13q22) CBF-AML was associated with hepatomegaly, splenomegaly and lymphadenopathy compared to t (8;21) (q22;22) (41.7% vs 22.7%, $p = 0.27$; 25% vs 13.6%, $p = 0.64$; 8.3%vs 4.5%, $p = 1$) respectively. inv (16) (p13q22) positive CBF-AML was also associated with anemia (HGB ≤ 8 g/dl) and leukocytosis $\geq 20 \times 10^9$/L compared to t (8;21)(q22;22) (91.7% vs 59.1%, $p = 0.06$; 83.8% vs 54.5%, $p = 0.14$) respectively.

In adults, t(8;21)(q22;22) was significantly associated with thrombocytopenia $\leq 20 \times 10^9$/L compared to inv (16) (50% vs 7.7%, $p = 0.01$). However, t(8;21)(q22;22) was significantly associated anemia (HGB ≤ 8 g/dl) with compared to inv (16) (p13q22) (72.7% vs 30.8%, $p = 0.03$) respectively. No significant difference was found in response to induction chemotherapy was found between in inv 16 compared to t(8;21) in pediatrics and adults ($p = 0.18$, $p = 0.19$ respectively, Table 1.

3.3. Effect of the initial clinical and laboratory characteristics on OS of CBF-AML

When we investigated the effect of different pretreatment clinical and laboratory parameters on the OS at 12 month period for adults and 6 months for pediatric CBF-AML, because the number of pediatric cases

Table 1
Initial patients characteristics for t (8;21) and inv(16) CBF AML patients.

CBF AML	Pediatric CBF-AML N = 34(%)		*p*-value	Adult CBF-AML N = 35(%)		*p* value
	inv 16 12 (35.4)	t(8;21) 22(64.7)		Inv 16 13(37)	t(8;21) 22(62.8)	
Age						
Mean ± SD	5.4 ± 5.3	8.01 ± 4.3	0.132	34.9 ± 12.7	30.5 ± 8.3	0.221
Gender						
Males	9(42.9)	12(57.1)	0.292	9(47.4)	10(52.6)	
females	3(23.1)	10(76.9)		4(25)	12(75)	0.29
Hepatomegaly	5(41.7)	5 (22.7)	0.27	4(30.8)	7(31.8)	1
Splenomegaly	3 (25)	3 (13.6)	0.641	4(30.8)	5(22.7)	0.698
Lymphadenopathy	1(8.3)	1(4.5)	1	2(15.4)	1(4.5)	0.541
FLT3-ITD positive	0	1(4.5)	1	0	2(9.1)	0.519
FLT3-TKD positive	1(100)	0	0.35	1(7.7))	2(9.1)	1
KIT mutations						
Wild	10(83.3)	19(86.4)		11(84.6)	16(72.7)	
mutants	2(16.7)	3(13.6)	1	2(15.4)	6(27.3)	0.68
FAB subtypes						
M1 + M2	2(16.7)	19(86.4)		4(30.8)	18(81.8)	
M4 + M5	10(83.3)	3(13.6)	<0.001	9(69.2)	4(18.2)	0.004
TLC						
$< 20 \times 10^9$ /L	2(16.7)	10(54.5)		6(46.2)	15(68.2)	
$\geq 20 \times 10^9$/L	10(83.8)	12(54.5)	0.14	7(53.8)	7(31.8)	0.288
Platelets						
$\leq 20 \times 10^9$ /L	5(41.7)	5(22.7)		1(7.7)	11(50)	
$> 20 \times 10^9$/L	7(58.3)	17(77.3)	0.271	12(92.3)	11(50)	0.01
HGB						
≤ 8 g/dl	11(91.7))	13(59.1)		4(30.8)	16(72.7)	
> 8 g/dl	1(8.3)	9(40.9)	0.06	9(69.2)	6(27.3)	0.03
BM blasts						
Mean ± SD	44.7 ± 13.9	54.9 ± 22.7	0.117	59.3 ± 25.8	49.6 ± 22.4	0.276
PB blasts						
Mean ± SD	32.9 ± 16.3	37.3 ± 19.4	0.490	47.3 ± 30.2	31.6 ± 17.2	0.103
Reponses to induction chemotherapy						
CR	3(37.5)	13(72.2)		4(50)	14(77.8)	
RD	5(62.5)	5(27.8)	0.18	4(50)	4(22.2)	0.197
OS						
6 months (%)	39.3	31.9		37	75	
median	0.8	2.5	0.772	-	-	0.91

Data are presented as n (%). FAB: French-American-British classification, FLT3 ITD: Fms-like Tyrosine Kinase Internal Tandem Duplication, FLT3 TKD: Fms-like Tyrosine Kinase Tyrosine Kinase Domain, HGB: Hemoglobin, PLT: Platelets count, TLC: Total Leukocyte Count, PB Blast: Peripheral Blood Blast, BM Blast:Bone Marrow Blast. CR: Complete Remission, RD: Resistant Disease, OS: Overall survival.

within the strata was too small at one year to be presented in the results. We have found that thrombocytopenia $\leq 20 \times 10^9$ /L had a significantly adverse effect on OS in adults ($p = 0.04$) CBF-AML, Table 2.

3.4. Prevalence and types of KIT mutations

The frequency of *KIT* mutations was higher in adults compared to pediatrics (22.9% and 14.7%, $p = 0.38$) respectively. *KIT* exon 8 mutations were positive (11.8% and 8.6%, $p = 0.66$) for pediatrics and adults respectively. Melting curve analysis revealed a single peak with a mean Tm at (80̊C) for wild cases, and a clear difference between wild and mutant cases for *KIT* exon 8 mutations. Fragment analysis and sequencing confirmed the insertion/deletion mutations. Sequencing confirmed positive cases, Table 3. *KIT* exon 17 was positive in (2.9% and 14.3%, $p = 0.095$) pediatrics and adults respectively.

3.5. Clinical and laboratory characteristics for KIT mutations

We have compared the initial laboratory and clinical characteristics for patients positive and negative for *KIT* mutations and found no significant difference was found between *KIT* mutated and unmutated CBF-AML in adults and pediatrics, Table 4.

4. Discussion

In the present study, we have primarily investigated CBF-AML as one group in (34 pediatrics and 35 adults) patients for different clinical, laboratory, and secondary molecular aberrations (*c-KIT* and *FLT-3*) that might affect the patient's outcome. Then we have separated each CBF-AML into two subtypes t(8;21) and inv (16) searching for the different role of each subtypes on the clinical outcome of CBF-AML. Based on the current controversy regarding the effect of *KIT*mutations on the prognosis of CBF-AML, we have analyzed *KIT* mutations status on CBF-AML as a one group because of the small number of cases.

When we considered all CBF-AML patients and compared pediatrics with adult CBF-AML, leukocytosis was significantly associated with pediatrics compared to adults (64.7% vs 40%, $p = 0.04$) respectively, similar results were previously reported [10,11].

FLT-3-ITD mutation is frequent in cytogenetically normal AML with adverse prognostic effect, however, it is relatively uncommon in CBF-AML with uncertain prognostic significance. In this study, the frequency of FLT-ITD mutations was low (2.9% and 5.7%) in pediatrics and adults respectively. These results were consistent with previous studies [7,12]. In contrast, *FLT-3 TKD D835* mutation has been associated with favorable prognostic effect in inv (16) positive CBF-AML [13,14]. In this study, the frequency of *FLT-3 TKD D835* mutation was (2.9% and 8.6%) for pediatrics and adults respectively and it was comparable to previous studies (6–24%) [15].

The frequency of *c-KIT* mutations was 14.2% and 23.5% for pediatrics and adults respectively, which was in the range reported by previous studies in pediatrics (11–41.5%) and adults (17–46%) [5,7,11].

Previous reports have implicated, older age, initial TLC, percentage of BM blasts (both are linked), and platelets count in influencing the

Table 2
The association between different prognostic factors and OS at 6 and 12 months in CBF-AML.

Prognostic factors	Pediatrics $N = 34$			Adults $N = 34$*		
	N	6 months	Median	6 months	12 months	Median
t(8;21) (q22.q22)						
Negative	12	39.3	0.8	13	37.0	37.0	1.6
Positive	22	31.9	2.5	21	75.0	62.9	-
p value		0.844			0.17		
FAB						
M1 = M2	21	28.7	2.5	21	76.5	62.6	–
M4 + M4	13	49.2	0.8	13	43.3	43.3	5
p value		0.77			0.09		
Inv 16						
Negative	22	31.9	2.5	21	75.0	62.9	–
Positive	12	39.3	0.8	13	37.0	37.0	
p value		0.77			0.09		
Platelets						
$\leq 20 \times 10^9$ /L	10	54.0	6	12	81.8	81.8	–
$> 20 \times 10^9$/L	24	30.0	2.3	22	51.8	39.3	8.7
p value		0.7			0.047		
TLC						
$< 20 \times 10^9$ /L	12	45.0	3	21	68.4	61.9	–
$\geq 20 \times 10^9$/L	22	32.0	2.3	13	54.9	44.0	8.7
p value		0.415			0.211		
HGB						
≤ 8 g/dl	24	41.2	3	19	70.1	55.2	-
> 8 g/dl	10	25.9	2.3	15	55.3	55.3	-
p value		0.235			0.758		
Hepatomegaly						
Negative	24	24.1	1.9	23	69.1	69.8	–
Positive	10	66.7	6.0	11	51.1	40.9	
p value		0.10			0.262		
Splenomegaly						
Negative	28	29.1	2.3	25	69.3	53.0	-
Positive	6	60	-	9	59.3	59.3	-
p value		0.183			0.832		

Data are presented as n (%). FAB: French-American-British classification,*FLT3* ITD: Fms-like Tyrosine Kinase Internal Tandem Duplication, *FLT3* TKD: Fms-like Tyrosine Kinase Tyrosine Kinase Domain, HGB: Hemoglobin, PLT: Platelets count, TLC: Total Leukocyte Count, PB Blast: Peripheral Blood Blast, BM Bone marrow blasts. *Data are presented for 34 adult patients only.

CBF-AML outcome. [1,16–18]. In this study, multivariant analysis revealed thrombocytopenia $\leq 20 \times 10^9$ /L as the only significant prognostic factor affecting OS in adults (81.8% vs 54.6%, p = 0.04). In accordance with Cancer and Leukemia Group B (CALGB), thrombocytopenia was significantly associated with t(8;21) compared to inv (16) in adults (50% vs 7.7%, $p = 0.01$). Lower OS was reported by the (CALGB) for CBF-AML t(8;21) positive patients presenting with thrombocytopenia [19]. **Appelbaum et al.** [18], found t(8;21) by itself, had a poorer outcome compared to inv (16) on OS, after adjusting for age and BM blasts. In this study, neither leukocytosis or the percentage of BM

We confirm previous reports showing the significant association between, t(8;21) (q22;q22) with FAB M1 + M2 and inv (16) with M4 + M5 subtypes in both adults and pediatrics ($p < 0.001$, $p = 0.004$) [1,15]. In addition, extramedullary manifestations (hepatosplenomegaly and lymphadenopathy) were more common with inv (16) compared to t(8;21) (q22;q22) (41.7% vs 22.7%, $p = 0.64$; 25% vs 13.6%, $p = 0.64$; 8.3%vs 4.5%, $p = 1$). In addition, leukocytosis was frequent with inv(16) compared to t(8;21), (83.8% vs 54.5%, $p = 0.14$) these finding were in accordance with previous studies [1,15,16,20]

In this study, *KIT* mutation status was not associated with gender, age, initial WBC count, platelet count, and percentage of BM blasts. Similar results were obtained by **Riera et al.** [10]. In contrast, [11,13,21] reported that *KIT* exon 17 was associated with leukocytosis in t(8;21) compared to inv(16). **Paschka et al.** [5] observed higher WBC and BM blasts in patients positive for *KIT* and *FLT3* mutations. Previous studies have also found an association between exon 8 mutations and exon 17 D816 with inv(16) and t(8;21) respectively [3,15,21]. Unfortunately, we could not analyze this association because of the small number of positive cases.

Few studies have addressed the prognostic effect of KIT mutations in pediatrics. In accordance with previous studies [3,22–24], we found no significant effect for *KIT* mutations in pediatric CBF-AML patient's outcome. On the other hand, **Shimada et al.** [21] and **Manara et al.**

Table 3
Sequencing analysis of *KIT* exon8 mutations.

Patient ID	Blast percentage	Cytogenetics	Fragment analysis	Nucleotide change	Amino acid change
65	75	t(8:21)	Deletion 6 bases	Del GACTTACGA ins TGC	L416_D419
2	67	Inv (16,16)	Deletion 6 bases	1346_1351delACTTAC	T417_Y418
53	20	t(8:21)	Insertion 5 bp	1352delinsTTCCT	R419Ffs*5
40	50	Inv (16,16)	Deletion 4 bp	1350_1353delACGA	Y418Sfs *4
23	79	Inv (16,16)	Deletion 6 bp	Del ACGACAGGCTCG insGCGC	Y418_V422
19	30	t(8:21)	Deletion 3 bp	1350_1352delACG	D419
31	62	t(8:21)	Insertion 5 bp	1351_1356delinsTTCCT	D419Sfs*5

Fs: frame shift mutation.

Table 4
Association of *KIT* mutations with prognostic factors in CBF-AML.

Variable	Pediatrics			Adults		
	KIT negative *N* = 29	*KIT* positive *N* = 5	*p* value	*KIT* negative *N* = 27	*KIT* positive *N* = 8	*p*- value
Sex						
Male: n (%)	16(55.2)	5(100)		14(51.9)	5(62.5)	
Female: n (%)	13(44.8)		0.132	13(41.8)	3(37.5)	0.700
FAB subtypes						
M1 + M2	18(62.1)	3(60)		16(59.3)	6(75)	
M4 + M5	11(37.9)	2(40)	1.000	11(40.7)	2(25)	0.683
Hepatomegaly						
negative: n (%)	21(72.4)	3(60)		20(74.1)	4(50)	
positive: n (%)	8(27.6)	2(40)	0.678	7(25.9)	4(50)	0.226
Splenomegaly						
negative: n (%)	24(82.8)	4(80)		22(81.5)	4(50)	
positive: n (%)	5(17.2)	1(20)	1.000	5(18.5)	4(50)	0.162
Lymphadenopathy						
negative: n (%)	27(93.1)	5(100)		2(7.4)	1(12.5)	
positive: n (%)	2(6.9)		1.000	2(7.4)	1(12.5)	0.553
t(8:21)						
t(8:21) negative	10(34.5)	2(40)		11(40.7)	2(25)	
t(8:21) positive	19(65.5)	3(60)	1.000	16(59.3)	6(75)	0.680
Inv (16)						
Inv16 wild	19(65.5)	3(60)		16(59.3)	6(75)	
Inv 16 positive	10(34.5)	2(40)	1.000	11(40.7)	2(25)	0.680
***FLT3*- ITD**						
FLT3 ITD wild	28(69.6)	5(100)		25(92.6)	8(100)	
FLT3 ITD mutant	1(3.4)	0	1.000	2(7.4)	0	1.000
***FT3*- TKD**						
FLT3 TKD wild	28(69.6)	5(100)		25(92.6)	7(87.5)	
FLT3 TKD mutant	1(3.4)	0	1.000	2(7.4)	1(12.5)	0.553
HGB (g/dL)						
≤ 8 g/dl	21(72.4)	3(60)		16(59.3)	4(50)	
> 8 g/dl	10(34.5)	2(40)	0.618	11(40.7)	4(50)	0.700
PLT ($x10^9$/L)						
< = 20 × 10^9/L	8(27.6)	2(40)		8(29.6)	4(50)	
> 20 × 10^9/L	8(27.6)	2(40)	0.618	8(29.6)	4(50)	0.402
TLC ($x10^9$/L)						
< 20,000	10(34.5)	2(40)		16(59.3)	5(62.5)	
> 20 × 10^9/L	19(65.5)	3(60)	1.000	11(40.7)	3(37.5)	1.000
Pb Blast (%)						
Mean ± SD	34.483 ± 18.13	43.2 ± 19.52	0.392	38.47 ± 25.5	34.12 ± 17.36	0.558
BM Blast (%)						
Mean ± SD	50.65 ± 20.03	55.2 ± 24.87	0.715	51.074 ± 24.21	60.62 ± 22.66	0.323
Response to induction chemotherapy						
CR	15(56.2)	1(33.3)		13(68.4)	2(28.6)	
RD	8(34.8)	2(66.7)	1.000	6(31.6)	5(71.4)	1.000

Data are presented as n (%). FAB: French-American-British classification, *FLT3* ITD**:** Fms-like Tyrosine Kinase Internal Tandem Duplication, *FLT3* TKD: Fms-like Tyrosine Kinase Tyrosine Kinase Domain, HGB: Hemoglobin, PLT: Platelets count, TLC: Total Leukocyte Count, PB Blast: Peripheral Blood Blast, BM Bone marrow blasts. CR: Complete Remission, RD: Resistant Disease, OS: Overall survival.

[25] found an adverse effect of *KIT* mutation on t(8;21) subtype of CBF-AML. (both were letters to the editors). The lack of prognostic effect for KIT mutations in pediatrics compared to adults could be related to the difference in treatment protocols or to the maturation stage of leukemic progenitors at which the mutation achieve clonal dominance [3].

In contrast to pediatrics, the effect of*KIT* mutations on the prognosis of adult CBF-AML was studied extensively. It was found that *KIT* mutations have no effect on the response to induction chemotherapy [5,11,26] but it has a different prognostic effects in t(8;21) and inv (16) CBF-AML subtypes. **Pashka et al.** [5]; **Care et al.** [13] found an adverse effect for *KITr* mutations in adults inv(16) AML. Other studies [11,12,2] found a prognostic effect for *KIT* exon 17 mutation in t(8;2) AML. Contrary to previous results, we and **Riera et al.** [10] found no difference between mutated and unmutated CBF-AML in adults.

5. Conclusion

The difference in the clinical and laboratory characteristics between inv(16) and compared t(8;21) positive AML, suggests dealing with these cytogenetic abnormalities as two separate entities and not as one group. t(8;21) is associated with thrombocytopenia, and it has an adverse effect on the OS of adult CBF-AML .inv (16) is associated with leukocytosis and extramedullary manifestations. *KIT* mutations are frequent in CBF-AML. FLT3 mutations are rare in CBF-AML. The prognostic effect of *KIT* mutations requires studying larger number of samples.

Funding

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit-sectors.

Disclosure

Passant Badr, Ghada M Elsayed, Dalia Negm Aldin, Bahia Y Riad, Nayera Hamdy declare that they have no conflict of interest.

Acknowledgments

All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take

responsibility for the integrity of the work and have given final approval to the version to be published. We are grateful for working at the National Cancer Institute, Cairo University, Egypt.

References

[1] M. Solh, S. Yohe, D. Weisdorf, C. Ustun, Core-binding factor acute myeloid leukemia: heterogeneity, monitoring, and therapy, Am. J. Hematol. 89 (12) (2014 Dec) 1121–1131, https://doi.org/10.1002/ajh.23821 Epub 2014 Aug 27.

[2] M. Malaise, D. Steinbach, S. Corbacioglu, Clinical implications of c-Kit mutations in acute myelogenous leukemia, Cur. Hematol. Malign Rep. 4 (2) (2009) 77–82.

[3] J.A.1 Pollard, T.A. Alonzo, R.B. Gerbing, P.A. Ho, R. Zeng, Y. Ravindranath, G. Dahl, N.J. Lacayo, D. Becton, M. Chang, H.J. Weinstein, B. Hirsch, S.C. Raimondi, N.A. Heerema, W.G. Woods, B.J. Lange, C. Hurwitz, R.J. Arceci, J.P. Radich, I.D. Bernstein, M.C. Heinrich, S. Meshinchi, Prevalence and prognostic significance of KIT mutations in pediatric patients with core bindingfactor AML enrolled on serial pediatric cooperative trials for de novo AML, Blood 115 (March (12)) (2010) 2372–2379, https://doi.org/10.1182/blood-2009-09-241075 25Epub 2010 Jan 7.

[4] W. Chen, H. Xie, H. Wang, L. Chen, Y. Sun, Z. Chen, Q. Li, Prognostic significance of KIT mutations in core-binding factor acute myeloid leukemia: a systematic review and meta-analysis, PLoS One 11 (January (1)) (2016) e0146614, , https://doi.org/10.1371/journal.pone.0146614 15eCollection 2016.

[5] P.1 Paschka, G. Marcucci, A.S. Ruppert, K. Mrózek, H. Chen, R.A. Kittles, T. Vukosavljevic, D. Perrotti, J.W. Vardiman, A.J. Carroll, J.E. Kolitz, Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv (16) and t(8;21): a cancer and leukemia group B Sstudy, J. Clin. Oncol. 24 (August (24)) (2006) 3904–3911 20.

[6] Fuster O, Barragán E, Bolufer P, Cervera J, Larráyoz MJ, Jiménez-Velasco A, Martínez-López J, Valencia A, Moscardó F, Sanz MA. Rapid detection of KIT mutations in core binding factor acute myeloid leukemia using high-resolution melting analysis, J. Mol. Diagn. 2009;11 September(5):458–63. Epub 2009 Jul 30. doi:10.2353/jmoldx.2009.090043.

[7] N. Boissel, H. Leroy, B. Brethon, N. Philippe, S. de Botton, A. Auvrignon, E. Raffoux, T. Leblanc, X. Thomas, O. Hermine, B. Quesnel, A. Baruchel, G. Leverger, H. Dombret, C. Preudhomme, Acute leukemiafrench association (ALFA); Leucémies Aiguës Myéloblastiques de l'Enfant (LAME) cooperative groups. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML), Leukemia 20 (June(6)) (2006) 965–970.

[8] Looijenga LH, de Leeuw H, van Oorschot M, van Gurp RJ, Stoop H, Gillis AJ, de Gouveia Brazao CA, Weber RF, Kirkels WJ, van Dijk T, von Lindern M, Valk P, Lajos G, Olah E, Nesland JM, Fosså SD, Oosterhuis JW. Stem cell factor receptor (C-KIT) codon 816 mutations predict development of bilateral testicular germ-cell tumors cancer res. 2003 15;63 November(22):7674–8.

[9] E.L. Kaplan, P. Meier, Nonparametric estimations from incomplete observations, J. Am. Stat. Assoc. 53 (1958) 457–481.

[10] L. Riera, F. Marmont, D. Toppino, C. Frairia, F. Sismondi, E. Audisio, et al., Core binding factor acute myeloidleukaemia and c-KIT mutations, Oncol. Rep. 29 (2013) 1859–1866.

[11] R. Cairoli, A. Beghini, G. Grillo, G. Nadali, F. Elice, C.B. Ripamonti, P. Colapietro, M. Nichelatti, L. Pezzetti, M. Lunghi, A. Cuneo, A. Viola, F. Ferrara, M. Lazzarino, F. Rodeghiero, G. Pizzolo, L. Larizza, E. Morra, Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study, Blood 107 (May(9)) (2006) 3463–3468 1Epub 2005 Dec 2.

[12] S.H. Park, H.J. Lee, I.S. Kim, J.E. Kang, E.Y. Lee, H.J. Kim, et al., Incidences and prognostic impact of c-KIT, WT1, CEBPA, and CBL mutations, and mutations associated with epigenetic modification in core binding factor acute myeloid leukemia: a multicenter study in a Korean population, Ann. Lab. Med. 35 (2015) 288–297.

[13] R.S. Care, P.J. Valk, A.C. Goodeve, F.M. Abu-Duhier, W.M. Geertsma- Kleinekoort, G.A. Wilson, M.A. Gari, I.R. Peake, B. Lowenberg, J.T. Reilly, Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias, Brit. J. Haematol. 121 (2003) 1365–2141.

[14] C. Allen, R.K. Hills, K. Lamb, C. Evans, S. Tinsley, R. Sellar, et al., The importance of relative mutant level for evaluating impact on outcome of KIT, FLT3, and CBL mutations in core-binding factor acute myeloid leukemia, Leukemia 27 (2013) 1891–1901.

[15] C.1 Ustun, G. Marcucci, Emerging diagnostic and therapeutic approaches in core binding factor acute myeloid leukaemia, Curr. Opin. Hematol. 22 (March(2)) (2015) 85–91, https://doi.org/10.1097/MOH.0000000000000124.

[16] B.F. Goemans, C.M. Zwaan, M. Miller, M. Zimmermann, A. Harlow, S. Meshinchi, A.H. Loonen, K. Hählen, D. Reinhardt, U. Creutzig, G.J. Kaspers, M.C. Heinrich, Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia, Leukemia 19 (September(9)) (2005) 1536–1542.

[17] H.A. Jung, C.H. Maeng, S. Park, S.J. Kim, K. Kim, J.H. Jang, C.W. Jung, Prognostic factor analysis in core-binding factor-positive acute myeloid leukemia, Anticancer Res. 34 (February(2)) (2014) 1037–1045.

[18] F.R. Appelbaum, K.J. Kopecky, M.S. Tallman, M.L. Slovak, H.M. Gundacker, H.T. Kim, G.W. Dewald, H.M. Kantarjian, S.R. Pierce, E.H. Estey, The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations, Br. J. Haematol. 135 (October(2)) (2006) 165–173 Epub 2006 Aug 25.

[19] G. Marcucci, K. Mrózek, A.S. Ruppert, K. Maharry, J.E. Kolitz, J.O. Moore, R.J. Mayer, M.J. Pettenati, B.L. Powell, C.G. Edwards, L.J. Sterling, J.W. Vardiman, C.A. Schiffer, A.J. Carroll, R.A. Larson, C.D. Bloomfield, Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): a cancer and leukemia group B study, J. Clin. Oncol. 23 (August(24)) (2005) 20.

[20] L.Y.1 Shih, D.C. Liang, C.F. Huang, Y.T. Chang, C.L. Lai, T.H. Lin, C.P. Yang, I.J. Hung, H.C. Liu, T.H. Jaing, L.Y. Wang, T.C. Yeh, Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-bindingfactor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples, Leukemia 22 (February(2)) (2008) 303–307 Epub 2007 Oct 25.

[21] A. Shimada, T. Taki, K. Tabuchi, A. Tawa, K. Horibe, M. Tsuchida, R. Hanada, I. Tsukimoto, Y. Hayashi, KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese ChildhoodAML Cooperative Study Group, Blood 107 (March(5)) (2006) 1806–1809 1Epub 2005 Nov 15.

[22] S. Nguyen, T. Leblanc, P. Fenaux, et al., A white blood cell index as the main prognostic factor in t(8;21) acute myeloidleukemia (AML): a survey of 161 cases from the French AML intergroup, Blood 99 (2002) 3517–3523.

[23] Hoyos M1, Nomdedeu JF, Esteve J, Duarte R, Ribera JM, Llorente A, Escoda L, Bueno J, Tormo M, Gallardo D, de Llano MP, Martí JM, Aventín A, Mangues R, Brunet S, Sierra J. Core binding factor acute myeloid leukemia: the impact of age, leukocyte count, molecular findings, and minimal residual disease, Eur. J. Haematol. 2013 ;91 September(3):209–18. Epub 2013 Jul 2. doi: 10.1111/ejh.12130.

[24] S. Schnittger, T.M. Kohl, T. Haferlach, W. Kern, W. Hiddemann, K. Spiekermann, C. Schoch, KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival, Blood 107 (2006) 1791–1799.

[25] E. Manara, V. Bisio, R. Masetti, V. Beqiri, R. Rondelli, G. Menna, et al., Core-binding factor acute myeloid leukemia in pediatric patients enrolled in the AIEOP AML 2002/01 trial: screening and prognostic impact of c-KIT mutations, Leukemia 28 (2014) 1132–1134, https://doi.org/10.1038/leu.2013.339 PMID: 24226631.

[26] S. Schwind, C.G. Edwards, D. Nicolet, K. Mrozek, K. Maharry, Y.Z. Wu, et al., inv (16)/t(16;16) acute myeloid leukemia with non-type A CBFB-MYH11 fusions associate with distinct clinical and genetic features and lack KIT mutations, Blood 121 (2013) 385.