Detection of KIT mutations in core binding factor acute myeloid leukemia

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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) 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.
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, analyzer 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 ≥ 20 × 10³/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 ≥ 20 × 10⁹/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 ≤ 20 × 10⁹/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
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 (80°C) 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-3-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
CBF-AML outcome. [1,16–18]. In this study, multivariate analysis revealed thrombocytopenia ≤ 20 × 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 blasts. We confirm previous reports showing the significant association between, t(8;21) (q22;q22) with FAB M1 + M2 and inv (16) with M4 + MS 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.

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### 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 | N 6 months | Median |
| **t(8;21) (q22;q22)** | 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 | - |
| M1 + 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** | | | | | | |
| ≤ 20 × 10^9 /L | 10 | 54.0 | 6 | 12 | 81.8 | 81.8 | - |
| > 20 × 10^9/L | 24 | 30.0 | 2.3 | 22 | 51.8 | 39.3 | 8.7 |
| **p value** | 0.7 | 0.047 |
| **TLC** | | | | | | |
| ≤ 20 × 10^9 /L | 12 | 45.0 | 3 | 21 | 64.8 | 61.9 | - |
| > 20 × 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.

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### Table 3

Sequencing analysis of KIT exon 8 mutations.

| Patient ID | Blast percentage | Cytogenetics | Fragment analysis | Nucleotide change | Amino acid change |
|------------|------------------|--------------|-------------------|-------------------|------------------|
| 65         | 75               | t(8;21)      | Deletion 6 bases   | Del GACTTAGA ins TGC | L416_D419 |
| 2          | 67               | Inv (16,16)  | Deletion 6 bases   | 1346,1351delACTTAC | T417,Y418 |
| 53         | 20               | t(8;21)      | Insertion 5 bp     | 1352delinsTTCTCT | R419Fs*5 |
| 40         | 50               | Inv (16,16)  | Deletion 4 bp      | 1350,1353delAGCA | Y418hs*4 |
| 23         | 79               | Inv (16,16)  | Deletion 6 bp      | Del AGAGACGGCTGGCinsG GCC | Y418,422 |
| 19         | 30               | t(8;21)      | Deletion 3 bp      | 1350,1353delACG | D419 |
| 31         | 62               | t(8;21)      | Insertion 5 bp     | 1351,1356delinsTTCTCT | D419Fs*5 |

Fs: frame shift mutation.
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 KIT mutations in adults inv(16) AML. Other studies \[11,12,2\] found a prognostic effect for KIT exon 17 mutation in t(8;2)

### 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 (53.2)  | 5 (100)| 0.132  | 14 (51.9)  | 5 (62.5)  | 0.700  |
| Female: n (%)             | 13 (44.8)  |        |        | 13 (41.8)  | 3 (37.5)  |        |
| 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) | 0.678  | 20 (74.1)  | 4 (50)   | 0.226  |
| positive: n (%)           | 8 (27.6)   | 2 (40) |        | 7 (25.9)   | 4 (50)   |        |
| 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  |
| Inv (16)                  |            |        |
| Inv16 wild                | 19 (65.5)  | 3 (60) |        | 16 (59.3)  | 6 (75)   | 0.680  |
| 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)| 1.000  | 25 (92.6)  | 8 (100) | 1.000  |
| FLT3 ITD mutant           | 1 (3.4)    | 0      |        | 2 (7.4)    | 0       |        |
| FLT3- TKD                 |            |        |
| FLT3 TKD wild             | 28 (69.6)  | 5 (100)|        | 25 (92.6)  | 7 (87.5)| 0.553  |
| FLT3 TKD mutant           | 1 (3.4)    | 0      | 1.000  | 2 (7.4)    | 1 (12.5)|        |
| 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 (x10^11/L)            |            |        |
| < 20 × 10^11/L            | 8 (27.6)   | 2 (40) | 0.618  | 8 (29.6)   | 4 (50)   | 0.402  |
| > 20 × 10^11/L            | 8 (27.6)   | 2 (40) |        | 8 (29.6)   | 4 (50)   |        |
| TLC (x10^9/L)             |            |        |
| < 20,000                  | 10 (34.5)  | 2 (40) |        | 16 (59.3)  | 5 (62.5)|        |
| > 20,000                  | 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)| 0.700  |
| 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 KIT 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.

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

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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. Malig. Rep. 4 (2009) 77–82.

[3] J.A.I Pollard, T.A. Alonzo, R.B. Gerbing, P.A. Ho, R. Zeng, Y. Ravindranath, G. Dahl, N.J. Lacayo, D. Beetson, M. Chang, H.J. Weinstein, B. Hirsch, S.C. Raimondi, N.A. Heerema, W.G. Woods, B.J. Lange, C. Hurwit, R.J. Arceci, J.P. Radich, I.D. Bernstein, M.C. Heinrich, S. Meschini, 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) 2373–2379, https://doi.org/10.1182/blood-2009-06-241075 Epub 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.I Paschka, G. Marcucci, A.S. Ruppert, K. Mrózek, H. Chen, R.A. Kittles, T. Vukosavljevic, D. Perrottii, 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 study, J. Clin. Oncol. 24 (August (24)) (2006) 3904–3911 20.

[6] Fuster O, Barragán E, Bolafer P, Cerera J, Larráyoz MJ, Jiménez-Velasco A, Martínez-López J, Valencia A, Moscardí F, Sane MA. Rapid detection of KIT mutations in core binding factor acute myeloid leukemia using high-resolution melting analysis, J. Mol. Diagn. 2009;11(September):548–63. Epub 2009 Jul 30. doi:10.2353/jmld.2009.090043.

[7] N. Beisul, H. Leroy, B. Brethon, N. Philippe, S. de Botton, A. Avryppen, E. Raffoux, T. Leblanc, X. Thomas, O. Hermine, B. Quenel, A. Baruchel, G. Legerer, H. Dombret, C. Preudhomme, Acute leukemia Denver association (ALFA); Leukémies Aigües Myeloblastiques de l’Enfant (LAME) cooperative groups. Incidence and prognostic impact of c-Kit mutations in adult acute myeloid leukemia (CBF-AML), Leukemia 20 (June(6)) (2006) 965–970.

[8] Loosjenga IH, de Leeuw H, van Oorschot M, van Gurp RJ, Stoop H, Gillis AJ, de Vries WR, Gouveia Brazao CA, Weber RF, Kirkels WJ, van Dijk T, von Lindern M, Valk P, Lajos A. Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia, Leukemia 19 (October(9)) (2005) 1536–1542.

[9] 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.

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

[11] 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(23)) (2005) 20.

[12] L.Y.I 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 re- ceptor 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.

[13] A. Shimada, T. Taki, K. Tabuchi, A. Tawa, K. Horibe, M. Tsukada, 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 Epub 2005 Nov 05.

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

[15] Hoyos MI, Nomdedeu JF, Esteve J, Duarte R, Ribera JM, Lorente A, Escoda L, Bueno J, Tomo M, Gallardo D, de Llanos MP, Marij JM, Aventin 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/ejha.12130.

[16] S. Schnittger, T.M. Kohl, H. Haferlach, W. Kern, W. Hiddemann, K. Spiesskerman, C. Schoch, KIT-DB16 mutations in AML:ETO-positive AML are associated with impaired event-free and overall survival, Blood 107 (May(9)) (2006) 3463–3468 Epub 2006 Dec 2.

[17] 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, Am. Lab. Med. 35 (2015) 288–297.