Old and new prognostic factors in acute myeloid leukemia with deranged core-binding factor beta

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Acute myeloid leukemia (AML) with deranged core-binding factor beta (CBFβ) is usually associated with a favorable prognosis with 50–70% of patients cured using contemporary treatments. We analyzed the prognostic significance of clinical features on 58 patients with CBFβ-AML aged <60 years. Increasing age was the only predictor for survival (P < 0.001), with an optimal cut-point at 43 years. White blood cells (WBCs) at diagnosis emerged as an independent risk factor for relapse incidence (P = 0.017), with 1.1% increase of hazard for each 1.0 × 109/L WBC increment. KIT mutations lacked prognostic value for survival and showed only a trend for relapse incidence (P = 0.069). Am. J. Hematol. 88:594–600, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Among acute myeloid leukemias (AMLs) with recurrent genetic abnormalities, patients with t(8;21)(q22;q22), inv(16)(p13q22), or t(16;16)(p13;q22), are referred to as core-binding factor (CBF)-AML. Although CBF-AML patients share a common molecular pathogenetic event, notably the creation of a fusion protein involving a CBF gene unit, these two types of AML differ with regard to morphologic presentation, immunophenotypic marker expression, prognostic factors, and response to treatments, and should be considered as distinct clinical entities [1,2].

In the inv(16)/t(16;16) group, the CBF-beta (CBFβ) gene located on 16q22 fuses to the MYH11 gene on 16p13, resulting in a chimeric protein. The translocation t(16;16)(p13;q22) is an equivalent rearrangement with lower incidence. Cytogenetically, the CBFβ–MYH11 rearrangement may be associated with trisomies of the chromosomes 8, 21, and 22 or with deletion of the chromosome 7 [3,4]. Patients with CBFβ-AML account for about 5–8% of adults with de novo AML and they are frequently associated with specific characteristics. This AML subset is morphologically associated with the French–American–British (FAB) M4 subtype with an abnormal eosinophil component (M4eo) and extramedullary involvement may be present [3–9].

Clinically, patients with CBFβ-AML are closely associated with a favorable outcome as compared with other AML subtypes [10–15]. High-complete remission (CR) rate and prolonged disease-free survival may be achieved when patients are treated with standard induction therapy followed by high-dose cytarabine (HD-AraC) post-remission therapies [16]. Despite these results, the outcome of CBFβ-AML patients does not appear to be as homogeneous as their cytogenetic definition, because only 54–74% are cured using contemporary treatment [17]. Recurrent disease occurs in 30–40% of patients, with a significant number of them subsequently dying from disease progression. Prognostic factors of relapse risk in CBFβ-AML subset are still a matter of debate. Female gender, older age, and low-platelet count have been reported as predictors for inferior outcome and/or shorter disease-free survival in patients enrolled in prospective trials [1,17,18]. Furthermore, higher white blood cells (WBCs) and low-platelet counts have been identified as bad predictor factors for CR achievements [19,20]. Conversely, nonrandom additional cytogenetic abnormalities such as trisomy +22, and male sex predicted better outcome [1,21].
in terms of both survival and relapse incidence (RI). This result contrasts with the observation done in AML with t(8;21)(q22;q22), suggesting differences in biology of CBF-AMLs.

Design and Methods
Patients’ characteristics, data collection, and treatment protocols
Fifty-eight patients aged less than or equal to 60 years with untreated AML presenting inv(16)(p13q22) or t(16;16)(p13;q22) diagnosed in eight Italian centers were included in this study (see Table I for patients’ characteristics).

Each patient gave his/her informed consent for collection of clinical data, the cryopreservation of bone marrow samples, and the performance of DNA-analysis for scientific purposes, in accordance with institutional guidelines.

Bone marrow samples from each patient were collected and cryopreserved at diagnosis and then centrally analyzed for KIT gene mutational status at the Department of Biology and Genetics for Medical Sciences, University of Milan, Italy.

For each patient, data regarding hematologic parameters, bone marrow morphology, immunophenotype, cytogenetic, molecular analysis, diagnosis of EML, treatment schedule, and outcome were recorded. The study started in January 2001. Until January 2010, patients’ data were periodically updated from the participating centers, centrally verified for consistency and completeness, and subsequently submitted for statistical analysis. The study design adhered to the Declaration of Helsinki and approval for this study was obtained from the Niguarda Hospital Review Board.

Patients were enrolled in intensive chemotherapy protocols, as previously described [30]. In brief, they received a standard induction therapy with an anthracycline-containing regimen, most commonly the “7+3” regimen with cytarabine in 7-day continuous intravenous infusion and three doses of anthracycline (idarubicin 12 mg/m²/day or daunorubicin 60 mg/m²/day) or the “ICE” schedule, including etoposide 100 mg/m²/day on days 1–5.

The post-remission chemotherapy consisted of three consolidation courses. In patients treated with the “7+3” regimen, the first cycle was with high-dose cytarabine (3,000 mg/m² every 12 hr for 3 consecutive days, with patients older than 50 years receiving a reduced dose at 2,000 mg/m²) and idarubicin 10 mg/m²/day on days 1 and 3, while patients treated with ICE schedule received a NOVIA course (mitoxantrone 12 mg/m²/day on days 1–4 and cytarabine 500 mg/m² every 12 hr for 6 doses). The second and third consolidation courses consisted of high-dose cytarabine.

The conditioning regimen for both autologous stem cell transplantation (ASCT) and allogeneic stem cell transplantation (allo-SCT) was with cyclophosphamide 60 mg/kg/day for 2 days and total-body irradiation in 6 fractions of 200 cGy (1,200 cGy) or busulfan 16 mg/kg over 4 days and cyclophosphamide 50 mg/kg over 4 days.

Definitions and criteria for treatment response
CR was defined as less than 5% of bone marrow blasts, regression of extramedullary disease, transfusion independency with peripheral neutrophil count greater than 1.0 × 10⁹/L and platelet count greater than 100 × 10⁹/L and disappearance of the cytogenetic and molecular markers [34,35]. Recurrent disease is defined as the reappearance of more than or equal to 5% blasts in the bone marrow or in the peripheral blood or as the appearance of a new extramedullary site of disease in patients with a previously documented CR.

Extramedullary disease was defined as any leukemic collection outside the bone marrow and its presence was documented either by histological, cytological, or radiological criteria.

Overall survival (OS) was calculated from the date of diagnosis until death, where all living patients were censored at the time of last contact. The duration of CR was calculated from the date of the first CR until the date of the first relapse. RI was calculated from the date of the first CR until the date of the first relapse, where patients were censored at the time of last contact or death not because of recurrent disease.

Screening of mutations in the coding region of KIT gene
Bone marrow samples were submitted for a centralized analysis for KIT gene mutations in exon 2, 8, 10, 11, and 17. Mutations of exon 17 were detected using sequencing and other sensitive assays such as enzymatic digestion with HincII for Asp816Val and with Tsp509I for Asn822Lys and ARMS (amplification refractory mutation system) polymerase chain reaction (PCR) for Asp816Tyr and Asp816His [22,36,37]. Direct sequencing of DNA and cDNA products was performed using Thermo Sequence Dye Terminator sequencing reaction and ABI Prism 3100 sequencing analyzer (Applied Biosystems, Warrington, United Kingdom).

Statistical analyses
All collected variables were submitted to usual descriptive methods. In particular, for continuous variables the distribution was first evaluated by the Shapiro–Wilks test, so that normally distributed variables were summarized with mean and standard deviation, while nonnormal variables were summarized with median and range.

The Pearson’s chi-square test with Yates’ correction for continuity and the Fisher’s exact test (if applicable) were used to check the association between categorical data, after crosstabulation. Comparisons of normally distributed continuous variables were carried out by Student’s t-test or by Welch test (in the case of nonhomogeneous variances between groups, previously verified by Levene’s test). The Mann–Whitney U test was used for comparison of continuous non-normally distributed variables.

The survival analysis was carried out using the Kaplan–Meier product limit method, followed by the log-rank test, to evaluate the possible differences in survival between groups. Cox univariate and multivariate regression models were also used to analyze the effects of continuous variables on survivorship. The optimal multivariate model was chosen.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n = 58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis (range, yr)</td>
<td>42 (15–60)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>40/18</td>
</tr>
<tr>
<td>Median WBC (range, × 10⁹/L)</td>
<td>24.7 (1.8–277)</td>
</tr>
<tr>
<td>Median marrow blast (range, %)</td>
<td>77.5 (26–95)</td>
</tr>
<tr>
<td>Extramedullary disease, no. (%)</td>
<td>7 (12.0)</td>
</tr>
<tr>
<td>Cytogenetic features</td>
<td></td>
</tr>
<tr>
<td>Without additional abnormalities (%)</td>
<td>43 (74.1)</td>
</tr>
<tr>
<td>No. abnormalities (%)</td>
<td>13* (22.4)</td>
</tr>
<tr>
<td>Including +8</td>
<td>2</td>
</tr>
<tr>
<td>Including +22</td>
<td>6</td>
</tr>
<tr>
<td>Including LOS</td>
<td>1</td>
</tr>
<tr>
<td>Structure abnormalities (%)</td>
<td>3* (5.2)</td>
</tr>
<tr>
<td>Including del(7q)</td>
<td>2</td>
</tr>
</tbody>
</table>

KIT mutational status

| KIT mutated cases, no. (%) | 15 (25.9) |
| Exon 17 | 12 |
| Exon 8 | 2 |
| Exon 10 | 1 |

WBC, white blood cells; LOS, losses of a sexual chromosome.

American Journal of Hematology

595
using a backward stepwise elimination after inserting all variables showing $P < 0.20$ at univariate analysis.

The receiver operating characteristics (ROC) curve was traced to analyze the role of patients' age on survivorship and to search for an optimal cut-off value for age itself. For all possible cut-off points, the total accuracy was considered together with sensitivity, specificity, positive predictive value and negative predictive value and the cut-off choice was made according to Youden.

Statistical analysis was done using Stata/SE 11.1 (The StataCorp, College Station, TX). Statistical significance was assumed for all tests with $P < 0.05$. The Bonferroni method was used to adjust significance in case of multiple comparisons.

**Results**

**Overall results of treatments**

Fifty-eight patients, aged between 15 and 60 years (median age: 42 years; male/female: 40/18), underwent treatment as described and were assessed for response. CR was obtained from 56 out of 58 (96.5%) patients. Primary refractory disease and one infectious complication during post-chemotherapy aplasia accounted for the two KIT-negative patients (aged 57 and 60 years, respectively) who did not achieve CR. A toxic death was subsequently recorded during the consolidation therapies. Twelve patients underwent ASCT instead of the third consolidation course and two KIT-negative patients received an allo-SCT in the first complete remission (CR1) from a sibling donor.

The median follow-up time for patients was 50 months based on the reverse Kaplan–Meier method. The estimated 5-year OS and RI resulted in 69.2% and 48.4%, respectively, with 32 patients alive in CR1 and 11 patients alive in second or subsequent CR (Figs. 1A and 2A; Table II).
TABLE II. Clinical Characteristics and Outcome of Patients with inv(16)/t(16;16) and Recurrent Disease

<table>
<thead>
<tr>
<th>Age (Yr)/sex</th>
<th>Cytotagetic at diagnosis</th>
<th>KIT status</th>
<th>WBC, ×10^9/L</th>
<th>EML</th>
<th>Status at AsSCT</th>
<th>Status at allo-SCT</th>
<th>Outcome</th>
<th>Survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>169.8</td>
<td>Absent</td>
<td>ND</td>
<td>ND</td>
<td>A/1st rel</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>26/M</td>
<td>46.XY.inv(16)(p13q22), (1;11)</td>
<td>V553I</td>
<td>7.6</td>
<td>Absent</td>
<td>ND</td>
<td>ND</td>
<td>97.9</td>
<td></td>
</tr>
<tr>
<td>29/F</td>
<td>46.XY.inv(16)(p13q22) D816V</td>
<td>11.1</td>
<td>Gastric mass</td>
<td>ND</td>
<td>2nd CR</td>
<td>A/2nd CR</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td>32/F</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>52.3</td>
<td>Absent</td>
<td>ND</td>
<td>2nd CR</td>
<td>A/2nd CR</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td>36/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>19.0</td>
<td>Absent</td>
<td>ND</td>
<td>2nd CR</td>
<td>A/2nd CR</td>
<td>74.8</td>
<td></td>
</tr>
<tr>
<td>36/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>30.4</td>
<td>Absent</td>
<td>ND</td>
<td>2nd CR</td>
<td>A/2nd CR</td>
<td>70.5</td>
<td></td>
</tr>
<tr>
<td>36/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>11.3</td>
<td>Absent</td>
<td>ND</td>
<td>A/2nd CR</td>
<td>ND</td>
<td>21.1</td>
<td></td>
</tr>
<tr>
<td>38/F</td>
<td>46.XY.inv(16)(p13q22) Exon 8</td>
<td>4.4</td>
<td>Absent</td>
<td>ND</td>
<td>2nd CR</td>
<td>A/2nd CR</td>
<td>49.6</td>
<td></td>
</tr>
<tr>
<td>39/M</td>
<td>46.XY.inv(16)(p13q22), +6</td>
<td>96.2</td>
<td>Absent</td>
<td>ND</td>
<td>2nd CR</td>
<td>A/2nd CR</td>
<td>64.3</td>
<td></td>
</tr>
<tr>
<td>42/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>11.7</td>
<td>Mesenteric mass</td>
<td>ND</td>
<td>1st CR</td>
<td>2nd CR</td>
<td>107.2</td>
<td></td>
</tr>
<tr>
<td>55/F</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>13.4</td>
<td>Absent</td>
<td>1st CR</td>
<td>ND</td>
<td>A/3rd CR</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>43/F</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>130.0</td>
<td>Absent</td>
<td>ND</td>
<td>2nd CR</td>
<td>D/TRM 2nd CR</td>
<td>27.1</td>
<td></td>
</tr>
<tr>
<td>46/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>23.8</td>
<td>Absent</td>
<td>ND</td>
<td>2nd CR</td>
<td>D/TRM 2nd CR</td>
<td>52.6</td>
<td></td>
</tr>
<tr>
<td>16/M</td>
<td>46.XY.inv(16)(p13q22) D816V</td>
<td>12.8</td>
<td>Absent</td>
<td>2nd CR</td>
<td>ND</td>
<td>D/TRM 2nd CR</td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td>16/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>27.7</td>
<td>Absent</td>
<td>ND</td>
<td>D/1st res rel</td>
<td>ND</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>47/M</td>
<td>46.XY.inv(16)(p13q22), +22,del(7)</td>
<td>12.3</td>
<td>Absent</td>
<td>ND</td>
<td>D/1st res rel</td>
<td>ND</td>
<td>29.8</td>
<td></td>
</tr>
<tr>
<td>54/F</td>
<td>48.XY. inv(16)(p13q22), +8,+21</td>
<td>49.8</td>
<td>Absent</td>
<td>ND</td>
<td>D/1st res rel</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>10.9</td>
<td>Ileal mass</td>
<td>ND</td>
<td>D/1st res rel</td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55/M</td>
<td>46.XY.inv(16)(p13q22) D816V</td>
<td>150.0</td>
<td>Absent</td>
<td>ND</td>
<td>D/1st res rel</td>
<td>14.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>58/F</td>
<td>46.XY.inv(16)(p13q22) D816V</td>
<td>122.0</td>
<td>Absent</td>
<td>ND</td>
<td>D/1st res rel</td>
<td>11.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>12.0</td>
<td>Absent</td>
<td>1st CR</td>
<td>ND</td>
<td>D/2nd rel</td>
<td>29.6</td>
<td></td>
</tr>
<tr>
<td>60/M</td>
<td>46.XY.inv(16)(p13q22) w/t</td>
<td>14.9</td>
<td>Absent</td>
<td>ND</td>
<td>D/2nd rel</td>
<td>22.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54/M</td>
<td>46.XY.inv(16)(p13q22) D816V</td>
<td>110.0</td>
<td>Absent</td>
<td>ND</td>
<td>D/2nd rel</td>
<td>26.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Allo-SCT, allogeneic stem cell transplantation; AsSCT, autologous stem cell transplantation; CR, complete remission; EML, extramedullary leukemia; WBC, white blood cells; ND, not determined; w/t, wild type; TRM, transplant related mortality.

Relapse incidence and survival after relapse

Twenty-four out of 58 patients who achieved CR experienced relapse, including 4 patients who received an AsSCT in CR1. The RI plot grew up rapidly to 43.6% within 17.7 months and reached 48.4% at 38.2 months (Fig. 2A). Twenty-three out of 24 patients underwent salvage chemotherapy, while 1 patient was lost at follow-up. The median survival time after relapse was 14.7 months (range: 1.1–92.4), with 17 (74%) patients achieving a second complete remission (CR2) and 6 (26%) dying for resistant relapse. Eleven patients underwent a stem cell transplantation (1 AsSCT, 10 allo-SCT) in CR2. Of them, eight allo-transplanted patients were alive and disease-free with a median CR2 duration of 30.8 months (range: 1.0–91.4), and three patients died for transplant-related mortality. The remaining six patients who entered the CR2 received intensive consolidation chemotherapy courses: four patients presented a second relapse and three of them subsequently died from disease progression (Table II). Overall, 11 out of 23 (47.8%) relapsed patients are still alive and disease-free, with a median CR2 duration of 19.0 months (range: 1.0–91.4).

Incidence of KIT mutations and correlation between KIT status and clinical characteristics

Mutational screening reported KIT gene mutations in 15 of 58 patients (25.9%): 12 (20.6%) patients showed a D816 missense mutation (TKD816), 2 (3.4%) patients presented an exon 8 in-frame deletion plus insertion mutations, and 1 (1.7%) patient had an exon 10 (V530I) transmembrane mutation (Table I). Patients with KIT gene mutations were classified as “KIT-Positive” (KIT+), while the remaining 43 patients who showed no mutations were classified as “KIT-Negative” (KIT−). Statistical analysis showed no significant difference in terms of age (P = 0.368), sex ratio (P = 0.756), and WBC count at diagnosis (P = 0.765) between KIT+ and KIT− patients.

Seven patients out of the 58 cases included in this study (12.0%) had EML at presentation. In all cases the EML manifested in the form of myeloid sarcoma involving a variety of sites (spinal masses, gastrointestinal tract, lungs) except for skin. The association between the KIT mutational status and EML turned out to be not significant (P = 0.360).

Treatment outcome by KIT mutational status

CR was achieved in 100.0% (15/15) of KIT+ patients after induction therapy. Recurrent disease was observed in 9 (60.0%) and 15 (34.9%) patients of KIT+ and KIT− groups, respectively. No difference was seen in terms of RI between KIT+ and KIT− patients (P = 0.166), with an estimated 5-year RI of 65.5% and 41.8%, respectively (Fig. 2B). Similarly, OS was not affected by KIT mutational status (P = 0.569), with an estimated 5-year OS of 62.3% and 72.1% for KIT+ and KIT− patients, respectively (Fig. 1B). Resistant relapses (four patients) and one transplant-related death accounted for the five KIT− deceased patients (Table III).

Prognostic factors for overall survival and relapse incidence

Cox univariate and multivariate regression models were performed to evaluate the role of different clinical variables as predictors for relapse or survival. The following potential prognostic parameters were evaluated, namely, age, sex, WBC count at diagnosis, EML, KIT status, and presence at standard cytogenetic of trisomy of chromosome 22. For continuous variables (age and WBC), an ROC curve analysis was performed toward survival in search of possible cut-off values. Age distribution showed an optimal cut-point at 43 years (AUC 0.827, sensitivity 93.3%, specificity 68.3; P = 0.0001), while no possible cut-off points for WBC were identified. In univariate analyses, only age, both as continuous or dichotomous variable with cut-off point set at 43 years, statistically significantly affected both RI and OS, while no other significant predictive parameters were identified.
A high-peripheral WBC count together with a raised serum LDH, the presence of hepatosplenomegaly, and EML, may reflect an increased tumor burden in AML even in the setting of “good risk” acute leukemias, such as acute prolymphocytic leukemia or CBF-AML [19,38–40]. In AML with inv(16), Delaunay et al. [20] reported that bad prognosis factors for CR achievement were a high WBC count, with an optimal cut-off point at 120 × 10^9/L, and lower platelet count. Martin et al. [19], in a small study, found that presenting WBC count had a significant negative influence on disease-free survival. Our data are substantially in line with reported data, therefore we found an increase of 1.1% hazard of relapse for each 1.0 × 10^9/L increment of WBC count. However, we were not able to identify any possible WBC cut-off value by means of the ROC analysis.

Activating KIT mutations are frequently found in CBF leukemia [41]. We recorded here an incidence of 25.9% of KIT mutations, with most patients showing D816 missense mutations (Table I). From this aspect, it is of interest that expression levels of both KIT mRNA and proteins is much higher in CBF-AML, with either wild type or mutant KIT, than in leukemia cells negative for CBF rearrangements. Moreover, we recently reported that CBF genetic abnormalities, in addition to directly targeting and down-regulating the expression of hematopoietic protein-coding genes containing AML1 consensus sequences, can target microRNA genes (Mir222/221) involved in the regulation of the KIT receptor leading to KIT overexpression in CBF-AML [42]. Furthermore, it has been postulated that mutations of the KIT gene may drive the WBC proliferation in CBF leukemia. Recently, Luck et al., showed that KIT mutations confer a distinct gene expression signature in CBF-AML and that one of the most significantly differentially expressed genes is LRP6 that is essential for noncanonical WNT5A signaling and thus for the maintenance of stem and progenitor cells [43,44]. Authors suggested that the different gene profiling may lead to an enhancement of proliferation in the KIT-mutated cases, which may be reflected in the higher blast counts of those patients [43]. The clinical observations that affected patients with t(8;21) appear to have a higher WBC count and WBC-index at presentation and a higher frequency of EML might support this hypotheses [30,39,45].

However, despite these reports on AML with t(8;21), we did not find any difference in WBC count (P = 0.765) and incidence of EML (P = 0.360) between the 15 KIT+ and the 43 KIT− cases harboring the inv(16)(t16;16) recruited in this study. Similarly, regarding the impact on outcome, this showed prognostic significance for OS (P < 0.00005) (Fig. 1C), whereas no statistical significance was found for all the other variables. When combined in the multivariate analyses, only age both as continuous or dichotomous variable was a significant part of the Cox model and proved to be an independent risk factor for OS (P < 0.001). Any increase of 1 year in age led to a 15% increase of the hazard ratio of 47.41 (95% CI; 4.87–461.39; P = 0.004), adjusting by WBC, EML, and KIT mutational status (Table IV).

In the multivariate Cox model with backward elimination of factors, WBC emerged as an independent risk factor for RI (P = 0.017) and any 1.0 × 10^9/L increment of WBC meant a 1.1% increase of the hazard of relapse (95% CI; 1.002–1.020; P = 0.017), adjusting by age and KIT mutational status (Table IV). KIT mutations showed a trend for RI but did not reach a significative value (P = 0.069).

Discussion

In this study, we have evaluated the impact of clinical and genetic features on the prognosis of de novo AML with inv(16)/t(16;16) in 58 patients with age less than or equal to 60 years, treated according to standard chemotherapy protocols. Overall, we observed a high CR rate (96.5%), a RI of 48.4% at 38.2 months after the first CR and, an estimated 5-year OS of 69.2%, according to outcome data reported in the recent literature. In this relative large cohort of homogeneously treated patients, we found that only WBC at presentation and age emerged as an independent risk factor for relapse (P = 0.017) and OS (P < 0.001), respectively.
study showed that KIT mutations did not reach a significant value as independent prognostic factor for relapse and survival neither in the multivariate nor in the Kaplan–Meier analysis, in contrast to those reported in adult patients with t(8;21) (Figs. 1B–2B; Tables III and IV) [24,26–28,30–32].

Accumulating evidence suggests that a high degree of similarity is identified between the two major subtypes of CBF leukemias. However, important differences on clinical and biological ground are reported [1,2]. A recent study using Drosophila as a model showed that AML1-ETO-expressing precursor cells express high levels of reactive oxygen species (ROS), and that ROS plays a central role in the proliferation of these precursors [46]. As for CBFj–MYH11 leukemia, gene expression profiling of AML-M4 subtype suggested a highly activated NF-kB pathway in inv(16) patients [47]. Given that these pathways, particularly Notch, Wnt, and Cox/PGE2 signaling, are essential for stem cell self-renewal, they could contribute to a different transforming activity of AML1-ETO and CBFj–MYH11 in CBF-AML.

Although our data suggest that a high WBC count is an unfavorable prognostic factor, because it increases the risk of relapse in multivariate analysis, it shows no significant effect on overall survival. In fact, after salvage and subsequent therapy including allo-SCT, we found that 11 out of 23 relapsed patients who underwent salvage chemotherapy are still alive and disease-free, with a median CR2 duration of 19 months.

In this study, only age as continuous or dichotomous value, with a best calculated cut-off point at 43 years, emerges as a prognostic factor affecting survival in both univariate or multivariate analysis. It is to be noted that, among the 30 patients aged 42 years or younger, we recorded only one death (transplant related), leading to a Kaplan–Meier plot of 95.5% for OS (P < 0.00005) (Table II; Fig. 1C). By contrast, focusing on 28 patients aged 43 years or older, we recorded 14 deaths (2 early deaths, 1 death in aplasia, 2 deaths for transplant-related complications, and 9 for first or second resistant relapse). Overall, 50% of patients aged 43 years or older are disease-free at 27.1 months (Table II; Fig. 2C).

Our data confirm that the strategy to perform an allelicrem SCT in CR >1 lead to encouraging results. In fact, of the 10 patients allo-transplanted while in second CR, 8 (80%) are alive and disease-free with a 4-year follow-up of 30.8 months. As in our study, Kuwatsuka et al., of 66 patients with inv(16) undergoing allo-SCT, reported an OS of 86% at 3 years in CR2 or CR3 and identified only age to be a significant prognostic factor. The Japanese study concluded that allo-SCT is not necessarily recommended for inv(16) in CR1 and that inv(16) patients who received an allo-SCT not in CR did significantly better than those with t(8;21) [2]. Furthermore, a French survey reported that age, with a best cut-off at 35 years, was the only factor for concurrent comorbidities in addition to different disease biology such as multidrug resistance protein (MDR1)-positive or stem cell phenotype adversely affecting both attainment of remission and refractory relapse risk [49,50].

Paschka et al. reported that in inv(16) patients the cumulative incidence of relapse (CIR) was higher for KIT-positive patients, especially if presenting exon 17 mutation, compared with KIT-negative patients (5-year CIR 80% vs. 29%; P = 0.002). Furthermore, the authors reported that KIT mutations predicted worse survival when adjusted for sex [24]. Anyway, it has to be noted that in the CALGB study the KIT-mutated patients were significantly older (median age: 38 vs. 49 years; P < 0.001) and were more frequently male (P < 0.05) compared with nonmutated patients. Moreover, in the reviewed literature, all the focused studies on the prognostic significance of KIT mutations in the CBFj–MYH11 adult patients have been unable to demonstrate any role of such mutations on survival; furthermore, to our knowledge, all studies but one [26] do not show any influence of KIT mutations on relapse [27,30–32].

All the results reported in these different studies are based on a relatively small population, principally because of the fact that AML is a rare disease and that the CBFj subtype accounts for about 5–8% of adults with de novo AML. In a 9-years period (January 2001–January 2010), we considered 58 patients aged less than or equal to 60 years belonging from 8 Italian centers. At present, this is one of the studies with the largest number of adult CBFj AML patients, second only to the one of Paschka and colleagues (counting 61 patients). Incrementing the number of patients surely would be of interest, but when it results in an excessive accrual time statistical analysis it is more likely to be biased.

In conclusion, while the prognostic significance of KIT mutations remains unclear with several studies yielding contrasting results [24,26,27,30–32], our data showed that only “old” prognostic factors, such as age and the WBC count at diagnosis, are important predictors of outcome in AML adult patients with inv(16)/t(16;16).

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References


7. Mariton P, Keating M, Kantarjian H, et al. Cytogenetic and clinical correlates in the proliferation of these precursors [46]. As for CBFj–MYH11 leukemia, gene expression profiling of AML-M4 subtype suggested a highly activated NF-kB pathway in inv(16) patients [47]. Given that these pathways, particularly Notch, Wnt, and Cox/PGE2 signaling, are essential for stem cell self-renewal, they could contribute to a different transforming activity of AML1-ETO and CBFj–MYH11 in CBF-AML.


