Clinical Characterization of Acute Myeloid Leukemia with Myelodysplasia-Related Changes as Defined by the 2008 WHO Classification System

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Abstract
Although some studies have validated the 2001 WHO classification of acute myeloid leukemia (AML), including the importance of multilineage dysplasia, others have suggested that multilineage dysplasia correlates with unfavorable cytogenetics but has no independent impact on prognosis. In 2008, the revised WHO classification has expanded this category into “AML with myelodysplasia-related changes” (AML-MRC). We evaluated the clinical, pathologic, cytogenetic, and molecular features of one hundred AML patients using the 2008 WHO criteria. Patients underwent genetic screening for NPM1, FLT3-ITD, FLT3-D835 and CEBPA mutations. Compared to patients with AML, not otherwise specified (AML-NOS), patients with AML-MRC were significantly older (p=0.0141), presented with a lower hemoglobin (p=0.044), more frequently expressed CD14 (p=0.048), and exhibited a decreased frequency of CEBPA mutations (p=0.001). Multivariate analysis indicated that patients with AML-MRC had a significantly worse OS, PFS and CR compared to AML-NOS (all p<0.0001). These data support the clinical, morphologic and cytogenetic criteria for this 2008 WHO AML category.
Introduction

The classification of acute myeloid leukemia (AML) has evolved from being based on morphologic and cytochemical findings, as included in the French-American-British (FAB) proposal\(^1\)\(^2\), to systems that incorporate cytogenetic abnormalities.\(^3\)\(^-\)\(^5\) In 2001, the WHO classification for tumors of hematopoietic and lymphoid tissues was proposed in an attempt to define more biologically homogeneous entities that have clinical relevance. As it relates to AML, this includes limited cytogenetic findings, presence of morphologic dysplasia and prior therapy\(^5\). Although later studies have validated this system\(^6\)\(^-\)\(^8\), including the importance of multilineage dysplasia, others have suggested that multilineage dysplasia correlates with unfavorable cytogenetics and has no independent impact on prognosis.\(^9\)\(^,\)\(^10\)

In 2008, a revision of the WHO classification has incorporated recently acquired genetic information into an updated classification scheme of AML.\(^11\) One of the revisions includes a new “AML with myelodysplasia-related changes” (AML-MRC) group. Patients are assigned to this group for any one of 3 reasons: 1) AML arising from previous myelodysplastic syndrome (MDS) or an MDS/myeloproliferative neoplasm, 2) AML with a specific MDS-related cytogenetic abnormality and/or 3) AML with multilineage dysplasia.\(^11\) The goal of the current study was to clinically characterize this newly defined AML-MRC subgroup as well as to evaluate frequent mutations present in AML including NPM1, FLT3 and CEBPA.

Study Design

Patients

One-hundred consecutive AML patients diagnosed at Stanford University Medical Center between 2005 and 2007 with adequate material for mutation analysis were studied. All cases were diagnosed with bone marrow aspirates, blood smears, trephine biopsies and/or flow cytometry. Clinical parameters, hemogram data and flow cytometry results at the time of diagnosis were reviewed. Clinical follow up information was obtained by retrospective review of the electronic charts. Cytogenetic risk group stratification was performed using Southwest Oncology Group (SWOG) criteria.\(^7\)\(^,\)\(^12\) This study has been approved by Stanford’s Institutional Review Board.

NPM1, FLT3 and CEBPA mutational analysis

The FLT3-ITD, FLT3-D835 and exon 12 NPM1 insertion mutations were detected by multiplex PCR followed by restriction enzyme detection and capillary electrophoresis.\(^13\)\(^-\)\(^15\) The entire coding region of CEBPA was PCR amplified and sequenced.\(^16\)\(^,\)\(^17\)

Statistical Analysis

Overall survival (OS), progression free survival (PFS) and complete response (CR) were defined by as previously described.\(^18\) These parameters were compared using Kaplan-Meier methods and log-rank test. Univariate and multivariate Cox proportional hazard models were performed. Quantitative factors were treated as continuous variables in these regression models. Categorical variables were compared using Fisher exact test.

Results and Discussion

Patient characteristics

The cases included 57 males and 43 females with a median age of 56 years (range 17-81). Follow up and therapy information was available for 90 patients. Most patients received idarubicin and cytarabine as induction therapy (81/90, 90%) and high dose or standard dose cytarabine for consolidation (75/90, 83%). Twelve patients underwent a bone marrow transplant.
Among the 90 patients with follow up, the median overall survival (OS) was 373 days (95% CI, 284-503) and the median progression free survival (PFS) was 254 days (95% CI, 222-349). Complete remission (CR) was achieved in 60 patients (67%). A univariate analysis showed that advanced age (> 60) predicted worse OS (p=0.001) and PFS (p=0.04). Stratification of patients into cytogenetic risk groups resulted in 9 patients with favorable, 65 with intermediate and 19 with unfavorable risk status and correlated with significant differences in OS (p=0.0001), PFS (p=0.0001) and achievement of CR (p=0.0001).

**WHO Classification**

Using the 2008 WHO criteria resulted in the distribution of AML subcategories listed in Table 1a. The percentage of patients encompassed by the AML-MRC category was 48%, as compared to prior reports of AML with multilineage dysplasia comprising 24-38%. Overall, 26 patients had a NPM1 mutation (16 of which were FLT3 mutated), 25 had FLT3-ITD alone, 8 had FLT3-D835 alone and 9 had a CEBPA mutation (3 of which were FLT3 mutated). The frequency of these mutations is within the range of prior studies. CEBPA mutations, associated with favorable prognosis, were significantly absent from AML-MRC (p=0.017) with no significant differences in the distribution of other mutations.

Comparison of the clinical outcome of the newly defined group AML-MRC with AML-NOS showed that AML-MRC had significantly worse OS, PFS and lower CR rate (p=0.0001) (Figure 1). Even after excluding the 14 patients with unfavorable cytogenetics from the AML-MRC group, the remaining AML-MRC patients had worse outcomes compared to all AML-NOS patients (OS, p=0.013; PFS, p=0.012; CR, p=0.0076). Among 65 patients with intermediate risk cytogenetics, the outcome difference between the AML-MRC and AML-NOS remained significant (OS, p=0.0292; PFS, p=0.0232), also indicating prognostic significance of multilineage dysplasia. This confirms the previously observed clinical significance of multilineage dysplasia, when strictly defined by the WHO criteria.

A multivariate Cox proportional hazard analysis, performed on the entire group, identified unfavorable cytogenetic risk group, advanced age (> 60 years), FLT3-ITD and AML-MRC status as significant predictors of worse OS (Table 1b). Checking the interaction terms in this Cox model confirmed that AML-MRC predicted poor survival, independent of age or cytogenetic status.

**AML with myelodysplasia related changes (AML-MRC)**

Patients with AML-MRC were significantly older (59 vs 51 years, p=0.0141) and had higher frequency of unfavorable cytogenetics (14/46 vs 3/36, p=0.014) compared to AML-NOS. The association of multilineage dysplasia with unfavorable cytogenetics has been previously reported; however, the difference in age could be attributed to the new definition of AML-MRC as some prior studies have not reported a significant age difference. Patients with AML-MRC presented with lower hematocrit (28 vs 33%, p=0.0138) and their blasts more frequently expressed CD14 as compared to AML-NOS (10/46 vs 4/36, p=0.048), with no other significant differences in antigen expression.

Within the group of 46 patients with AML-MRC, a low platelet count (< 20 K/uL) correlated with worse OS (p=0.0456) and shorter PFS (p=0.0294). A wild-type NPM1/mutated FLT3 pattern in AML-MRC resulted in significantly worse PFS (p=0.0385) compared to other AML-MRC cases. Presence of FLT3-D835 mutation alone in this category also correlated with worse OS (p=0.0265) compared to wild type FLT3 cases. Although the importance of this mutation has been controversial, Whitman et al recently showed that FLT3-D835 mutation correlates with worse clinical outcome in younger adults with AML.
The clinical outcome of patients with a history of MDS was not significantly different from the remaining cases of AML-MRC (OS, p=0.249; PFS, p=0.265), consistent with prior studies. Presence of MDS-related cytogenetic abnormalities correlated with a significantly worse OS (p=0.002) and PFS (p=0.001). Of the 14 patients with MDS-related cytogenetic abnormalities, 7 had morphological dysplasia. Further analysis showed that 32 patients with multilineage dysplasia in the absence of cytogenetic abnormalities have a better outcome than 7 patients with MDS-related cytogenetic abnormalities but without dysplasia (OS, p=0.0529; PFS, p=0.0226). However, the group with dysplasia still had worse outcomes compared to all AML-NOS patients (OS, p=0.013; PFS, p=0.012; CR, p=0.0076) suggesting that while the absence of cytogenetic abnormalities in AML-MRC indicates a possible better prognosis, the presence of multilineage dysplasia, as defined by the WHO, retains prognostic significance.

Conclusion
The newly defined WHO category of AML-MRC exhibits a significantly worse clinical outcome compared to AML-NOS and is predictive of worse overall survival in the multivariate analysis of AML patients, independent of age or cytogenetic risk group. These findings support the clinical, morphologic and cytogenetic criteria for this 2008 WHO AML category.

Authorship
Contribution: O.K.W. and D.A.A. designed the research, analyzed results and wrote the manuscript; K.S., L.M., performed experiments; M.S. and J.G. collected the clinical data; L.R. performed statistical analysis; J.L.Z., J.M. and J.G. assisted with writing the manuscript

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References
10. Wandt H, Schakel U, Kroschinsky F et al. MLD according to the WHO classification in AML has no correlation with age and no independent prognostic relevance as analyzed in 1766 patients. Blood 2008;111:1855-1861.


Table 1a. 2008 WHO classification of 100 patients with AML

<table>
<thead>
<tr>
<th>Type and Description</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with recurrent genetic abnormalities</td>
<td>10</td>
</tr>
<tr>
<td>AML with t(8;21)(q22;q22);(RUNX1-RUNX1T1)</td>
<td>3</td>
</tr>
<tr>
<td>AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);(CBFB-MYH11)</td>
<td>3</td>
</tr>
<tr>
<td>APL with t(15;17)(q22;q12);(PML-RARA)</td>
<td>3</td>
</tr>
<tr>
<td>AML with t(9;11)(p22;q23);(MLLT3-MLL)</td>
<td>0</td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34);(DEK-NUP214)</td>
<td>1</td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21q26.2);(RPN1-EVI1)</td>
<td>0</td>
</tr>
<tr>
<td>AML (megakaryoblastic) with t(1;22)(p13;q13);(RBMI5-MKLI)</td>
<td>0</td>
</tr>
<tr>
<td>Provisional entity: AML with mutated NPM1</td>
<td>26*</td>
</tr>
<tr>
<td>Provisional entity: AML with mutated CEBPA</td>
<td>9*</td>
</tr>
</tbody>
</table>

AML with myelodysplasia-related changes

- Prior history of myelodysplastic syndrome (MDS) 16
- MDS-related cytogenetic abnormality 14
- Multilineage dysplasia 41

Therapy-related myeloid neoplasms

- Acute myeloid leukemia, not otherwise specified 39
  - AML with minimal differentiation 3
  - AML without maturation 7
  - AML with maturation 9
  - Acute myelomonocytic leukemia 7
  - Acute monoblastic/monocytic leukemia 9
  - Acute erythroid leukemia 3
  - Acute megakaryoblastic leukemia 1
  - Acute basophilic leukemia 0
  - Acute panmyelosis with myelofibrosis 0

*The provisional entities were classified in other relevant categories

Table 1b. Multivariate Cox proportional hazard analysis of 90 patients with AML

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Overall Survival (OS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytogenetic Risk Group</td>
<td>0.001</td>
<td>2.825</td>
<td>1.517-5.264</td>
</tr>
<tr>
<td>Age &lt; or &gt; 60</td>
<td>0.037</td>
<td>2.112</td>
<td>1.010-4.416</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>0.047</td>
<td>1.983</td>
<td>1.007-3.905</td>
</tr>
<tr>
<td>AML-MRC</td>
<td>0.041</td>
<td>1.919</td>
<td>1.011-3.650</td>
</tr>
</tbody>
</table>
Figure 1: A. Overall Survival and B. Progression Free Survival for patients with AML-NOS and AML-MRC
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