

Brief report

Clinical characterization of acute myeloid leukemia with myelodysplasia-related changes as defined by the 2008 WHO classification system

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Although some studies have validated the 2001 World Health Organization (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 100 AML patients using the 2008 WHO criteria. Patients underwent genetic screening for *NPM1*, *FLT3-ITD*, *FLT3-D835*, and *CEBPA* mutations. Compared with patients with AML, not otherwise specified, patients with AML-MRC were significantly older ($P = .014$), presented with a lower hemoglobin ($P = .044$), more frequently expressed CD14 ($P = .048$), and exhibited a decreased frequency of *CEBPA* mutations

($P = .001$). Multivariate analysis indicated that patients with AML-MRC had a significantly worse overall survival, progression-free survival, and complete response compared with AML-not otherwise specified (all $P < .001$). These data support the clinical, morphologic, and cytogenetic criteria for this 2008 WHO AML category. (Blood. 2009;113:1906-1908)

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 proposal,^{1,2} to systems that incorporate cytogenetic abnormalities.³⁻⁵ In 2001, the World Health Organization (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*.

Methods

Patients

A total of 100 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 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 polymerase chain reaction followed by restriction enzyme detection and capillary electrophoresis.¹³⁻¹⁵ The entire coding region of *CEBPA* was polymerase chain reaction amplified and sequenced (Snaddon et al,¹⁶ and J.-Y. Ahn, K.S., O.K.W., S.D. Boyd, and D.A.A., J Mol Diag, manuscript in review).

Statistical analysis

Overall survival (OS), progression-free survival (PFS), and complete response (CR) were defined as previously described.¹⁷ These parameters were compared using Kaplan-Meier methods and log-rank test. Univariate and multivariate Cox proportional hazard models were performed.

Submitted October 6, 2008; accepted December 18, 2008. Prepublished online as Blood First Edition paper, January 8, 2009; DOI 10.1182/blood-2008-10-182782.

Presented in abstract form at the 50th annual meeting of the American Society of Hematology, San Francisco, CA, December 6, 2008 (publication no. 922).

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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 men and 43 women with a median age of 56 years (range, 17-81 years). Follow-up and therapy information was available for 90 patients. Most patients received idarubicin and cytarabine as induction therapy (81 of 90, 90%) and high-dose or standard-dose cytarabine for consolidation (75 of 90, 83%). Twelve patients underwent a bone marrow transplantation.

Among the 90 patients with follow-up, the median OS was 373 days (95% confidence interval, 284-503 days) and the median PFS was 254 days (95% confidence interval, 222-349 days). CR was achieved in 60 patients (67%). A univariate analysis showed that advanced age (> 60 years) predicted worse OS ($P = .001$) and PFS ($P = .04$). 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 ($P = .001$), PFS ($P = .001$), and achievement of CR ($P = .001$).

WHO classification

Using the 2008 WHO criteria resulted in the distribution of AML subcategories listed in Table 1. The percentage of patients encompassed by the AML-MRC category was 48%, compared with prior reports of AML with multilineage dysplasia comprising 24% to 38%.^{6-8,18} Overall, 26 patients had an *NPM1* mutation (16 were *FLT3* mutated), 25 had *FLT3-ITD* alone, 8 had *FLT3-D835* alone, and 9 had a *CEBPA* mutation (3 were *FLT3* mutated). The

Table 1. 2008 WHO classification of 100 patients with AML

Type and description	No. of cases
AML with recurrent genetic abnormalities	10
AML with t(8;21)(q22;q22);(<i>RUNX1-RUNX1T1</i>)	3
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);(<i>CBFB-MYH11</i>)	3
APL with t(15;17)(q22;q12);(<i>PML-RARA</i>)	3
AML with t(9;11)(p22;q23);(<i>MLL3-MLL</i>)	0
AML with t(6;9)(p23;q34);(<i>DEK-NUP214</i>)	1
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2);(<i>RPN1-EVI1</i>)	0
AML (megakaryoblastic) with t(1;22)(p13;q13);(<i>RBM15-MKL1</i>)	0
Provisional entity: AML with mutated <i>NPM1</i>	26*
Provisional entity: AML with mutated <i>CEBPA</i>	9*
AML with myelodysplasia-related changes	48
Prior history of myelodysplastic syndrome (MDS)	16
MDS-related cytogenetic abnormality	14
Multilineage dysplasia	41
Therapy-related myeloid neoplasms	3
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.

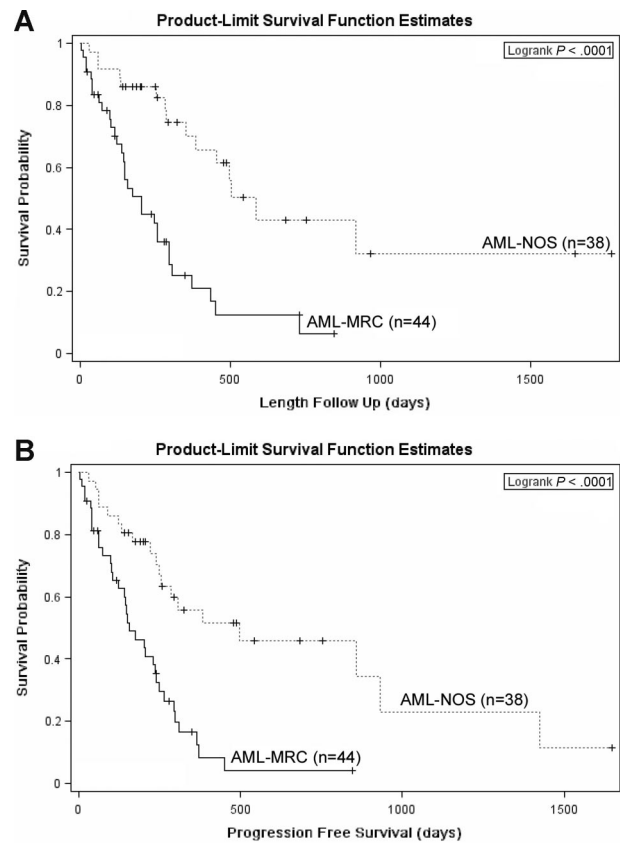


Figure 1. Survival data. Overall survival (A) and progression-free survival (B) for patients with AML-NOS and AML-MRC.

frequency of these mutations is within the range of prior studies.¹⁹ *CEBPA* mutations, associated with favorable prognosis,²⁰ were significantly absent from AML-MRC ($P = .017$) with no significant differences in the distribution of other mutations.

Comparison of the clinical outcome of the newly defined group AML-MRC with AML-not otherwise specified (NOS) showed that AML-MRC had significantly worse OS, PFS, and lower CR rate ($P = .001$; 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 with all AML-NOS patients (OS, $P = .013$; PFS, $P = .012$; CR, $P = .008$). Among 65 patients with intermediate-risk cytogenetics, the outcome difference between the AML-MRC and AML-NOS remained significant (OS, $P = .029$; PFS, $P = .023$), also indicating prognostic significance of multilineage dysplasia. This confirms the previously observed clinical significance of multilineage dysplasia,⁶⁻⁸ 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 2). Checking the interac-

Table 2. Multivariate Cox proportional hazard analysis of 90 patients with AML

	Variable	P	Hazard ratio	95% CI
Overall survival (OS)	Cytogenetic risk group	.001	2.825	1.517-5.264
	Age < or > 60 y	.037	2.112	1.010-4.416
	<i>FLT3-ITD</i>	.047	1.983	1.007-3.905
	AML-MRC	.041	1.919	1.011-3.650

tion terms in this Cox model confirmed that AML-MRC predicted poor survival, independent of age or cytogenetic status.

AML-MRC

Patients with AML-MRC were significantly older (59 vs 51 years, $P = .014$) and had higher frequency of unfavorable cytogenetics (14 of 46 vs 3 of 36, $P = .014$) compared with AML-NOS. The association of multilineage dysplasia with unfavorable cytogenetics has been previously reported^{7,9,10}; 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.¹⁰ Patients with AML-MRC presented with lower hematocrit (28% vs 33%, $P = .014$) and their blasts more frequently expressed CD14 compared with AML-NOS (10 of 46 vs 4 of 36, $P = .048$), with no other significant differences in antigen expression.

Within the group of 46 patients with AML-MRC, a low platelet count ($< 20\,000/\mu\text{L}$) correlated with worse OS ($P = .046$) and shorter PFS ($P = .029$). A wild-type *NPM1*/mutated *FLT3* pattern in AML-MRC resulted in significantly worse PFS ($P = .038$) compared with other AML-MRC cases. The presence of *FLT3-D835* mutation alone in this category also correlated with worse OS ($P = .026$) compared with wild type *FLT3* cases. Although the importance of this mutation has been controversial,¹⁹ Whitman et al recently showed that *FLT3-D835* mutation correlates with worse clinical outcome in younger adults with AML.²¹

The clinical outcome of patients with a history of MDS was not significantly different from the remaining cases of AML-MRC (OS, $P = .249$; PFS, $P = .265$), consistent with prior studies.⁷ The presence of MDS-related cytogenetic abnormalities correlated with a significantly worse OS ($P = .002$) and PFS ($P = .001$). Of the

14 patients with MDS-related cytogenetic abnormalities, 7 had morphologic 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, $P = .053$; PFS, $P = .023$). However, the group with dysplasia still had worse outcomes compared with all AML-NOS patients (OS, $P = .013$; PFS, $P = .012$; CR, $P = .008$), suggesting that, whereas 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.

In conclusion, the newly defined WHO category of AML-MRC exhibits a significantly worse clinical outcome compared with AML-NOS and is predictive of worse OS 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. and L.M. performed experiments; M.S. and J.G. collected the clinical data; L.R. performed statistical analysis; and J.L.Z., J.D.M., and J.G. assisted with writing the manuscript.

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

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