Reproducibility and prognostic significance of morphologic dysplasia in *de novo* acute myeloid leukemia

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The 2008 WHO classification of acute myeloid leukemia includes a category of acute myeloid leukemia with myelodysplasia-related changes; however, the significance of multilineage dysplasia alone is controversial and its reproducibility has not been evaluated in acute myeloid leukemia. We performed an in-depth analysis of morphologic dysplasia in 159 *de novo* acute myeloid leukemia cases lacking myelodysplasia-related cytogenetic abnormalities. Using the 2008 WHO criteria, there were 89 acute myeloid leukemia—not otherwise specified (56%) and 43 acute myeloid leukemia with myelodysplasia-related changes (27%), while 27 cases were ambiguous as to myelodysplasia-related changes status due to limited maturing cells (acute myeloid leukemia—not evaluable, 17%). On multivariable analysis, neither acute myeloid leukemia with myelodysplasia-related changes nor acute myeloid leukemia—not evaluable showed significantly different event-free survival compared with acute myeloid leukemia—not otherwise specified in the 137 patients treated with induction chemotherapy. When individual dysplastic features were analyzed, only micromegakaryocytes and hypogranulated myeloid cells emerged as factors significantly associated with shorter event-free survival in a multivariable analysis that included the other significant covariates of age, white blood count, platelet count, abnormal karyotype and stem-cell transplantation. Our findings indicate that the current 2008 WHO definition of multilineage dysplasia in acute myeloid leukemia in its current form is not optimal, and that the use of a more restricted definition of morphologic dysplasia results in more relevant risk stratification that is independent of other conventional prognostic factors. *Modern Pathology* (2015) **28, 965–976; doi:10.1038/modpathol.2015.55; published online 15 May 2015**

Acute myeloid leukemia is a heterogeneous disease, with varied patient outcome that reflects both patient-related and disease-related factors. The karyotype at the time of diagnosis provides critical prognostic information, but 40–50% of patients with acute myeloid leukemia have normal or non-specific cytogenetic findings.^{1,2} An increasing number of gene mutations have been found that risk-stratify acute myeloid leukemia patients with normal karyotype. Internal tandem duplications of the *FLT3* gene (*FLT3*-ITD) represent one of the most frequently encountered mutations and confer adverse prognosis,^{3,4} while

NPM1 mutations, found in 35% of cytogenetically normal acute myeloid leukemia, are associated with a favorable outcome in the absence of *FLT3*-ITD mutation.⁵

The recent 2008 WHO classification incorporates underlying cytogenetic and molecular genetic abnormalities into acute myeloid leukemia subtyping.⁶ This classification divides acute myeloid leukemia into groups with recurrent cytogenetic abnormalities, acute myeloid leukemia with myelodysplasia-related changes, therapy-related myeloid neoplasms, and acute myeloid leukemia—not otherwise specified. Acute myeloid leukemia with *NPM1* and *CEBPA* mutations are provisional entities. The category of acute myeloid leukemia with myelodysplasia-related changes encompasses acute myeloid leukemia occurring after a prior myelodysplastic syndrome or myelodysplastic/myeloproliferative neoplasm, as well as those cases with

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specific myelodysplastic syndrome-associated cytogenetic abnormalities and/or significant morphologic multilineage dysplasia; the latter is defined as the presence of $\geq 50\%$ dysplastic cells in two or three hematopoietic lineages.⁶ Acute myeloid leukemia with myelodysplasia-related changes comprises a large proportion of acute myeloid leukemia cases: applying the 2008 WHO criteria results in up to 48% of all acute myeloid leukemia cases being considered as acute myeloid leukemia with myelodysplasia-related changes by meeting one, two or all three of the aforementioned criteria.⁷ The frequency of morphologically-defined multilineage dysplasia ranges from 30 to 38% in all types of acute myeloid leukemia and is about 25% in all *de novo* acute myeloid leukemia.⁸

Although there is general agreement on the poor prognostic impact of a prior myeloid neoplasm or myelodysplastic syndrome-related cytogenetics, the clinical significance of morphologic dysplasia in acute myeloid leukemia has been debated.^{8–15} In earlier studies, Goasguen *et al*¹¹ found that the presence of dysgranulopoiesis was associated with a significant decrease in the ability to achieve a complete remission, and dysplastic changes of granulocytes and megakaryocytes were associated with worse event-free survival in a series by Gahn *et al*.¹² Tamura *et al*¹³ found that the presence of trilineage dysplasia was associated with lower disease-free survival. A study by Arber *et al*¹⁴ suggested that the less rigid criteria of only two cells lines (rather than trilineage dysplasia) used in the 2001 WHO definition of acute myeloid leukemia with multilineage dysplasia retained prognostic significance.

Other studies applying the WHO criteria for acute myeloid leukemia with myelodysplasia-related changes found that morphologic dysplasia alone had no prognostic importance outside of its association with unfavorable karyotype.^{8–10} Miesner *et al*⁸ found that acute myeloid leukemia with myelodysplasia-related changes lacking prior myelodysplastic syndrome or associated cytogenetic abnormalities had a lower incidence of *FLT3* mutations, but a similar outcome as acute myeloid leukemia—not otherwise specified (57%). Falini *et al*¹⁶ showed that *NPM1* mutations in acute myeloid leukemia were frequently associated with multilineage dysplasia, but that this did not confer worse clinical outcome. Another study found no significance of dysplasia in *NPM1*-mutated acute myeloid leukemia, but in patients with wild-type *NPM1*, those with multilineage dysplasia showed an inferior response to induction and, in younger patients, a lower 5-year survival, suggesting that the prognostic relevance of multilineage dysplasia in acute myeloid leukemia might depend on *NPM1* mutation status.¹⁷ Most recently, Devillier *et al*. compared acute myeloid leukemia with myelodysplasia-related changes to acute myeloid leukemia—not otherwise specified patients in a group younger than 66 years of age and found no difference in relapse-free survival or overall survival;

however, the acute myeloid leukemia with myelodysplasia-related changes group included patients with prior myelodysplastic syndrome and associated cytogenetic abnormalities.¹⁵ A recent study examined *de novo* intermediate-risk acute myeloid leukemia patients with wild-type *NPM1* and found that multilineage dysplasia had an adverse impact on outcome using a definition of $>50\%$ of the cells in one lineage plus $>30\%$ in at least one other lineage, which is less strict than the 2008 WHO definition of acute myeloid leukemia with myelodysplasia-related changes.¹⁸ This was also the first study to note that a significant subset of patients (21.5%) did not have enough residual hematopoiesis to assess for dysplasia.¹⁸

The reproducibility of scoring dysplasia in acute myeloid leukemia has not been evaluated to our knowledge, and could in part explain the varied results obtained in prior studies examining the prognostic significance of multilineage dysplasia. In myelodysplastic syndrome, where the threshold for dysplasia in each lineage is lower (10% rather than 50% for acute myeloid leukemia with myelodysplasia-related changes), studies have observed a moderate reproducibility for identifying dysplasia and noted that the degree of agreement could be improved if the features for dyserythropoiesis were refined.¹⁹ Specific dysplastic features have shown strong correlation with a diagnosis of myelodysplastic syndrome compared with non-neoplastic processes, and certain specific dysplastic findings have also been associated with outcome in myelodysplastic syndrome patients.²⁰ In contrast, although the same dysplastic features are used to define both acute myeloid leukemia with myelodysplasia-related changes and myelodysplastic syndrome, the spectrum of dysplastic changes seen in acute myeloid leukemia is not well-documented. Neither the 50% dysplasia threshold nor the specific dysplastic features that should be counted have been validated with respect to association with clinical outcome in acute myeloid leukemia. Thus, it is unclear if the current morphologic definition of acute myeloid leukemia with myelodysplasia-related changes is optimal to define a cohort with worse clinical outcome. The goal of this study was to evaluate reproducibility and prognostic significance of morphologic dysplasia assessment in *de novo* acute myeloid leukemia lacking specific recurrent or myelodysplasia-associated karyotype abnormalities.

Materials and methods

Patients

Cases of newly diagnosed *de novo* acute myeloid leukemia were identified from the pathology archives of Brigham and Women's Hospital and Massachusetts General Hospital between 2009 and 2013. All cases had bone marrow aspirate smear and

biopsy slides available for review that were diagnosed as acute myeloid leukemia prior to any therapy being administered. Only cases with adequate karyotype, available *NPM1* and *FLT3* mutation information, and clinical follow-up information were included. Patients who had received any prior cytotoxic therapy, had a prior diagnosis of any myeloid neoplasm, or had karyotype abnormalities of acute myeloid leukemia with myelodysplasia-related changes or acute myeloid leukemia with recurrent genetic abnormalities⁶ were excluded. Only cases with biopsies containing at least 5 mm in length of hematopoietic marrow and with aspirates containing at least 1000 intact hematopoietic cells were included.

Morphology Assessment

Slides from each case (consisting of both bone marrow biopsy and aspirate smears) were viewed in a blinded manner by three hematopathologists (OKW, RPH, and OP) who scored dysplasia in each lineage in increments of 10%. A minimum of 10 megakaryocytes (on biopsies and/or aspirate smears) and 20 erythroids and 20 myeloid elements (in aspirate smears) were required, otherwise a lineage was designated as 'not evaluable'. Specific dysplastic features in each lineage were also scored on a semi-quantitative scale ($< 10\%$ cells showing the feature = 0, 10–25% = 1, 26–50% = 2, 51–75% = 3, and $> 75\%$ = 4). Specific dysplastic features scored in the erythroid lineage included megaloblastoid change, multinucleation, nuclear irregularities, and pyknosis. Dysgranulopoiesis features scored included abnormal nuclear shape (including pseudo Pelger–Huet anomaly with unilobate or bilobed nuclei, as well as nuclear hypersegmentation with six or more lobes) and hypogranulation. Dysmegakaryopoiesis features scored included micromegakaryocytes (mononucleated forms with a diameter of 7–10 μm), forms with two or multiple separated, rounded nuclear lobes, and forms with hypolobated or monolobated nuclei but normal size. Criteria for each dysplastic feature were those described for myelodysplastic syndrome in a recent study.¹⁹ The diagnostic pathology reports were reviewed to determine the frequency of an acute myeloid leukemia with myelodysplasia-related changes diagnosis based on the original case review.

According to the 2008 WHO classification criteria, the acute myeloid leukemia with myelodysplasia-related changes group was defined as cases with an average dysplasia percentage score among the three observers of $\geq 50\%$ in at least two hematopoietic lineages. Cases with at least 2 lineages showing $< 50\%$ dysplastic cells formed the acute myeloid leukemia—not otherwise specified group; for the purposes of this study, cases with *NPM1* mutation (a provisional acute myeloid leukemia with recurrent genetic abnormalities entity in the 2008 WHO Classification) were included within the acute

myeloid leukemia—not otherwise specified group. Cases in which determination of acute myeloid leukemia with myelodysplasia-related changes or acute myeloid leukemia—not otherwise specified could not be made, comprised the acute myeloid leukemia—not evaluable group. In addition to cases lacking two or three evaluable lineages, cases were assigned to this group if one lineage was not evaluable and another had $\geq 50\%$ dysplastic cells.

Clinical Data

The complete blood count and WBC differential results at the time of acute myeloid leukemia diagnosis were recorded. Type of treatment, including date of any allogeneic stem-cell transplant, date of relapse, or disease refractoriness to two induction regimens, and status at last follow-up were recorded for each patient. Complete remission and overall survival were determined as defined by clinical standards.²¹ Event-free survival was defined as the time from diagnosis to either relapse or death for any reason, whichever occurred first. Patients who were alive and relapse free were censored at the time that they were last known to be relapse free.

Statistical Analysis

Fisher's exact test and Kruskal–Wallis test were used to compare categorical and continuous variables; for multiple comparisons between acute myeloid leukemia groups, one-way ANOVA and Fisher's exact test with Bonferroni's adjustment were used. Event-free survival from diagnosis was estimated using the method of Kaplan and Meier. Multivariable subsequent to univariate Cox proportional hazards regression models were used to assess the impact of the patient groups along with other risk factors on event-free survival. For multivariable analyses including both patients with and without stem-cell transplant, stem-cell transplant was included as a time-dependent variable. A P -value < 0.05 was considered statistically significant. The Kendall's coefficient of concordance W with a correction for ties was used to compare the three observers' scores of dysplasia percentages, acute myeloid leukemia group categorization, and individual dysplastic feature scores. To assess the optimal cutoff points for dysplasia in each lineage and specific morphologic features, the method of recursive partitioning was used, using the criteria of $P < 0.01$ and at least 10 patients in each partition group to be considered statistically significant.

Results

Diagnosis of Acute Myeloid Leukemia with Myelodysplasia-Related Changes

The total number of acute myeloid leukemia cases that met study criteria and were evaluated by the observers was 159. By averaging the dysplasia

Table 1 Types of dysplastic changes seen in acute myeloid leukemia with myelodysplasia-related changes groups and inter-rater agreement of specific dysplastic feature scoring

<i>Dysplastic finding (average of the 3 observers)</i>	<i>Acute myeloid leukemia—not otherwise specified (N = 89)</i>	<i>Acute myeloid leukemia with myelodysplasia- related changes (N = 43)</i>	<i>Acute myeloid leukemia—not evaluable (N = 27)</i>	<i>Kendall's coefficient of concordance W</i>
<i>Overall % dysplastic erythroids, median (range)</i>	13.3 (0–76.7)	66.7 (6.7–90)	16.7 (10–40)	—
Megaloblastoid changes, <i>n</i> (%) with average score $\geq 1/\geq 3$	24 (27%)/1 (1%)	29 (67%)/4 (9%)	2 (7%)/0 (0%)	0.689
Multinucleation, <i>n</i> (%) with average score $\geq 1/\geq 3$	13 (15%)/0	27 (63%)/4 (9%)	1 (4%)/0	0.716
Nuclear irregularities, <i>n</i> (%) with average score $\geq 1/\geq 3$	49 (55%)/1 (1%)	38 (88%)/18 (42%)	4 (15%)/0	0.803
Pyknosis, <i>n</i> (%) with average score $\geq 1/\geq 3$	3 (3%)/2 (2%)	3 (7%)/0	0/0	0.762
Erythroids not evaluable, <i>n</i> (%)	6 (7%)	3 (7%)	22 (81%)	—
<i>Overall % dysplastic myeloids, median (range)</i>	6.7 (0–83.3)	65 (3.3–93.3)	6.7 (0–73.3)	—
Abnormal nuclear shape, <i>n</i> (%) with average score $\geq 1/\geq 3$	17 (19%)/1 (1%)	33 (77%)/8 (19%)	2 (7%)/0	0.738
Hypogranular cytoplasm, <i>n</i> (%) with average score $\geq 1/\geq 3$	34 (38%)/2 (2%)	34 (79%)/17 (40%)	4 (15%)/2 (7%)	0.805
Myeloids not evaluable, <i>n</i> (%)	6 (7%)	3 (7%)	16 (59%)	—
<i>Overall % dysplastic megakaryocytes, median (range)</i>	16.7 (0–86.7)	76.7 (0–100)	66.7 (0–93.3)	—
Micromegakaryocytes, <i>n</i> (%) with average score $\geq 1/\geq 3$	16 (18%)/0	37 (86%)/11 (26%)	8 (30%)/2 (7%)	0.773
Separated nuclear lobes, <i>n</i> (%) with average score $\geq 1/\geq 3$	24 (27%)/2 (2%)	31 (72%)/11 (26%)	7 (26%)/0	0.836
Hypolobated nuclei, <i>n</i> (%) with average score $\geq 1/\geq 3$	47 (53%)/2 (2%)	35 (81%)/7 (16%)	6 (22%)/0	0.619
Megakaryocytes not evaluable, <i>n</i> (%)	9 (10%)	2 (5%)	17 (63%)	—

percentages from all 3 observers, the median erythroid dysplasia percentage was 20% (range 0–90%), the median myeloid dysplasia score was 10% (range 0–93%), and the median megakaryocyte dysplasia score was 37% (range 0–100%). Erythroid, myeloid, and megakaryocytic lineages were not evaluable due to insufficient cells in 31 (19%), 25 (16%), and 28 (18%) cases, respectively. The Kendall's coefficient of concordance *W* among the three observers' dysplasia percentage scores were 0.906, 0.855, and 0.911 for erythroid, myeloid, and megakaryocyte lineages, respectively.

Following the 2008 WHO Classification criteria using the average dysplasia percentages for each lineage, 89 were acute myeloid leukemia—not otherwise specified (56%), 43 acute myeloid leukemia with myelodysplasia-related changes (27%), and 27 acute myeloid leukemia—not evaluable (17%). Kendall's coefficient of concordance *W* among pathologists with respect to acute myeloid leukemia—not otherwise specified, acute myeloid leukemia with myelodysplasia-related changes, and acute myeloid leukemia—not evaluable assignment was 0.939. Only 10 cases were diagnosed as acute myeloid leukemia with myelodysplasia-related changes in the original pathology reports, representing 5/43 (12%) acute myeloid leukemia with myelodysplasia-related changes and 5/89 (6%) acute myeloid leukemia—not otherwise specified cases as characterized in this study. The dysplasia scoring data for the acute myeloid leukemia—not otherwise

specified, acute myeloid leukemia with myelodysplasia-related changes, and acute myeloid leukemia—not evaluable groups are shown in Table 1. The most frequent dysplastic changes observed in acute myeloid leukemia with myelodysplasia-related changes were nuclear irregularities in the erythroid lineage, hypogranulation in the myeloid lineage, and micromegakaryocytes and separated nuclear lobes in the megakaryocyte lineage. Examples of the typical dysplastic changes observed in bone marrow aspirates and biopsies are shown in Figure 1.

Patient Characteristics

Comparison of clinical and genetic features between acute myeloid leukemia—not otherwise specified, acute myeloid leukemia with myelodysplasia-related changes, and AML—not evaluable groups is shown in Table 2. Acute myeloid leukemia—not evaluable presented with higher WBC, peripheral blood, and bone marrow blast percentage, and higher rate of *FLT3*-ITD mutation compared with acute myeloid leukemia—not otherwise specified (Table 2). Acute myeloid leukemia with myelodysplasia-related changes had lower hemoglobin level compared with acute myeloid leukemia—not otherwise specified. Abnormal karyotypes were seen in 28/159 cases (18%). The most common abnormalities were +8 (eight cases), +11 (four cases), and +13 (four cases); other trisomies were seen in three cases,

Figure 1 Examples of typical morphologic dysplastic features found in acute myeloid leukemia with myelodysplasia-related changes. (a) Erythroid cells frequently show irregular nuclear contours (Wright–Giemsa, $\times 100$). (b) Myeloid cells show hypogranular cytoplasm as well as nuclear hypoblobation, including pseudo Pelger–Huet forms (Wright–Giemsa, $\times 100$). (c) Megakaryocytes in the bone marrow biopsy often include forms with hypoblobated nuclei and smaller micromegakaryocytes (H&E, $\times 50$). (d) A subset of cases had megakaryocytes with widely separated, rounded nuclear lobes (Wright–Giemsa, $\times 100$). H&E, hematoxylin and eosin.

chromosome losses in two cases, ring chromosome in one case, and balanced or unbalanced translocations in five cases. There were no significant differences in the blast immunophenotypes (expression of CD34, CD13, CD33, CD117, CD64, CD7, CD11b, or CD19) between the three groups (data not shown).

Clinical Outcome Analysis

For analysis of clinical outcome, only the 137 patients (73 acute myeloid leukemia—not otherwise specified, 41 acute myeloid leukemia with myelodysplasia-related changes, and 23 acute myeloid leukemia—not evaluable) who received induction chemotherapy were analyzed. With 75 events (35 in acute myeloid leukemia—not otherwise specified, 27 in acute myeloid leukemia with myelodysplasia-related changes, and 13 in acute myeloid leukemia—not evaluable) observed among those patients, on univariate analysis, acute myeloid

leukemia with myelodysplasia-related changes patients had inferior event-free survival compared with acute myeloid leukemia—not otherwise specified patients ($P=0.038$). There was no significant difference in event-free survival or overall survival between acute myeloid leukemia—not evaluable and acute myeloid leukemia—not otherwise specified patients (Figures 2a and b). There was no significant association of any of the three groups with achievement of complete remission ($P=0.18$). Of the 137 patients, 61 underwent stem-cell transplant in first complete remission, and this group had significantly longer event-free survival compared with the patients who did not receive stem-cell transplant in first complete remission ($n=76$, $P<0.0001$). The associations of variables with event-free survival in all 137 patients treated with induction are shown in Table 3. Stepwise multivariable analysis (with stem-cell transplant considered as a time-dependent variable) showed that abnormal karyotype, age, white blood count, and stem-cell transplant, but

Table 2 Comparison of clinical and genetic features of the WHO acute myeloid leukemia with myelodysplasia-related changes groups

	Acute myeloid leukemia—not otherwise specified (n = 89)	Acute myeloid leukemia with myelodysplasia-related changes (n = 43)	Acute myeloid leukemia—not evaluable (n = 27)
Age at diagnosis, median (range)	59 (19–87)	55 (20–82)	62 (20–89)
Bone marrow blast %, median (range)	60 (20–95)	54 (21–92)	84.5 (25–94) ^a
Peripheral blood blast %, median (range)	15 (0–98)	19.5 (0–91)	49.5 (0–98) ^a
White blood count (×10 ⁹ /l), median (range)	6.1 (0.5–214)	14.5 (0.8–228)	33 (1.2–300) ^b
Hemoglobin (g/dl), median (range)	9.9 (3.8–15)	8.6 (4–12.8) ^b	8.8 (5–13)
Platelet count (×10 ⁹ /l), median (range)	107 (2–248)	73 (16–305)	60 (12–187) ^b
<i>NPM1</i> mutated, N (%)	30 (34%)	18 (42%)	14 (52%)
<i>FLT3</i> -ITD mutated, N (%)	16 (18%)	10 (23%)	13 (48%) ^b
<i>NPM1</i> mutated/ <i>FLT3</i> wild-type, N (%)	22 (25%)	14 (33%)	5 (19%)
Abnormal karyotype, N (%)	19 (21%)	6 (14%)	3 (11%)

^aStatistically different from both acute myeloid leukemia with myelodysplasia-related changes and acute myeloid leukemia—not otherwise specified at $\alpha = 0.05$. ^bStatistically different from acute myeloid leukemia—not otherwise specified at $\alpha = 0.05$.

not the WHO-defined acute myeloid leukemia with myelodysplasia-related changes grouping, were independently associated with event-free survival (Table 4).

To determine if the WHO-defined grouping had prognostic significance in the patients treated or not treated with stem-cell transplant, we performed univariate analyses in patients who did or did not receive stem-cell transplant in first complete remission. For patients who did not receive stem-cell transplant in first complete remission, acute myeloid leukemia—not evaluable had significantly inferior event-free survival compared with acute myeloid leukemia—not otherwise specified ($P = 0.005$), while event-free survival of acute myeloid leukemia with myelodysplasia-related changes patients was not significantly different from acute myeloid leukemia—not otherwise specified ($P = 0.1$; Figure 2c). For patients who underwent stem-cell transplant in first complete remission, there was no significant difference in event-free survival between acute myeloid leukemia with myelodysplasia-related changes and acute myeloid leukemia—not otherwise specified ($P = 0.98$) or between acute myeloid leukemia—not evaluable and acute myeloid leukemia—not otherwise specified ($P = 0.21$; Figure 2d). On stepwise multivariable Cox regression analysis of patients, who did not undergo stem-cell transplant in first complete remission, age > 64 ($P = 0.01$) and *FLT3*-ITD mutation ($P = 0.0002$) were significantly associated with shorter event-free survival when compared with patients ≤ 64 and patients without *FLT3*-ITD mutation, but not acute myeloid leukemia with myelodysplasia-related changes ($P = 0.11$) or acute myeloid leukemia—not evaluable ($P = 0.09$) when compared with acute myeloid leukemia—not otherwise specified patients. For patients who underwent stem-cell transplant in first complete remission, stepwise multivariable analysis showed that only abnormal karyotype ($P = 0.014$) was significantly associated with shorter event-free survival compared with those with normal karyotype.

Identification of Specific Morphologic Features Associated with Event-Free Survival

Since we did not find a significant independent effect of acute myeloid leukemia with myelodysplasia-related changes status on event-free survival, we examined dysplasia in each lineage separately. We also used recursive partitioning to determine if dysplasia cutoffs other than 50% (in 10% increments) were superior with respect to influence on event-free survival in the 137 patients treated with induction therapy. Using the 50% WHO cutoff, erythroid dysplasia was not significantly associated with event-free survival, nor was there any superior cutoff that met the recursive partitioning criteria. Using the 50% WHO cutoff, myeloid dysplasia was not significantly associated with event-free survival ($P = 0.20$); a cutoff of $> 60\%$ was optimal, but was only borderline associated with inferior event-free survival ($P = 0.052$). Using the 50% WHO cutoff, megakaryocytic dysplasia was borderline associated with event-free survival ($P = 0.10$) and there was no superior cutoff that met recursive partitioning criteria.

We then examined each specific dysplastic feature in univariate analysis for event-free survival. Among all the dysplastic features evaluated (listed in Table 1), only micromegakaryocytes (average score ≥ 3 , corresponding to $> 50\%$ micromegakaryocytes, $P = 0.004$) and hypogranulated myeloid cells (average score ≥ 3 , corresponding to $> 50\%$ hypogranulated myeloids, $P = 0.008$) were associated with adverse event-free survival. Using the presence of $> 50\%$ micromegakaryocytes or hypogranulated myeloid cells to define a new group (acute myeloid leukemia micromegakaryocytes/hypogranular), there were 28 acute myeloid leukemia micromegakaryocytes/hypogranular cases, 31 cases in which micromegakaryocytes/hypogranular status was unassessable due to insufficient megakaryocytes and/or myeloids, and 78 cases with both $\leq 50\%$ micromegakaryocytes and hypogranular myeloids. Acute myeloid leukemia micromegakaryocytes/hypogranular patients had a significantly shorter event-free survival compared with both patients with

Figure 2 Kaplan–Meier analysis of outcome of acute myeloid leukemia patients treated with induction chemotherapy according to acute myeloid leukemia with myelodysplasia-related changes status. **(a)** Among all patients, event-free survival is shorter in acute myeloid leukemia with myelodysplasia-related changes patients (AML-MRC, $n=41$) than acute myeloid leukemia—not otherwise specified patients (AML—NOS, $n=73$, $P=0.039$), while there is no significant difference in event-free survival between acute myeloid leukemia—not evaluable (AML—NE, $n=23$) and acute myeloid leukemia—not otherwise specified patients ($P=0.29$). **(b)** Among all patients, there is no significant difference in overall survival between acute myeloid leukemia—not otherwise specified, acute myeloid leukemia with myelodysplasia-related changes, and acute myeloid leukemia—not evaluable patients. **(c)** Among the 76 patients not receiving allogeneic stem-cell transplant in first remission, event-free survival is shorter in acute myeloid leukemia—not evaluable patients ($n=11$) than acute myeloid leukemia—not otherwise specified patients ($n=36$, $P=0.004$), while there is no significant difference in event-free survival between acute myeloid leukemia with myelodysplasia-related changes ($n=29$) and acute myeloid leukemia—not otherwise specified patients ($P=0.11$). **(d)** Among the 61 patients receiving stem-cell transplant in first remission, there is no significant difference in event-free survival between acute myeloid leukemia—not otherwise specified, acute myeloid leukemia with myelodysplasia-related changes, and acute myeloid leukemia—not evaluable patients.

$\leq 50\%$ micromegakaryocytes/hypogranular myeloids ($P=0.001$) and the combined group of patients with $\leq 50\%$ micromegakaryocytes/hypogranular and unassessable micromegakaryocyte/hypogranular status ($P=0.002$; Figure 3a), and also shorter overall survival compared with patients with $\leq 50\%$ micromegakaryocytes/hypogranular myeloids ($P=0.006$; Figure 3b). There was no significant association of acute myeloid leukemia micromegakaryocytes/hypogranular status and achievement of complete remission ($P=0.21$). Results of stepwise multivariable Cox regression using acute myeloid leukemia micromegakaryocytes/hypogranular status as a variable are shown in Table 5. After adjusting for other covariates, acute myeloid leukemia micromegakaryocytes/hypogranular status

was associated with inferior event-free survival (HR 2.6 (1.5–4.6), $P=0.0008$), while the non-assessable group was not significantly associated with event-free survival (HR 1.7 (0.9–3.1), $P=0.11$) compared with the patients with $\leq 50\%$ micromegakaryocytes/hypogranular myeloids.

Discussion

Previous studies of acute myeloid leukemia have found conflicting results regarding the significance of morphologic dysplasia in acute myeloid leukemia.^{8–18} Early studies found that trilineage dysplasia correlated with worse overall survival

Table 3 Results of the univariate Cox proportional hazard regression for patients who received induction chemotherapy (N=137)

Factor	N	Hazard ratio (95% CI)	P-value
<i>WHO acute myeloid leukemia classification</i>			0.108
Acute myeloid leukemia—not otherwise specified	73	Reference	—
Acute myeloid leukemia with myelodysplasia-related changes	41	1.709 (1.032–2.83)	0.038
Acute myeloid leukemia—not evaluable	23	1.406 (0.743–2.66)	0.295
<i>Gender</i>			0.6823
Male	69	Reference	—
Female	68	0.909 (0.577–1.433)	—
<i>NPM1 mutation</i>			0.4954
Absent	83	Reference	—
Present	54	0.848 (0.529–1.361)	—
<i>FLT3-ITD mutation</i>			0.2198
Absent	102	Reference	—
Present	35	1.372 (0.828–2.275)	—
<i>NPM1 and FLT3-ITD status</i>			0.7577
other <i>NPM1/FLT3</i> combinations	100	Reference	—
<i>NPM1</i> mutation present, <i>FLT3</i> -ITD absent	37	0.923 (0.553–1.54)	—
<i>Karyotype status</i>			0.0912
Normal	115	Reference	—
Abnormal	22	1.633 (0.924–2.884)	—
<i>Acute myeloid leukemia—FAB subtype</i>			0.8992
M0	12	Reference	—
M1	30	0.665 (0.278–1.592)	—
M2	22	0.995 (0.41–2.412)	—
M4/M5/M5a	62	0.929 (0.429–2.011)	—
M6	1	—	—
Unknown/missing	10	—	—
Age at diagnosis		1.022 (1.003–1.041)	0.0236
White blood count		1.004 (1–1.007)	0.0403
Peripheral blood blasts		1.002 (0.995–1.009)	0.5377
Hemoglobin		0.964 (0.865–1.075)	0.5121
Platelet count		1.003 (0.999–1.006)	0.1366
Average % erythroid dysplasia score among 3 pathologists (0–100%)		0.997 (0.987–1.007)	0.6063
<i>Average % erythroid dysplasia score among 3 pathologists (< 50% vs ≥ 50%)</i>			0.5554
< 50%	80	Reference	—
≥ 50%	29	0.838 (0.465–1.509)	—
Not assessable/missing	28	—	—
Average % myeloid dysplasia score among 3 pathologists (0–100%)		1.006 (0.998–1.015)	0.1229
<i>Average % myeloid dysplasia score among 3 pathologists (< 50% vs ≥ 50%)</i>			0.1972
< 50%	82	Reference	—
≥ 50%	35	1.41 (0.836–2.378)	—
Not assessable/missing	20	—	—
<i>Average % myeloid dysplasia score among 3 pathologists (< 60% vs ≥ 60%)</i>			0.0515
< 60%	90	Reference	—
≥ 60%	27	1.741 (0.996–3.043)	—
Not assessable/missing	20	—	—
Average % megakaryocyte dysplasia score among 3 pathologists (0–100%)		1.007 (0.999–1.015)	0.0927
<i>Average % megakaryocyte dysplasia score among 3 pathologists (< 50% vs ≥ 50%)</i>			0.1035
< 50%	65	Reference	—
≥ 50%	49	1.524 (0.918–2.533)	—
Not assessable/missing	23	—	—

and event-free survival in acute myeloid leukemia,^{11–14} and provided the basis for creating the entity of acute myeloid leukemia with multilineage dysplasia in the 2001 WHO Classification.^{19,22} However, later studies reported conflicting results,^{8–15} although some of these studies were not limited to *de novo* acute myeloid leukemia and most did not include examination of bone marrow biopsies, which has been shown to facilitate the identification of dysplastic changes among erythroid cells and megakaryocytes in acute myeloid leukemia with myelodysplasia-related changes.²³ Most recently,

Rozman *et al*¹⁸ suggested that a broader definition of dysplasia with a lower threshold than the WHO criteria may be more clinically meaningful. Our retrospective study of 159 patients with *de novo* acute myeloid leukemia lacking specific cytogenetic abnormalities shows that the identification of morphologic dysplasia in acute myeloid leukemia is reproducible, with high concordance among the three hematopathologists.

Unlike prior studies, we graded the percentage of dysplastic elements from 0 to 100% and also evaluated the frequency of specific types of dysplasia

Table 4 Final model after multivariable Cox regression analysis for event-free survival of all acute myeloid leukemia patients treated with induction ($n = 137$)

	P-value	HR	95% CI
Treatment with stem-cell transplantation (time varying)	0.0028	0.442	0.259–0.755
Abnormal karyotype	0.0408	1.83	1.026–3.264
Age (continuous)	0.0054	1.027	1.008–1.047
White blood count (continuous)	0.0047	1.005	1.002–1.009

Figure 3 Kaplan–Meier analysis of outcome of 137 acute myeloid leukemia patients treated with induction chemotherapy according to presence of micromegakaryocytes and/or hypogranulation. (a) Event-free survival is shorter in acute myeloid leukemia patients with $>50\%$ micromegakaryocytes and/or hypogranular myeloids (AML-MH, $n = 28$) than in acute myeloid leukemia patients with $\leq 50\%$ micromegakaryocytes and hypogranular myeloids (AML-N, $n = 78$, $P = 0.003$), while there is no significant difference in event-free survival between patients with acute myeloid leukemia with $\leq 50\%$ micromegakaryocytes/hypogranular and those with unassessable micromegakaryocytes/hypogranulation (AML-UA, $n = 31$, $P = 0.21$). (b) Overall survival is shorter in patients with acute myeloid leukemia with $>50\%$ micromegakaryocytes and/or hypogranular myeloids than in those with $\leq 50\%$ micromegakaryocytes/hypogranular ($P = 0.006$), while there is no significant difference in overall survival between patients with $\leq 50\%$ micromegakaryocytes/hypogranular and those with unassessable micromegakaryocytes/hypogranulation ($P = 0.27$).

to examine the prognostic relevance of individual morphologic dysplastic findings. We found that acute myeloid leukemia with myelodysplasia-related changes, as defined by 2008 WHO Classification, was present in 27% of *de novo* acute myeloid leukemia cases lacking specific cytogenetic abnormalities, a rate similar to prior studies.^{9,18} We found that the spectrum of dysplastic changes seen in acute myeloid leukemia with myelodysplasia-related changes is similar to that observed in myelodysplastic syndrome. Despite a high concordance between the hematopathologists in scoring acute myeloid leukemia with myelodysplasia-related changes in this retrospective review, the number of acute myeloid leukemia cases designated as acute myeloid leukemia with myelodysplasia-related changes in diagnostic pathology reports was significantly lower (only 6% as compared with 27% identified by the blinded retrospective review). This finding suggests that the WHO diagnosis of acute myeloid leukemia with myelodysplasia-related changes based on morphologic dysplasia is not being

uniformly applied in clinical practice, even in two large academic medical centers during a time range of 2009–2013 (after publication of the 2008 WHO Classification that mandated the application of these criteria).

Our study also showed that a high frequency of acute myeloid leukemia cases (17% in our cohort) did not have sufficient maturing hematopoietic elements present for evaluation of dysplasia, similar to findings reported by Rozman *et al.*¹⁸ It is unclear in the current WHO Classification whether these cases should be designated as acute myeloid leukemia with myelodysplasia-related changes or as acute myeloid leukemia—not otherwise specified. This non-evaluable group has significantly higher white blood count, peripheral blood and bone marrow blasts, *FLT3*-ITD mutation incidence, and shorter event-free survival (in patients who did not undergo stem-cell transplant in first complete remission) compared with acute myeloid leukemia—not otherwise specified, consistent with a more aggressive form of acute myeloid leukemia. However, on

Table 5 Final model after multivariable Cox regression analysis for event-free survival of all acute myeloid leukemia patients treated with induction (*n* = 137)

	P-value	HR	95% CI
>50% micromegakaryocytes and/or >50% hypogranulated myeloids ^a (<i>N</i> = 28)	0.0008	2.631	1.496–4.627
Unassessable with respect to micromegakaryocytes and hypogranulated myeloids ^a (<i>N</i> = 31)	0.1102	1.663	0.891–3.104
Treatment with stem-cell transplantation (time varying)	0.0083	0.477	0.275–0.826
Abnormal karyotype	0.0224	1.982	1.102–3.564
Age (continuous)	0.0251	1.022	1.003–1.042
White blood count (continuous)	0.0017	1.006	1.002–1.010
Platelet count (continuous)	0.0238	1.004	1.001–1.008

^aCompared to reference group with ≤50% micromegakaryocytes and ≤50% hypogranulated myeloids.

multivariable analysis, acute myeloid leukemia—not evaluable was not significantly associated with shorter event-free survival, suggesting that the poorer outcome of this group in the univariate analysis reflects its association with higher age, white blood count, and *FLT3*-ITD mutation. Acute myeloid leukemia with *FLT3*-ITD mutation has higher bone marrow blast counts compared with acute myeloid leukemia lacking *FLT3*-ITD,²⁴ resulting in smaller numbers of maturing elements that are required to distinguish acute myeloid leukemia with myelodysplasia-related changes from acute myeloid leukemia—not otherwise specified.

We found that the WHO-defined acute myeloid leukemia with myelodysplasia-related changes designation had no significant bearing on event-free survival or overall survival on multivariable analysis. Even when considering acute myeloid leukemia with myelodysplasia-related changes and acute myeloid leukemia—not evaluable together as one group, there was no association with event-free survival in multivariable analysis that included *FLT3*-ITD status and age (data not shown). We examined the dysplasia percentage in each lineage independently and explored cutoffs for dysplasia other than the WHO-defined threshold of 50%, but did not find cutoffs that were significantly superior to the WHO acute myeloid leukemia with myelodysplasia-related changes threshold; in particular, erythroid dysplasia appeared to have no significant association with event-free survival. We also found that the vast majority of individual morphologic dysplastic features in each hematopoietic lineage had no association with event-free survival. However, the presence of >50% micromegakaryocytes and >50% hypogranulated myeloid cells were both associated with adverse event-free survival, an effect that was independent of *FLT3* and *NPM1* mutation status. Although this retrospective study included a substantial proportion of patients treated with stem-cell transplant, we found that the impact of these dysplastic features was also independent of stem-cell transplant. In a study by Verburgh *et al*²⁰ on myelodysplastic syndrome without excess blasts, micromegakaryocytes were also found to have a particularly strong impact

on prognosis compared with other features of dysmegakaryopoiesis. In an early small study comparing *de novo* acute myeloid leukemia with trilineage dysplasia to acute myeloid leukemia arising from myelodysplastic syndrome, the rate of micromegakaryocytes was higher in acute myeloid leukemia arising from myelodysplastic syndrome.²⁵

Our data suggest that only certain specific dysplastic features (micromegakaryocytes and hypogranulated neutrophils) may be prognostically relevant in *de novo* acute myeloid leukemia with intermediate-risk cytogenetics and that a more restrictive definition of dysplastic changes defining acute myeloid leukemia with myelodysplasia-related changes may be warranted. Such a more limited and simplified definition of acute myeloid leukemia with myelodysplasia-related changes may increase its applicability, as we found that the WHO Classification diagnosis of acute myeloid leukemia with myelodysplasia-related changes based on morphology alone does not appear to be consistently applied in practice. To our knowledge, this is the first study to explore the optimal cutoff of dysplastic changes in each lineage and to examine the effect of specific dysplastic morphologies on the outcome of *de novo* acute myeloid leukemia. The results of our study support those of Rozman *et al*,¹⁹ and suggest that morphologic dysplasia does have clinical significance and should continue to be identified in pathology reports. Our study included patients with *NPM1* mutation and had a higher proportion of patients who underwent stem-cell transplant, which may explain our more restricted definition of morphologic dysplasia (micromegakaryocytes and hypogranulated myeloid cells) compared with the more broad definition encompassing all three lineages suggested in the study by Rozman *et al*.¹⁹ Our study also incorporated findings in bone marrow trephine biopsies, which are important in evaluating megakaryocytic dysplasia.²⁰

Mutational profiling has an increasing role in the risk stratification of acute myeloid leukemia, and specific mutations such as *ASXL1* have been associated with morphologic dysplasia in acute myeloid leukemia.²⁶ Comprehensive mutational analysis may

define a biologically relevant group of myelodysplastic syndrome-related acute myeloid leukemia that is independent of morphologic dysplasia or myelodysplastic syndrome history.²⁷ Our study did not examine the effect of additional mutations other than *FLT3* and *NPM1*, and thus we could not determine if our morphologic findings were independent of other additional mutations known to have prognostic significance in acute myeloid leukemia. However, next-generation sequencing is not yet widely available in all centers, and optimal risk stratification for all myeloid diseases ideally incorporates a combination of clinical features, karyotype, molecular features, as well as morphology. In the context of this multimodality approach, we propose that the finding of frequent micromegakaryocytes or hypogranular myeloid cells can define adverse-risk morphology in *de novo* acute myeloid leukemia that lacks specific cytogenetic abnormalities.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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