Comparison of genetic and clinical aspects in patients with acute myeloid leukemia and myelodysplastic syndromes all with more than 50% of bone marrow erythropoietic cells

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ABSTRACT

Background
The World Health Organization separates acute erythroid leukemia (erythropoiesis in ≥50% of nucleated bone marrow cells; ≥20% myeloblasts of non-erythroid cells) from other entities with increased erythropoiesis – acute myeloid leukemia with myelodysplasia-related changes (≥20% myeloblasts of all nucleated cells) or myelodysplastic syndromes – and subdivides acute erythroid leukemia into erythroleukemia and pure erythroid leukemia subtypes. We aimed to investigate the biological/genetic justification for the different categories of myeloid malignancies with increased erythropoiesis (≥50% of bone marrow cells).

Design and Methods
We investigated 212 patients (aged 18.5-88.4 years) with acute myeloid leukemia or myelodysplastic syndromes characterized by 50% or more erythropoiesis: 108 had acute myeloid leukemia (77 with acute erythroid leukemia, corresponding to *erythroid/myeloid erythroleukemia*, 7 with pure erythroid leukemia, 24 with acute myeloid leukemia with myelodysplasia-related changes) and 104 had myelodysplastic syndromes. Morphological and chromosome banding analyses were performed in all cases; subsets of cases were analyzed by polymerase chain reaction and immunophenotyping.

Results
Unfavorable karyotypes were more frequent in patients with acute myeloid leukemia than in those with myelodysplastic syndromes (42.6% *versus* 13.5%; $P<0.0001$), but their frequency did not differ significantly between patients with acute erythroid leukemia (39.0%), pure erythroid leukemia (57.1%), and acute myeloid leukemia with myelodysplasia-related changes (50.0%). The incidence of molecular mutations did not differ significantly between the different categories. The 2-year overall survival rate was better for patients with myelodysplastic syndromes than for those with acute myeloid leukemia ($P<0.0001$), without significant differences across the different acute leukemia subtypes. The 2-year overall survival rate was worse in patients with unfavorable karyotypes than in those with intermediate risk karyotypes ($P<0.0001$). In multivariate analysis, only myelodysplastic syndromes *versus* acute myeloid leukemia ($P=0.021$) and cytogenetic risk category ($P=0.002$) had statistically significant effects on overall survival.

Conclusions
The separation of acute myeloid leukemia and myelodysplastic syndromes with 50% or more erythropoietic cells has clinical relevance, but it might be worth discussing whether to replace the subclassifications of different subtypes of acute erythroid leukemia and acute myeloid leukemia with myelodysplasia-related changes by the single entity, *acute myeloid leukemia with increased erythropoiesis ≥50%*.

Key words: acute myeloid leukemia, acute erythroid leukemia, cytogenetics, myelodysplastic syndrome, prognosis.

Citation: Bacher U, Haferlach C, Alpermann T, Kern W, Schnittger S, and Haferlach T. Comparison of genetic and clinical aspects in patients with acute myeloid leukemia and myelodysplastic syndromes all with more than 50% of bone marrow erythropoietic cells. Haematologica 2011;96(9):1284-1292. doi:10.3324/haematol.2011.043687

Manuscript received on March 7, 2011. Revised version arrived on May 9, 2011. Manuscript accepted May 10, 2011.

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Introduction

Acute erythroid leukemia (AEL), which represents only 2-5% of all cases of adult acute myeloid leukemia (AML), can be separated according to the World Health Organization (WHO) classification[1] into two subtypes: *erythroleukemia, erythroid/myeloid* leukemia is characterized by 50% or more of nucleated bone marrow cells and at least 20% myeloblasts of all non-erythroid cells in the bone marrow. *Pure erythroid leukemia* (pEL) is restricted to cases with 80% or more of erythropoiesis without relevant myeloblast counts. Both subtypes belong to the WHO category "*AML, not otherwise specified*" (AML-NOS).[1] Cytogenetic alterations were described in 50-80% of cases of AEL, with high rates of prognostically adverse alterations, i.e. of chromosome 5 or 7 or complex karyotypes.[2] Outcomes of AEL were described to be poor, but allogeneic hematopoietic stem cell transplantation seemed to improve survival.[3]

AEL must be separated by cytomorphological criteria from other malignant subtypes of hematologic disorders with increased erythropoiesis (≥50%) in the bone marrow: cases with at least 50% of erythropoietic cells and at least 20% of myeloblasts of all nucleated cells and myelodysplasia-related changes due to multilineage dysplasia (two or three hematopoietic lineages), typical cytogenetic alterations, or preceding myelodysplastic syndrome (MDS) or myeloproliferative neoplasm are classified as AML with myelodysplasia-related changes (AML-MRC).[4] In contrast, cases with at least 50% of erythropoiesis but less than 20% of myeloblasts related to non-erythroid cells do not fulfill the criteria for AEL but are most consistent with a classification of MDS when the WHO criteria are applied (Table 1).[5] Recently, Hasserjian *et al.* suggested that AEL is part of the continuum of MDS and AML with erythroid hyperplasia, in which karyotype rather than an arbitrary blast cut-off is prognostically relevant,[2] and it has been discussed whether other parameters, such as cytogenetics or the degree of dysplasia might provide a better basis than percentages of erythroblasts and myeloblasts for therapeutic decisions in these patients.[6] Furthermore, AEL shows similarities to AML-MRC[4] with regards to the high frequency of a preceding MDS, presence of multilineage dysplasia, and types of cytogenetic alterations.[2]

The distinction of AEL from MDS or AML-MRC with at least 50% of erythropoietic cells is, therefore, still under discussion. With the aim of clarifying the biological and genetic justification of the different WHO categories, we studied the morphological characteristics, cytogenetic and molecular genetic profiles, and clinical outcomes of 212 patients with AML or MDS in whom at least 50% of all nucleated cells in the bone marrow were erythroid cells.

Design and Methods

Patients

The study cohort consisted of 212 consecutive patients with at least 50% of erythropoietic cells in the bone marrow and a diagnosis of AML or MDS according to the 2008 WHO criteria.[5] There were 73 females and 139 males with a median age of 68.8 years (range, 18.5-88.4 years). In order to be included in the retrospective analysis karyotype and bone marrow cytomorphology had to be available in parallel. Patients in the WHO category "*AML with recurrent genetic abnormalities*" or suffering from other hematologic entities (e.g. myeloproliferative neoplasms) were excluded from the study. Cases fulfilling the criteria of increased erythropoiesis (≥50% of all nucleated cells) and therapy-related AML or therapy-related MDS were included in the analysis but were not considered as a separate category solely based on the history of therapy-related disease in order to make comparison possible with other studies on the subject.[2] Bone marrow samples were sent between August 2005 and March 2010 to the MLL Munich Leukemia Laboratory. Patients gave informed consent to laboratory analysis of their samples and to the use of their data for research purposes. The study was approved by the Bavarian Medical Association (*Bayerische Landesärztekammer*) and performed in accordance with the Declaration of Helsinki. Samples were investigated by a comprehensive diagnostic work-up including cytomorphology, immunophenotyping by multiparameter flow cytometry, cytogenetics, and molecular genetics. Clinical follow-up data were available for 167 patients.

Table 1. Categorization of the different AML and MDS entities with ≥50% of bone marrow erythropoietic cells according to the 2008 WHO classification. (*for separation from AEL, patients with ≥50% of bone marrow erythropoietic cells and ≤19% of myeloblasts in relation to non-erythroid cells in the bone marrow are best compatible with a diagnosis of MDS; **according to the 2001 WHO classification).

Entity	Morphological correlate	Erythropoiesis (Bone marrow)	Myeloblasts (Bone marrow)	Additional characteristics
Erythroleukemia (erythroid/ myeloid)	FAB "M6a" **	Erythroid precursors ≥50% of all nucleated cells	≥20% of non-erythroid cells, but <20% of nucleated cells	-
Pure erythroid leukemia (AML-NOS)	"M6b" **	Immature erythroid precursors (pro-erythroblasts) ≥80% of all nucleated cells	No significant increase	-
AML-MRC (with ≥50% of bone marrow erythropoietic cells)	-	Erythroid precursors ≥50% of all nucleated cells	≥20% of all nucleated cells	Multilineage dysplasia /MDS-related cytogenetics/ history of MDS
MDS (with ≥50% of bone marrow erythropoietic cells)*	-	Erythroid precursors ≥50% of all nucleated cells	≤19% of non-erythroid cells	RA, RCMD, RARS, MDS-U, t-MDS, RAEB-1/2

AML-NOS: AML, not otherwise specified; RA: refractory anemia; RCMD: refractory cytopenia with multilineage dysplasia; RARS: refractory anemia with ring sideroblasts; RAEB: refractory anemia with excess blasts; t-MDS: therapy-related MDS (may also be categorized separately according to the 2008 WHO classification); MDS-U: unclassifiable MDS.

Cytomorphology

May-Grünwald-Giemsa staining of bone marrow smears, combined with myeloperoxidase, non-specific esterase, and – in the case of MDS – iron staining, was performed on samples from all patients.[7] Cytomorphological evaluation and classification of cases based on WHO criteria was done by one researcher (UB) and independently validated by another (TH). Cases fulfilling the category "*erythroleukemia, erythroid/myeloid*" were called "*acute erythroid leukemia, AEL*" in this study. Dysplasia was assessed following the definitions of Goasguen *et al.*[8] and the WHO 2008 classification.[4] Five hundred bone marrow cells were evaluated for each case.

Cytogenetics

Chromosome banding analysis combined with fluorescence *in situ* hybridization was performed in all 212 patients.[9] All patients were categorized into groups with "favorable", "intermediate", and "unfavorable" cytogenetics according to the revised Medical Research Council[10] criteria for AML. In accordance with the WHO classification, the following cytogenetic abnormalities assigned patients to the category "*AML with an MDS-related cytogenetic abnormality*": complex karyotype, -7/del(7q), -5/del(5q), i(17q)/t(17p), -13/del(13q), del(11q), del(12p)/t(12p), del(9q), idic(X)(q13), and different reciprocal rearrangements, e.g. t(3;21)(q26.2;q22.1).

Molecular mutation analysis and immunophenotyping

Mutation analysis, using previously described methods, was performed for *NPM1* mutations (116 cases investigated),[11] *FLT3*-ITD (n=123),[12] *FLT3*-TKD (n=69),[13] *MLL*-PTD (n=124),[14] and *NRAS* mutations (n=82).[15] Immunophenotyping by multiparameter flow cytometry[16] was performed in 124 cases.

Statistical analysis

Mean differences were analyzed using the t-test. A χ^2 or Fisher's exact test was applied in the case of contingency tables. Overall survival was defined as the time from diagnosis to death or last follow-up. The probabilities of overall survival were estimated using the Kaplan-Meier method. The log-rank test was used to compare risk factor categories in survival analysis. Cox proportional hazard regression models were applied investigate the risk factors affecting time to events. All tests were two-sided, accepting *P* values of 0.05 or below as indicating a statistically significant difference. Statistical analyses were performed using SPSS software version 19.0.0 (Chicago, IL, USA).

Results

Cytomorphological classification

Based on cytomorphological evaluation, the so-defined "AML cohort" consisted of 108 patients with different subtypes of AML (AEL, pEL, and AML-MRC): 77 patients had AEL (corresponding to the WHO category "*erythroid/myeloid erythroleukemia*"); 7 patients were classified as having pEL,[1] and in 24 patients myeloblasts accounted for at least 20% of all nucleated cells and these patients, therefore, fulfilled the criteria of AML-MRC either due to multilineage dysplasia (n=18), MDS-related cytogenetics (n=5) or a history of MDS (n=1).[4] The other 104 patients (the "MDS cohort") had different subtypes of MDS: according to the 2008 WHO classification 17 had refractory anemia, 18 had refractory anemia with ring sideroblasts, 46 had refractory cytopenia with multilineage dysplasia, 21 had refractory anemia with excess of blasts (RAEB)-1 and two had RAEB-2 (based on the presence of ≥10% of bone marrow blasts). Thus, only 23/104 patients (22.1%) had advanced MDS, i.e. RAEB-1/-2 (Table 2). Applying the 2008 WHO criteria,[4] MDS-related cytogenetic alterations were found in 16 MDS patients (15.4% of the MDS cohort).

Clinical characteristics

We first compared the clinical parameters of the AML and MDS cohorts. The male-to-female ratio (AML: 2.0; MDS: 1.8) and median age (AML: 68.8 years; MDS: 68.0 years) were similar. Median white blood cell count, platelet count, and hemoglobin concentration were significantly lower in the AML cohort (P=0.022, P<0.0001, and P=0.001, respectively). When the different AML subgroups were compared, the male-to-female ratio was higher in patients with pEL than in those with AEL or AML-MRC (6.0 *versus* 2.0 *versus* 1.7, respectively; P=n.s.). Neither median age nor peripheral blood counts showed significant differences across the AML subgroups (exact values and ranges are shown in Table 2).

History of disease

Considering the total cohort, 171 patients (80.7%) had *de novo* disease, 15 (7.1%) had secondary disease, and 26 (12.3%) had therapy-related disease. In more detail, in the AML cohort, 78/108 (72.2%) had *de novo* disease, 15 (13.9%) had secondary disease, and 15 had a history of previous chemotherapy or radiotherapy (13.9%). In the MDS cohort, 93/104 (89.4%) had *de novo* disease, and 11 had therapy-related MDS (10.6%). Thus, not surprisingly, *de novo* disease was significantly more frequent in the MDS cohort (P=0.002; Table 2).

Cytogenetics

Aberrant karyotypes were detected in 97 (45.8%) of the whole cohort of 212 patients. Aberrant karyotypes were more frequent in the AML cohort than in the MDS one (63/108; 58.3%; *versus* 34/104; 32.7%, respectively; P=0.0002). In contrast, no significant differences were detected across the different AML subgroups regarding the distribution of aberrant karyotypes: AEL: 42/77, 54.5%; pEL: 5/7, 71.4%; AML-MRC: 16/24; 66.7%. Unfavorable karyotypes (according to MRC criteria)[10] were detected in 60/212 patients (28.3%). Unfavorable karyotypes were more frequent in AML than in MDS (46/108, 42.6% *versus* 14/104, 13.5%; P<0.0001), but were similarly distributed in the various AML cohorts (AEL: 30/77, 39.0%; pEL: 4/7, 57.1%; AML-MRC: 12/24, 50.0%; P=n.s.) (Tables 2 and 3, Figure 1).

Molecular analysis

NPM1 mutations were more frequent in the AML cohort than in the MDS one (19/82, 23.2% *versus* 3/34, 8.8%) but the difference was not statistically significant. *FLT3*-ITD was detected in 4/83 (4.8%) investigated cases of AML but in none of 40 investigated cases of MDS (P=n.s.). *FLT3*-TKD (AML: 2/47, 4.3%; MDS: 1/22, 4.5%), *NRAS* mutations (AML: 2/43, 4.7%; MDS: 1/39, 2.6%), and *MLL*-PTD (AML: 10/83, 12.0%; MDS: 1/41, 2.4%) occurred at similar low frequencies in AML and MDS. The mutations did not differ significantly across the AEL, pEL, and AML-MRC subgroups. *FLT3*-ITD was seen in 2/59 (3.4%) AEL, in 2/18 (11.1%) AML-MRC and in 0/6 pEL. *NPM1* mutations were detected in 15/57 (26.3%) AEL, in

1/6 (16.7%) pEL, and in 3/19 (15.8%) AML-MRC (Table 2, Figure 1).

Multiparameter flow cytometry

The comparison of immunophenotypes between the groups revealed a lower expression of CD11b in patients with AML-MRC (n=10) than in patients with AEL/pEL (n=49) (mean expression, 18% *versus* 31%, *P*=0.016) and a higher expression of CD34 in the patients with AML-MRC (45% *versus* 14%, *P*=0.014). No significant differences were found for the other antigens analyzed.

Survival outcomes

Survival according to morphological subtypes

The 2-year overall survival rate in the total cohort was 54.8%. The 2-year overall survival rate was significantly better in the MDS patients than in the AML cohort (73.3% *versus* 37.4%; *P*<0.0001) (Figure 2A) but did not differ significantly between patients with advanced MDS (RAEB-1/2) and those with other MDS subtypes without a blast increase (59.9% *versus* 68.9%; Figure 2B). Furthermore, the 2-year overall survival rates were not significantly different between the AEL, pEL, and AML-MRC subgroups (38.5% *versus* 30.0% *versus* 33.4%, respectively; Figure 2C). The median overall survival was 14.5 months for patients with AEL, 8.8 months for those with pEL, and 9.3 months for patients with AML-MRC (Table 4).

Survival according to cytogenetics

The 2-year overall survival rate was lower in patients with aberrant karyotypes than in those with a normal karyotype (32.8% *versus* 73.6%; *P*<0.0001). When cytoge-

Table 2. Clinical, cytogenetic, and molecular genetic characteristics of the 212 patients with different AML or MDS entities and ≥50% of bone marrow erythropoietic cells. Ranges are given in brackets. Cytogenetic risk stratification was done according to the revised MRC[10] criteria (*comparison of MDS and AML cohorts, **comparison of AEL *versus* pEL *versus* AML-MRC and of AEL/pEL *versus* AML-MRC).

Parameter	Total cohort	MDS total	AML total	P*	AEL	pEL	AML-MRC	P**
Numbers of patients	212	104	108	-	77	7	24	-
Clinical characteristics								
males: females (ratio)	139:73 (1.9)	67:37 (1.8)	72:36 (2.0)	n.s.	51:26 (2.0)	6:1 (6.0)	15:9 (1.7)	n.s.
median age (years)	68.8 (18.5–88.4)	68.0 (18.5-88.4)	68.8 (20.0–84.9)	n.s.	68.8 (25.7–84.9)	69.8 (49.1–71.5)	68.4 (20.0–82.5)	n.s.
median WBC (×10⁹/L)	3.2 (7.0–27.0)	3.9 (0.7–27.0)	2.8 (0.9–17.8)	0.022	3.0 (0.9–17.8)	3.8 (1.2–4.4)	2.1 (0.9–16.6)	n.s.
median platelets (×10⁹/L)	80 (6 -527)	80 (6-527)	60 (8-330)	<0.0001	66 (11-330)	43 (18-74)	47 (8-237)	n.s.
median Hb (g/dL)	9.1 (5.0-15.0)	9.7 (6.0-15.0)	8.9 (5.0-15.0)	0.001	8.8 (5.0-15.0)	8.3 (6.0-9.0)	9.1 (5.0-13.0)	n.s.
Clinical outcomes								
median 2-year OS rate	54.8%	73.3%	37.4%	<0.0001	38.5%	30.0%	33.4%	n.s.
History of disease								
de novo	171 (80.7%)	93 (89.4%)	78 (72.2%)	0.002	56 (72.7%)	3 (42.9%)	19 (79.2%)	
secondary	15 (7.1%)	-	15 (13.9%)	(de novo *vs.*	11 (14.3%)	2 (28.6%)	2 (8.3%)	n.s.
therapy-related	26 (12.3%)	11 (10.6%)	15 (13.9%)	others)	10 (13.0%)	2 (28.6%)	3 (12.5%)	
Cytogenetics (n=212)								
normal karyotypes	115 (54.2%)	70 (67.3%)	45 (41.7%)	0.0002	35 (45.5%)	2 (28.6%)	8 (33.3%)	n.s.
aberrant karyotypes	97 (45.8%)	34 (32.7%)	63 (58.3%)		42 (54.5%)	5 (71.4%)	16 (66.7%)	
intermediate karyotypes	152 (71.7%)	90 (86.5%)	62 (57.4%)	<0.0001	47 (61.0%)	3 (42.9%)	12 (50.0%)	n.s.
unfavorable karyotypes	60 (28.3%)	14 (13.5%)	46 (42.6%)		30 (39.0%)	4 (57.1%)	12 (50.0%)	
Molecular mutations								
NPM1 mutated	22/116 (19.0%)	3/34 (8.8%)	19/82 (23.2%)	n.s.	15/57 (26.3%)	1/6 (16.7%)	3/19 (15.8%)	n.s.
FLT3-ITD positive	4/123 (3.3%)	0/40 (0.0%)	4/83 (4.8%)	n.s.	2/59 (3.4%)	0/6 (0.0%)	2/18 (11.1%)	n.s.
FLT3-TKD positive	3/69 (4.3%)	1/22 (4.5%)	2/47 (4.3%)	n.s.	1/34 (2.9%)	0/3 (0.0%)	1/10 (10.0%)	n.s.
NRAS mutated	3/82 (3.7%)	1/39 (2.6%)	2/43 (4.3%)	n.s.	1/30 (3.3%)	0/3 (0.0%)	1/10 (10.0%)	n.s.
MLL-PTD positive	11/124 (8.9%)	1/41 (2.4%)	10/83 (12.0%)	n.s.	7/59 (11.9%)	0/6 (0.0%)	3/18 (16.7%)	n.s.

n: number; WBC: white blood cell count; OS: overall survival; Hb: hemoglobin; n.s.: not significant.

Table 3. Cytogenetic risk stratification according to revised MRC[10] criteria in the total cohort. Patients with recurrent cytogenetic abnormalities (WHO, 2008) were excluded from the analysis.

Revised MRC criteria	Cytogenetic subcategories	Number of patients (%) Total	MDS total	AML total	AEL	pEL	AML-MRC
Intermediate risk (n=52)	normal karyotype	115 (54.2%)	70 (67.3%)	45 (41.7%)	35 (45.5%)	2 (28.6%)	8 (33.3%)
	+8	10 (4.7%)	5 (4.8%)	5 (4.6%)	3 (3.9%)	1 (14.3%)	1 (4.2%)
	-Y sole	8 (3.8%)	8 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	del(20q)	4 (1.9%)	3 (2.9%)	1 (0.9%)	1 (1.3%)	0 (0.0%)	0 (0.0%)
	other non-complex	15 (7.1%)	4 (3.8%)	11 (10.2%)	8 (10.4%)	0 (0.0%)	3 (12.5%)
Unfavorable risk (n=60)	complex alterations	50 (23.6%)	12 (11.5%)	38 (35.2%)	24 (31.2%)	4 (57.1%)	10 (41.7%)
	-7/add(7q)	8 (3.8%)	1 (1.0%)	7 (6.5%)	5 (6.5%)	0 (0.0%)	2 (8.3%)
	del(5q)/-5/add(5q)	2 (0.9%)	1 (1.0%)	1 (0.9%)	1 (1.3%)	0 (0.0%)	0 (0.0%)
Total		**212 (100.0%)**	**104 (100.0%)**	**108 (100.0%)**	**77 (100.0%)**	**7 (100.0%)**	**24 (100.0%)**

netic risk was categorized according to the revised MRC criteria[10] for the whole cohort (n=212), unfavorable karyotypes were associated with an inferior 2-year overall survival compared to intermediate karyotypes (median overall survival 7.6 months *versus* 70.4% at 2 years; $P<0.0001$; Figure 3A). The same held true when the MDS and AML cohorts were considered separately: patients with unfavorable karyotypes had lower 2-year overall survival rates than patients with intermediate karyotypes (MDS: median 8.4 months *versus* 80.9% at 2 years; $P<0.0001$; Figure 3B; AML: median 6.2 months *versus* 56.3%; $P<0.0001$; Figure 3C; Table 4). Interestingly, patients with AML and intermediate cytogenetics had a significantly worse median 2-year overall survival rates than patients with MDS and intermediate cytogenetics (56.3% *versus* 77.0%; $P=0.007$; Figure 4A). In contrast, median overall survival did not differ significantly between AML and MDS patients with unfavorable karyotypes (6.2 and 8.4 months, respectively) (Figure 4B).

Survival according to molecular markers

Neither *NPM1* nor *MLL*-PTD mutation status had a significant impact on prognosis: 2-year overall survival rates in patients with *NPM1*mut and *NPM1*wt were 63.6% and

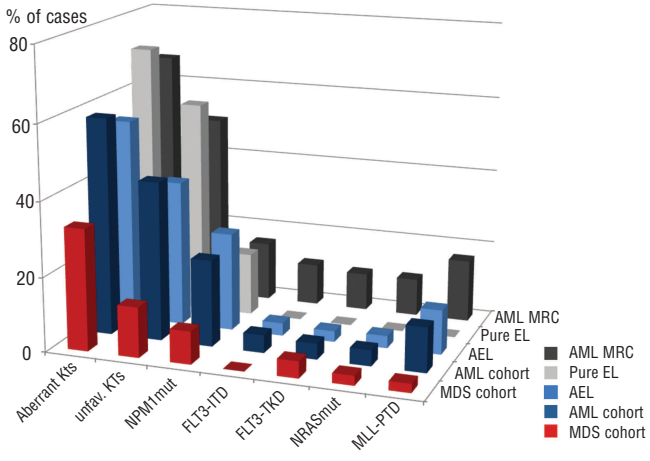

Figure 1. Frequencies of aberrant and unfavorable karyotypes and the different molecular mutations in the MDS and AML cohorts and in the different AML subgroups (AEL, pEL, and AML-MRC) with ≥50% of bone marrow erythropoietic cells.

Table 4. Median 2-year overall survival (OS) rates and median OS in months in the patients with AML and MDS with ≥50% of bone marrow erythropoietic cells (*comparing median OS in the AML and MDS cohorts; **revised MRC[10] criteria).

Parameter	N. of patients	Median 2-year OS rate (%)	Median OS (months)	*P*
Hematologic entities				
total cohort	212	54.8	47.7	
MDS total	104	73.3	n.r.	
early MDS	81	68.9	n.r.	n.s.
MDS-RAEB	23	59.9	n.r.	
AML total	108	37.4	13.3	<0.0001*
AEL	77	38.5	14.5	n.s.
pEL	7	30.0	8.8	n.s.
AML-MRC	24	33.4	9.3	n.s.
Cytogenetics (n=212)				
normal karyotypes	115	73.6	n.r.	<0.0001
aberrant karyotypes	97	32.8	13.3	
intermediate karyotypes**	152	70.4	n.r.	<0.0001
unfavorable karyotypes**	60	not reached	7.6	
Molecular mutations				
NPM1 mutations (n=116)				
NPM1 mutated	22	63.6	n.r.	n.s.
NPM1 wildtype	94	52.5	25.1	n.s.
MLL-PTD (n=124)				
MLL-PTD positive	11	35.7	19.5	n.s.
MLL-PTD negative	113	53.1	n.r.	n.s.
History of disease (n=212)				
de novo	171	60.2	47.7	0.010
secondary	15	29.2	8.8	0.010
therapy-related	26	40.6	21.7	n.s.

n: number; n.r.: not reached; n.s.: not significant; RAEB: refractory anemia with excess blasts.

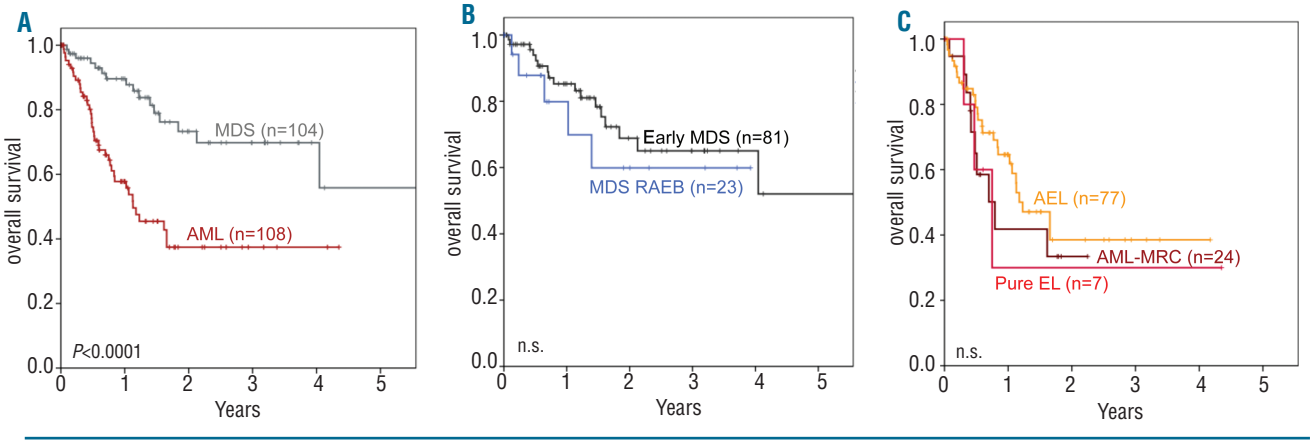

Figure 2. Overall survival in the different hematologic entities with ≥50% of bone marrow erythropoietic cells comparing (A) the MDS (n=104) *versus* AML (n=108) cohort ($P<0.0001$), (B) early MDS subtypes (n=95) *versus* MDS-RAEB (n=23) (n.s.) and (C) the different AML subgroups (AEL, n=77; pure EL, n=7; AML-MRC, n=24; n.s.).

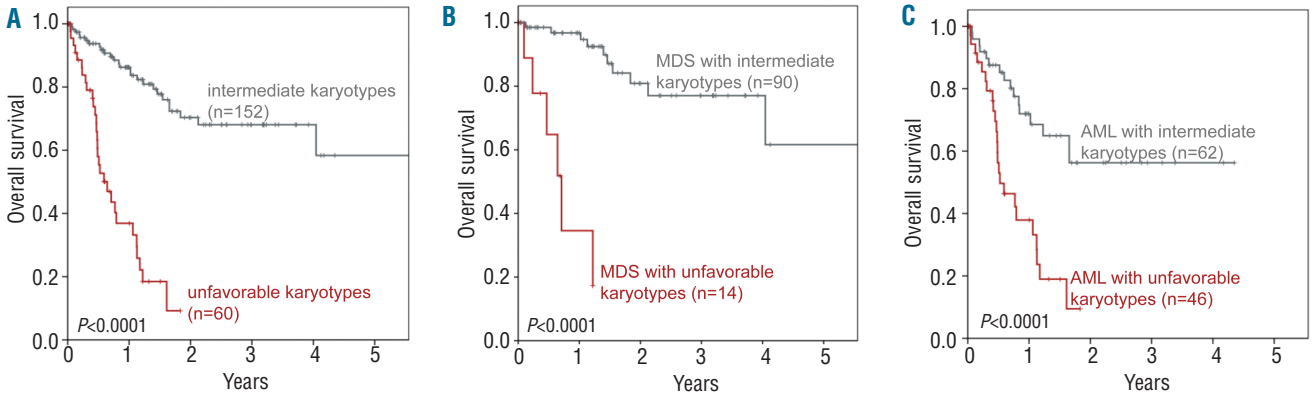

Figure 3. Overall survival depending on the cytogenetic risk group (MRC revised criteria). Unfavorable karyotypes were associated with significantly inferior overall survival compared with intermediate karyotypes. (A) in all patients (n=212; *P*<0.0001), (B) in the MDS cohort (n=104; *P*<0.0001), and (C) in the AML cohort (n=108; *P*<0.0001).

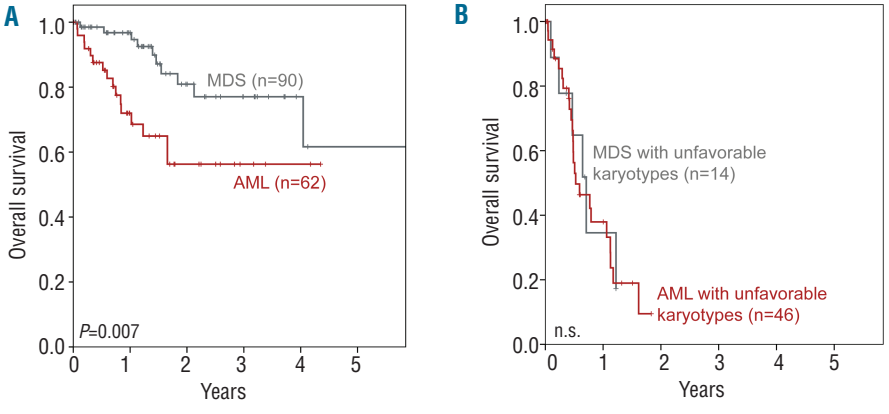

Figure 4. Overall survival depending on the cytogenetic risk group (MRC revised criteria). (A) When patients with intermediate risk (n=152) were considered, MDS patients (n=90) had better outcomes than those with AML (n=62; *P*=0.007); (B) in the unfavorable cytogenetic risk group (n=60), outcomes of patients with MDS (n=14) and AML (n=46) did not differ significantly.

52.5%, respectively, while those in *MLL*-PTD positive and negative patients were 35.7% and 53.1%, respectively (Table 4).

Survival according to history of disease

In the total cohort, the 2-year overall survival rate was significantly better in patients with *de novo* disease than in those with secondary disease (60.2% *versus* 29.2%; *P*=0.010; Figure 5A), but no significant difference was found when compared to therapy-related disease (40.6%; *P*=n.s.) or between patients with secondary and therapy-related disease (*P*=n.s.). Neither in the MDS (Figure 5B) nor in the AML cohort (Figure 5C) did the history of disease have a significant impact on 2-year overall survival rates (Table 4).

Univariate and multivariate analyses

When we analyzed various clinical, cytogenetic, and molecular genetic parameters with regards to their ability to predict overall survival, only age (*P*=0.002), platelet count (*P*=0.009), classification as MDS *versus* AML (*P*<0.0001), cytogenetic risk category (*P*<0.0001), and the history of the disease (*P*=0.039) were associated with a significantly worse outcome. The white blood cell count, hemoglobin concentration, *FLT3*-ITD, *NPM1*, *FLT3*-TKD, *MLL*-PTD, and *NRAS* mutations did not have a significant impact on overall survival. In multivariate analysis, classification as MDS *versus* AML (*P*=0.021) and cytogenetic risk category (*P*=0.002) were the only parameters independently related to worse outcomes (Table 5).

Discussion

Hematologists encounter many difficulties when trying to assign patients with AML and MDS and at least 50% of erythropoietic cells in the bone marrow to the correct WHO category, and the separation of cases into the AEL and AML-MRC categories, for example, in patients with complex karyotypes, leaves room for discussion. In this study we investigated 212 patients with different subtypes of AML and MDS, but all with 50% or more of erythropoietic cells in the bone marrow, who were classified based on the 2006 WHO criteria[5] with some modifications: patients with therapy-related AML or MDS were not considered in a separate category, but included in the cohort if they fulfilled the criterion of at least 50% of erythropoietic cells. This was done in order to facilitate comparisons with previous studies on myeloid malignancies with increased erythropoiesis (e.g. the study by Hasserjian *et al.*[2]), although therapy-related disorders might alterna-

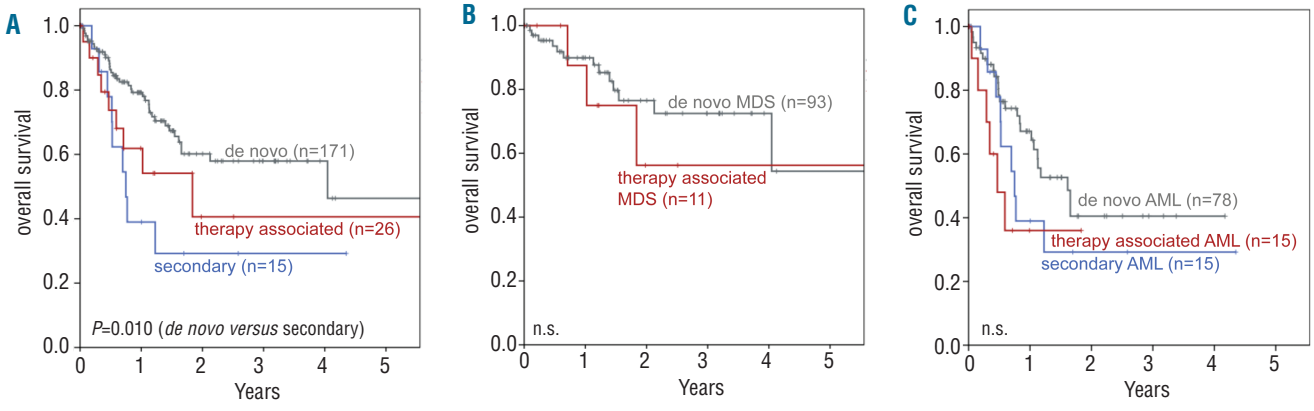

Figure 5. Overall survival depending on the history of disease, (A) in all patients (n=212). Patients with *de novo* disease had significantly better outcomes than patients with secondary disease (*P*=0.010), (B) in the MDS cohort (n=104; *P*=n.s.), and (C) in the AML cohort (n=108; n.s.).

tively be considered as a separate category. For better correlation we also, like Hasserjian *et al.*,[2] categorized cases fulfilling the morphological criteria of AEL accordingly, irrespective of cytogenetics, but admit that a classification of cytogenetically unfavorable cases as AML-MRC was possible, strictly following the WHO criteria.[4] In our study, the WHO category "*erythroleukemia, erythroid/myeloid*" was termed "*acute erythroid leukemia, AEL*".

The median overall survival in our AEL cohort was 14.5 months, which was better than that in the studies by Hasserjian *et al.*[2] and Santos *et al.*[17] who reported median overall survivals of 8-9 months. Only Kasyan *et al.* described a more favorable median overall survival of 19 months in patients with AEL.[18] The lower rate of unfavorable karyotypes (according to the revised MRC criteria)[10] in our AEL patients (39% compared to around 60% in the cohorts of Hasserjian *et al.*[2] and Santos *et al.*[17]) might, in part, explain this difference. Second, more than 70% of our patients with AEL had *de novo* disease, whereas the rates of *de novo* AEL were much lower in the studies by Hasserjian *et al.*[2] and Santos *et al.*,[17] ranging from 31-25%. These differences in the frequencies of *de novo* disease and cytogenetic risk profiles might be due to the fact that patients in our analysis were not part of a defined clinical study but were unselected and sent from different hematology centers for diagnostic purposes. Indeed, Domingo-Claros *et al.* documented that only 11% of a cohort of patients with different acute erythroid malignancies had secondary disease,[19] and Wells *et al.* reported secondary disease in 12% of AML FAB M6 patients in a population-based study,[20] suggesting that *de novo* AEL might not be an infrequent phenomenon.

Characterizing the molecular marker profile in AEL, we found a strikingly low rate of *FLT3*-ITD (3.4%) which was considerably less than in the overall cohort of patients with AML.[12,21] Similar, Hasserjian *et al.* reported a 6% *FLT3*-ITD mutation rate in AEL,[2] and Thiede *et al.* found 4.0% of *FLT3*-ITD mutated cases in FAB M6 patients within a large study focusing on *NPM1* mutations in AML.[22] *NPM1* mutations were also less frequent in our AEL cohort (26.3%) than in the overall AML cohort.[22,23] Thiede *et al.* described only 4.0% of *NPM1* mutated cases in AML FAB M6 patients.[22] In a previous analysis including 401 patients with normal karyotype AML, we found a 12% *NPM1* mutation rate in a subgroup of 25 FAB M6 patients.[24] In contrast, the frequency of *MLL*-PTD in our AEL cohort (11.9%) was similar to that described among AML cases as a whole.[25,26]

In a next step, we compared the AEL subgroup with other AML subtypes and at least 50% of erythropoietic cells in the bone marrow, i.e. pEL and AML-MRC. These three subgroups had overlapping poor median 2-year overall survival rates, a high frequency of unfavorable karyotypes, low *NPM1* mutation and *FLT3*-ITD rates, and similar patterns of expression of the majority of antigens determined by immunophenotyping. In contrast, the AML cohort differed significantly from the MDS cohort with regards to lower peripheral blood counts, worse median 2-year overall survival (*P*<0.0001) and higher frequencies of unfavorable karyotypes (*P*<0.0001).

Table 5. Analysis of prognostic parameters regarding overall survival in univariate and multivariate analyses in the total cohort of 212 patients with ≥50% of bone marrow erythropoietic cells (*revised MRC criteria).

Parameter	*P*	
	Univariate analysis	Multivariate analysis
Age	0.002	n.s.
Sex	n.s.	-
White blood cell count	n.s.	-
Platelet count	0.009	n.s.
Hemoglobin concentration	n.s.	-
MDS *versus* AML	<0.0001	0.021
Cytogenetic risk group*	<0.0001	0.002
Secondary *versus de novo*	0.039	n.s.
FLT3-ITD	n.s.	-
NPM1 mutations	n.s.	-
FLT3-TKD	n.s.	-
MLL-PTD	n.s.	-
NRAS mutations	n.s.	-

n.s.: not significant.

In multivariate analysis, only the distinction of MDS *versus* AML (P=0.021) and the cytogenetic risk category (P=0.002) retained prognostic significance. The cytogenetic risk group was prognostically relevant also when AML and MDS were investigated separately. As in our study, Hasserjian *et al.* found that cytogenetic risk group was strongly prognostic when they performed histopathological and cytomorphological evaluation of 124 cases of AEL and compared them with cases of MDS or AML-MRC all characterized by at least 50% of erythropoiesis.[2] Nevertheless, it should be noted that the WHO guidelines alternatively allow cases fulfilling the morphological criteria of AEL but with unfavorable cytogenetics to be classified as AML-MRC,[4] which would result in a decreased proportion of cases with unfavorable karyotypes within the remaining AEL cases. In contrast to Hasserjian *et al.*[2] who found prognosis to be independent of blast percentage in the specific setting of erythroid hyperplasia, we found that the survival of our patients with different categories of AML was significantly worse than that of the patients with MDS and at least 50% of erythropoietic bone marrow cells. Based on a previous characterization of the MDS cohort studied by Hasserjian *et al.* (published by Wang *et al.*), aberrant karyotypes were more frequent in the Hasserjian and Wang MDS cohorts, and advanced MDS-RAEB was slightly more frequent when compared to our study composition.[2,27] This might explain why the AML and MDS patients in the cohort described by Hasserjian *et al.* had comparable clinical profiles.

In conclusion, based on our data the specific description of cases as AML or MDS with 50% or more of erythroid precursors according to 2008 WHO classification seems to have clinical relevance, as patients with AML had worse outcomes, which was in part explained by different cytogenetic risk profiles. However, the suggested separation of AML into the different subtypes, erythroleukemia (erythroid/myeloid), pEL, and AML-MRC with 50% or more erythropoiesis, seems arbitrary. These AML subtypes show no significant differences regarding clinical characteristics, cytogenetic or molecular genetic risk profiles, or survival outcomes. Given the difficulties which arise in research studies and in daily practice in correctly applying the WHO criteria to cases with these specific cytomorphological characteristics, an easier to use definition for such patients would be helpful. It could, therefore, be interesting to investigate whether a combined group of AML with "*increase of erythropoiesis ≥50% in the bone marrow*" might facilitate definitions for clinical studies and the development of therapeutic strategies for these patients. Of note, cytogenetics is the most important prognostic parameter for patients with myeloid malignancies and increased erythropoiesis and it is essential to carry out cytogenetic studies at diagnosis in order to guide clinical decisions.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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