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ORIGINAL REPORT

Impact of Pretransplantation Minimal Residual Disease, As Detected by Multiparametric Flow Cytometry, on Outcome of Myeloablative Hematopoietic Cell Transplantation for Acute Myeloid Leukemia

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ABSTRACT

Purpose
Allogeneic hematopoietic cell transplantation (HCT) benefits many patients with acute myeloid leukemia (AML) in first remission. Hitherto, little attention has been given to the prognostic impact of pretransplantation minimal residual disease (MRD).

Patients and Methods
We retrospectively studied 99 consecutive patients receiving myeloablative HCT for AML in first morphologic remission. Ten-color multiparametric flow cytometry (MFC) was performed on bone marrow aspirates before HCT. MRD was identified as a cell population showing deviation from normal antigen expression patterns compared with normal or regenerating marrow. Any level of residual disease was considered MRD positive.

Results
Before HCT, 88 patients met morphologic criteria for complete remission (CR), whereas 11 had CR with incomplete blood count recovery (CRi). Twenty-four had MRD before HCT as determined by MFC. Two-year estimates of overall survival were 30.2% (range, 13.1% to 49.3%) and 76.6% (range, 64.4% to 85.1%) for MRD-positive and MRD-negative patients; 2-year estimates of relapse were 64.9% (range, 42.0% to 80.6%) and 17.6% (range, 9.5% to 27.9%). After adjustment for all or a subset of cytogenetic risk, secondary disease, incomplete blood count recovery, and abnormal karyotype pre-HCT, MRD-positive HCT was associated with increased overall mortality (hazard ratio [HR], 4.05; 95% CI, 1.90 to 8.62; $P < .001$) and relapse (HR, 8.49; 95% CI, 3.67 to 19.65; $P < .001$) relative to MRD-negative HCT.

Conclusion
These data suggest that pre-HCT MRD is associated with increased risk of relapse and death after myeloablative HCT for AML in first morphologic CR, even after controlling for other risk factors.

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INTRODUCTION

The optimal treatment for acute myeloid leukemia (AML) in first remission remains unclear. Recent meta-analyses suggest a benefit of allogeneic hematopoietic cell transplantation (HCT) for patients with poor- or intermediate-risk disease,[1-3] although methodologic issues have clouded some of these analyses.[4] The decision to undergo transplantation rests on the relative risks of relapse and nonrelapse mortality (NRM) compared with those of chemotherapy and depends on the recognition of factors that predict HCT outcome, such as cytogenetics, age, HLA disparities, and comorbidities.[5,6] So far, the prognostic impact of minimal residual disease (MRD) detection by multiparametric flow cytometry (MFC) at the time of HCT is unknown. By using technology adaptable by most clinical laboratories, MFC enables the detection of small numbers of occult AML cells that persist during therapy.[7-9] It is conceivable that these minute populations of persistent AML cells increase the likelihood of adverse outcome, particularly disease recurrence, after HCT. However, it is unclear what role pretransplantation MRD plays, if any, on outcome of AML after allogeneic HCT. Herein, we retrospectively address this question in the patients with AML in first remission undergoing myeloablative HCT between May 2006 and September 2009 at our center.

PATIENTS AND METHODS

Study Cohort

Patients of all ages, identified from our computerized database, were included in this study if they had AML in first remission or if they met criteria for first remission except that they had MRD at the time of HCT, underwent myeloablative conditioning, had either a matched sibling or unrelated donor, and received the first transplantation. We used the 2008 WHO criteria to define AML and Southwest Oncology Group/Eastern Cooperative Oncology Group (SWOG/ECOG) criteria to assign cytogenetic risk.[10,11] Cytogenetic analysis was performed with the G-banding method. Treatment response criteria were used as proposed by an International Working Group.[12] The HCT-specific comorbidity index (HCT-CI) and the pretransplantation assessment of mortality (PAM) score were calculated as described previously.[13-15] All patients were treated on institutional review board–approved protocols and gave consent in accordance with the Declaration of Helsinki. Follow-up was current as of June 11, 2010.

MFC Detection of MRD

Ten-color MFC was performed on bone marrow aspirates as previously described.[16,17] The panel consisted of three tubes as follows: (1) HLA-DR-Pacific Blue (PB), CD15-fluorescein isothiocyanate (FITC), CD33-Phycoerythrin (PE), CD19-PE-Texas Red (PE-TR), CD117-PE-Cy5, CD13-PE-Cy7, CD38-Alexa 594 (A594), CD34-allophycocyanin (APC), CD71-APC-A700 and CD45-APC-H7; (2) HLA-DR-PB, CD64-FITC, CD123-PE, CD4-PE-TR, CD14-PE-Cy5.5, CD13-PE-Cy7, CD38-A594, CD34-APC, CD16-APC-A700 and CD45-APC-H7; and (3) CD56-Alexa 488, CD7-PE, CD5-PE-Cy5, CD33-PE-Cy7, CD38-A594, CD34-APC and CD45-APC-H7. All antibodies were obtained from Beckman-Coulter (Fullerton, CA) or Becton Dickinson (BD Biosciences, San Jose, CA). Up to 1 million events per tube were acquired on a custom-built LSR II flow cytometer (BD Biosciences), and data compensation and analysis were performed by using noncommercial software developed in our laboratory. MRD was identified as a cell population showing deviation from the normal patterns of antigen expression seen on specific cell lineages at specific stages of maturation compared with either normal or regenerating marrow.[18] When identified, the abnormal population was quantified as a percentage of the total $CD45^+$ white blood cell events. Any level of residual disease was considered MRD positive: MRD levels were less than 0.01% in two patients, between 0.01% to 0.1% in eight patients, and greater than 0.1% in 14 patients (range, 0.007% to 3%; median, 0.29%).

Statistical Analyses

Unadjusted probabilities of overall survival (OS) and disease-free survival (DFS) were estimated by using the Kaplan-Meier method, and probabilities of NRM and relapse were summarized by using cumulative incidence estimates. NRM was defined as death without prior relapse and was considered a competing risk for relapse, whereas relapse was a competing risk for NRM. All outcomes were treated as time-to-event end points. Outcomes between MRD-positive and MRD-negative groups were compared by using Cox regression. All models were adjusted for all or for subsets of the following factors: peripheral blood counts at the time of HCT (complete remission [CR] versus CR with incomplete peripheral blood count recovery [CRi]), karyotype at time of HCT (normal *v* abnormal), cytogenetic risk group at time of AML diagnosis (unfavorable *v* favorable/intermediate), and presence of secondary AML (no *v* yes). Additional models included the PAM score, a validated predictor of all-cause mortality during the first 2 years after HCT,[15] and the HCT-CI, a validated predictor of NRM.[13,14] The limited number of events for some outcomes limits our ability to adjust for each of these factors in a single model. Categorical patient characteristics were compared by using Fisher's exact test, and continuous characteristics were compared with the two-sample *t* test. No adjustments were made for multiple comparisons, and all two-sided *P* values from the regression models were derived from the Wald test. Statistical analyses were performed with STATA (StataCorp LP, College Station, TX).

RESULTS

Patient Characteristics

We identified 100 patients undergoing first myeloablative HCT from a matched-related or an unrelated donor for AML in first remission, of whom 99 patients had pre-HCT MFC studies available. All 99 patients had less than 5% bone marrow blasts and thus met the morphologic criterion for leukemia-free state and CR. Seventy-five patients had no MRD by flow cytometry (ie, MRD negative), and 24 had flow cytometric evidence of MRD (ie, MRD positive). The characteristics of the study population, induction and consolidation chemotherapies, donors, and transplantations are summarized in Tables 1 and 2; detailed information on the 24 MRD-positive patients is provided in Table 3. The time between MFC study and HCT was similar between MRD-positive patients (median, 25.5 days; range, 11 46 days) and MRD-negative patients (median, 24.0 days; range, 14 to 68 days; $P = .65$). MRD-positive patients more likely had AML with unfavorable versus favorable/intermediate cytogenetics ($P = .14$), had a higher prevalence of secondary AML ($P < .01$), and less often received consolidation chemotherapy containing high-dose cytarabine (HIDAC; $P < .007$) or any type of consolidation therapy ($P = .05$). The median duration of first morphologic remission before HCT was shorter for MRD-positive patients (median, 102 days; range, 16 to 169) than MRD-negative patients (median, 129 days; range, 26 to 367 days; $P = .02$). The mean PAM score was higher in the MRD-positive group (mean, 24.8; range, 18 to 32) compared with the MRD-negative group (mean, 23.8; range, 18 to 34), although the difference was not statistically significant ($P = .25$). By comparison, the mean HCT-CI was slightly lower in the MRD-positive group (mean, 2.5; range, 0 to 7) compared with the MRD-negative group (mean, 2.6; range, 0 to 6; $P = .74$).

Relationship Between MRD Status, Peripheral Blood Counts, and Cytogenetics at Time of HCT

Among the MRD-negative patients, 68 (90.7%) had pre-HCT peripheral blood counts that met criteria for CR, whereas seven patients had incomplete blood count recovery (ie, platelet counts $< 100{,}000/\mu L$ with absolute neutrophil count $> 1{,}000/\mu L$ in all seven patients) and were thus classified as CRi (Table 1). Among the MRD-positive patients, a higher proportion had either neutrophils less than $1{,}000/\mu L$ and/or platelets less than $100{,}000/\mu L$ than among the MRD-negative patients, but the different was not statistically significant (four [16.7%] of 24 *v* seven [9.3%] of 75; $P = .45$). Conventional cytogenetic studies were attempted in 97 patients, and data were obtained in 96. Ninety of these had at least 20 metaphases available for analysis; three additional patients had 19 metaphases available, and one patient had 12 and 16 metaphases each available for analysis. In one patient case, only six metaphases were available for cytogenetic analysis, but this patient's result was nevertheless included as a previously known cytogenetic abnormality was revealed. In 11 patients, these cytogenetic studies revealed an abnormal karyotype; in eight patients, cytogenetic abnormalities were consistent with those found at initial AML diagnosis, whereas new abnormalities were found in the other three patients. Not surprisingly, MRD-positive patients were more likely to have abnormal cytogenetic studies than MRD-negative patients (26.1% *v* 6.8%; $P < .03$). Furthermore, patients with abnormal cytogenetic studies were significantly more likely to have either

Table 1. Pretransplantation Demographic and Clinical Characteristics of Study Cohort

Parameter	MRD Negative (n = 75)		MRD Positive (n = 24)		All (N = 99)	
	No.	%	No.	%	No.	%
Age, years						
Median	43.4		47.4		45.3	
Range	0.6-69.5		10.1-66.8		0.6-69.5	
Sex						
Male	31		14		45	
Female	44		10		54	
WBC at diagnosis, $\times 10^3/\mu L$						
Median	9.1		3.65		6.9	
Range	0.2-227.0		0.3-180.0		0.2-227.0	
Cytogenetic risk group						
Favorable	2	2.7	0	0	2	2.0
Intermediate	29	38.7	6	25.0	35	35.4
Unfavorable	36	48.0	17	70.8	53	53.5
Unknown or missing	8	10.7	1	4.2	9	9.1
Secondary AML		30.7		62.5		38.4
No. of induction courses						
1	42	56	12	50.0	54	54.5
2	30	40.0	9	37.5	39	39.4
≥ 3	1	1.3	3	8.3	4	4.0
Missing data	2	2.7	0	0	2	2.0
Type of consolidation therapy						
None	13	17.3	9	37.5	22	22.2
HIDAC-containing	53	70.7	9	37.5	62	62.6
Not HIDAC-containing	8	10.7	6	25.0	14	14.1
Missing data	1	1.3	0	0	1	1.0
No. of consolidation courses						
0	13	17.3	9	37.5	22	22.2
1	49	65.3	10	41.7	59	59.6
2	6	8.0	4	16.7	10	10.1
3	6	8.0	1	4.2	7	7.1
Missing data	1	1.3	0	0	1	1.0
≥ 1 course of consolidation therapy		82.4		62.5		77.5
Duration of first remission, days						
Median	129		102		125	
Range	26-367		16-169		16-367	
Peripheral blood counts before HCT						
ANC $>$ 1,000/μL and platelets $\geq$ 100,000/μL	68	90.7	20	83.3	88	88.9
ANC $<$ 1,000/μL and/or platelets $<$ 100,000/μL	7	9.3	4	16.7	11	11.1
Routine cytogenetics before HCT						
Normal karyotype	68	90.7	17	70.8	85	88.7
Abnormal karyotype	5	6.7	6	25.0	11	11.1
Missing or inadequate data	2	2.7	1	4.2	3	3.0
HCT-CI						
Mean	2.6		2.5		2.6	
Range	0-6		0-7		0-7	
PAM score*						
Mean	23.8		24.8		24.0	
Range	18-34		18-32		18-34	
Source of stem cells						
Bone marrow	17	22.7	8	33.3	25	25.3
Cord blood	10	13.3	2	8.3	12	12.1
Peripheral blood	48	64.0	14	58.3	62	62.6
CMV seropositive before HCT		64.9		62.5		64.3

Abbreviations: MRD, minimal residual disease; AML, acute myeloid leukemia; HIDAC, high-dose cytarabine; HCT, hematopoietic cell transplantation; ANC, absolute neutrophil count; PAM, pretransplantation assessment of mortality; CI, comorbidity index; CMV, cytomegalovirus.

*Among 94 patients, five pediatric patients had missing pulmonary function tests that contributed to PAM.

Table 2. Donor and Transplant Recipient Demographic and Clinical Characteristics of Study Cohort

Parameter	MRD Negative (n = 75)		MRD Positive (n = 24)		All (N = 99)	
	No.	%	No.	%	No.	%
Donor type						
Related	30	40	6	25	36	36.4
Unrelated	45	60	18	75	63	63.6
Donor age, years						
Median	40.1		34.6		39.3	
Range	5.7-64.4		19.1-59.3		5.7-64.4	
Donor sex						
Male	43		16		59	
Female	25		8		33	
Unknown	7		0		7	
Patient/donor sex						
Male/male	17	22.7	10	41.7	27	27.3
Female/female	16	21.3	4	16.7	20	20.2
Male/female	9	12.0	4	16.7	13	13.1
Female/male	26	34.7	6	25.0	32	32.3
Unknown	7	9.3	0	0	7	7.1
Donor CMV seropositive		40		40.9		40.2
Patient/donor CMV serostatus						
Positive/positive	20	26.7	7	29.2	27	27.3
Negative/negative	15	20.0	7	29.2	22	22.2
Positive/negative	23	30.7	6	25.0	29	29.3
Negative/positive	6	8.0	2	8.3	8	8.1
Unknown	11	14.7	2	8.3	13	13.1
Conditioning regimen						
Chemotherapy ± radiolabeled antibody	50	66.7	16	66.7	66	66.7
TBI ± radiolabeled antibody	25	33.3	8	33.3	33	33.3
T-cell depletion		9.3		0		7.1
GVHD prophylaxis						
Calcineurin inhibitor + methotrexate	63	84.0	18	75.0	81	81.8
Calcineurin inhibitor + MMF	12	16.0	5	20.8	17	17.2
Other	0	0	1	4.2	1	1.0
Nucleated cell dose, $\times 10^8$/kg						
Bone marrow						
Median	3.5		2.8		3.1	
Range	0.9-14.0		1.3-4.2		0.9-14.0	
Peripheral blood						
Median	10.9		8.7		10.8	
Range	4.0-42.3		4.6-26.7		4.0-42.3	
Cord blood						
Median	0.3		1.1		0.3	
Range	0.2-0.6		1.1-1.1		0.2-1.1	

NOTE. Three patients (n = 2, MRD-negative patients; n = 1, MRD-positive patient) received donor lymphocyte infusions as part of a treatment strategy for overt acute myeloid leukemia relapse (n = 2) and for a decline in T-cell donor chimerisms (n = 1).

Abbreviations: MRD, minimal residual disease; CMV, cytomegalovirus; TBI, total body irradiation; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil.

low neutrophil or platelet counts before HCT (36.4% *v* 5.8%; $P < .01$). However, this difference was largely explained by a higher likelihood of low neutrophil and/or platelet counts in patients with abnormal cytogenetic studies among the MRD-negative patients (60.0% *v* 4.4%; $P < .004$). In contrast, among MRD-positive patients, the likelihood of low neutrophil and/or platelet counts before HCT was similar among patients with normal and abnormal cytogenetic studies (11.8% *v* 16.7%, respectively; $P = 1.00$). Together, according to the response criteria proposed by the International Working Group,[12] 68 patients were MRD negative and met criteria for cytogenetic CR (n = 65) or CRi, (n = 3), whereas five patients only met morphologic criteria for CR (n = 2) or CRi (n = 3) but did not meet cytogenetic criteria for remission.

OS, DFS, Relapse, and NRM

There were a total of 33 deaths, 27 relapses, and 13 NRM events; these contributed to the probability estimates for OS, DFS, relapse, and NRM stratified by MRD status and are shown in Figure 1. The median follow-up after HCT among survivors was 776 days (range, 111 to 1,445 days). The 2-year estimates of OS for MRD-positive and MRD-negative patients were 30.2% (range, 13.1% to 49.3%) and 76.6% (range, 64.4% to 85.1%), respectively, and the 2-year estimates

Table 3. Detailed Characteristics of MRD-Positive Patients

Patient	Age (years)	Sex	Cytogenetic Risk	Secondary AML	Consolidation*	CR Status	Cytogenetics at HCT	Pre-HCT MRD Level (%)	Immunophenotypic Profile of Abnormal AML Cells	Post-HCT MRD Level (%)†
1	51.7	M	Unfavorable	No	HIDAC × 1	CR	Normal	0.007	$CD13^-$, $CD34^+$, $CD38^-$, DR-negative	0
2	47.9	F	Unfavorable	No	HIDAC × 2	CR	Normal	0.007	$CD15^+$, $CD34^+$, $CD38^-$, $CD56^+$, DR-negative	0.004
3	54.2	M	Unfavorable	No	HIDAC × 2	CR	Normal	0.01	$CD4^+$, $CD34^+$, $CD38^-$	0
4	66.8	M	Intermediate	Yes	None	CR	Normal	0.02	$CD5^+$, $CD56^+$	0
5	45.3	F	Unfavorable	Yes	HIDAC × 1	CR	Normal	0.03	$CD7^+$, $CD13^+$, $CD33^-$, $CD38^-$, DR-negative	0
6	46.9	M	Unfavorable	Yes	Other × 1*	CR	Normal	0.04	$CD34^+$, $CD38^-$	0
7	63.1	F	Unfavorable	No	HIDAC × 1	CR	Normal	0.04	$CD7^+$, $CD33^+$, $CD34^-$, DR-positive	0
8	20.2	F	Unfavorable	Yes	HIDAC × 1	CR	Abnormal	0.06	$CD7^+$, $CD33^-$, $CD34^+$, $CD38^-$, $CD123^-$	0.85
9	18.2	F	Unfavorable	Yes	Other × 3	CR	Normal	0.1	$CD34^+$, $CD38^-$	0.02
10	65.0	M	Unfavorable	Yes	None	CR	Abnormal	0.1	$CD4^+$, $CD5^+$, $CD7^+$, $CD38^-$, $CD117^+$, DR-positive	0.04
11	50.7	M	Unfavorable	Yes	None	CR	Normal	0.25	$CD5^+$, $CD7^+$, $CD33^+$, $CD34^+$, $CD56^+$, $CD117^+$, DR-negative	0
12	11.1	M	Unfavorable	Yes	Other × 1	CR	Abnormal	0.28	$CD4^+$, $CD13^-$, $CD33^-$, $CD34^-$, $CD38^-$	5.7
13	54.5	F	Unfavorable	Yes	HIDAC ×1	CR	Abnormal	0.3	$CD4^+$, $CD33^+$, $CD34^+$, $CD38^-$, $CD56^+$, $CD123^+$	0
14	36.1	F	Unfavorable	No	Other × 2	CR	Normal	0.3	$CD7^+$, $CD13^-$, $CD34^+$, $CD38^+$, $CD45^-$, $CD56^+$ DR-positive	0
15	62.8	F	Unfavorable	Yes	Other × 1	CR	Normal	0.4	$CD7^+$, $CD33^-$, $CD38^-$	0
16	20.2	M	Unfavorable	No	Other × 1; HIDAC × 1	CR	Normal	0.4	$CD4^+$, $CD7^+$, $CD33^+$, $CD38^-$, $CD123^+$, DR-positive	0
17	10.1	M	Intermediate	No	None	CRi	Normal	0.5	$CD13^-$, $CD19^+$, $CD34^+$, $CD117^+$, DR-positive	0
18	18.2	M	Intermediate	No	None	CR	Normal	0.7	$CD13^-$, $CD33^+$, $CD34^-$, $CD117^+$, DR-positive	0.03
19	63.9	M	Unfavorable	Yes	Other × 1	CRi	Abnormal	0.84	$CD7^+$, $CD33^+$, $CD64^+$, $CD123^+$	0
20	57.9	M	Intermediate	Yes	None	CRi	Normal	1.2	$CD7^+$, $CD13^-$, $CD15^+$, DR-positive	0
21	35.1	M	Intermediate	Yes	None	CRi	Missing	1.3	$CD7^+$, $CD33^+$, $CD34^-$, $CD38^+$	0
22	26.7	F	Unfavorable	No	HIDAC × 1	CR	Normal	1.6	$CD4^+$, $CD13^-$, $CD34^-$, $CD38^-$, $CD45^-$, $CD64^+$, $CD123^+$, DR-negative	0.06
23	37.9	F	Intermediate	Yes	None	CR	Abnormal	2.8	$CD7^+$, $CD13^+$, $CD33^+$	0
24	56.0	M	Unknown	Yes	None	CR	Normal	3.0	$CD7^+$, $CD15^+$, $CD34^-$, $CD38^-$, $CD45^-$, DR-negative	0.08

NOTE. Positive and negative with regard to immunophenotype describe abnormally increased or decreased antigen expression on the abnormal blasts relative to their normal counterparts.

Abbreviations: MRD, minimal residual disease; AML, acute myeloid leukemia; CR, complete remission; HCT, hematopoietic cell transplantation; HIDAC, high-dose cytarabine; CRi, CR with incomplete blood count recovery; DR, HLA-DR.

*Regimens used for consolidation are separated into those containing HIDAC and those that did not include HIDAC (ie, other).

†Post-HCT MRD denotes MRD results from bone marrow examinations obtained a median of 28 days (range, 26-46 days) after HCT.

for DFS were 9.0% (range, 1.6% to 24.9%) and 74.8% (range, 62.8% to 83.4%), respectively. The estimates of relapse at 2 years were 64.9% (range, 42.0% to 80.6%) for MRD-positive patients and 17.6% (range, 9.5% to 27.9%) for MRD-negative patients, and the 2-year estimates of NRM were also higher for MRD-positive patients compared with MRD-negative patients (26.0% [range, 0.6% to 44.6%] *v* 10.1% [4.4% to 18.7%]). A longer interval between MFC assessment and HCT could theoretically allow time for reappearance of disease in MRD^- patients, resulting in misclassification. However, among MRD-negative patients, the estimates for OS, DFS, relapse, and NRM were similar for those with an interval between MFC assessment and HCT of $\geq$ 25 days and for those whose interval was less than 25 days, arguing against this being a significant bias in our study (data not shown).

We then developed uni- and multivariate regression models for OS, DFS, relapse, and NRM by using MRD status (positive *v* negative), pre-HCT karyotype (abnormal *v* normal), pre-HCT peripheral blood count recovery (CR *v* CRi), as well as other established predictors for AML outcome (cytogenetics at AML diagnosis, presence of secondary disease, PAM score, and the HCT-CI[13,14]). In the entire cohort, the unadjusted hazard of MRD positive versus MRD negative for overall mortality was 5.03 (range, 2.51 to 10.07; $P < .001$), the unadjusted hazard of relapse was 9.81 (range, 4.39 to 21.94; $P < .001$), the unadjusted hazard of failure for DFS was 8.29 (range, 4.31 to 15.93; $P < .001$), and the unadjusted hazard for NRM was 5.93 (range, 1.91 to 18.39; $P = .002$). When the analysis was restricted to the six MRD-positive and 29 MRD-negative patients with intermediate-risk cytogenetics, the unadjusted hazards of MRD positive versus MRD negative were similarly increased for overall mortality (HR, 9.47), relapse (HR, 24.35), and failure for DFS (HR, 9.08).

In the entire cohort, the unadjusted hazard of abnormal pre-HCT karyotype of overall mortality was 6.16 (range, 2.66 to 14.26; $P < .001$), the unadjusted hazard of relapse was 1.90 (range, 0.56 to 6.45; $P = .31$), the unadjusted hazard of failure for DFS was 3.81 (range, 1.71 to 8.49; $P < .002$), and the unadjusted hazard for NRM was 9.60 (range, 2.96 to 31.13; $P < .001$). The unadjusted hazard of pre-HCT CRi status of overall mortality was 2.19 (range, 0.90 to 5.33; $P = .08$), the unadjusted hazard of relapse was 1.65 (range, 0.57 to 4.78; $P = .36$), the unadjusted hazard of failure for DFS was 2.00 (range, 0.88 to 4.53; $P = .10$), and the unadjusted hazard for NRM was 2.79 (range, 0.77 to 10.15; $P = .12$). After adjustment for various covariates as summarized in Table 4, the hazard ratios of MRD positive versus MRD negative were 4.05 (range, 1.90 to 8.62; $P < .001$) for overall mortality, 8.49 (range, 3.67 to 19.65; $P < .001$) for relapse, and

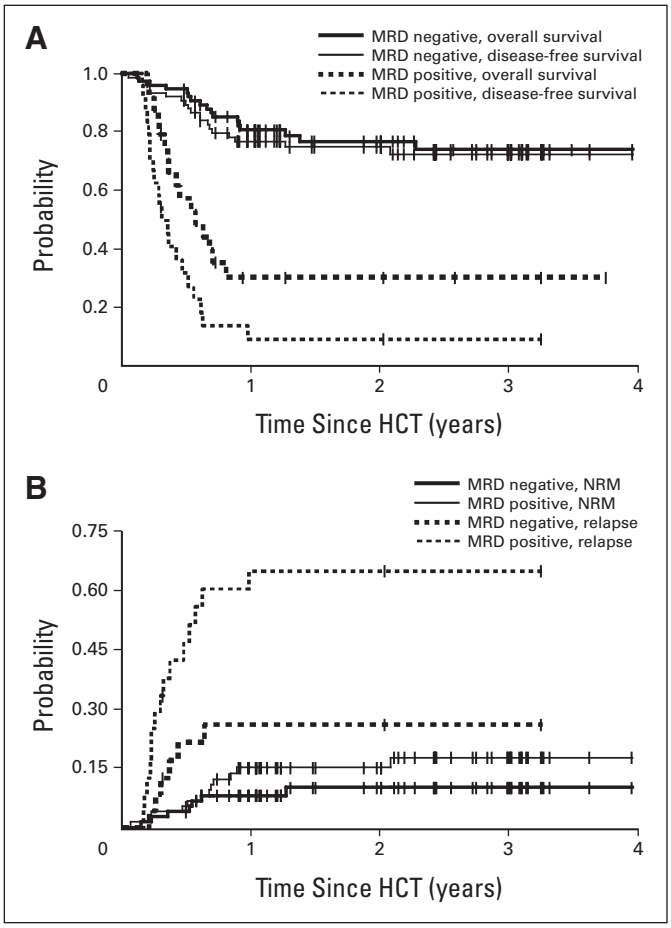

Fig 1. Overall survival (OS), disease-free survival (DFS), relapse, and nonrelapse mortality (NRM). Estimates of the probability of (A) OS and DFS as well as (B) relapse and NRM for patients with acute myeloid leukemia in first morphological remission, with negative versus positive multiparametric flow cytometry results for pre-hematopoietic cell transplantation (HCT) minimal residual disease (MRD).

7.06 (range, 3.54 to 14.07; $P < .001$) for failure for DFS, respectively. With only 13 NRM events, we allowed only one factor (peripheral blood counts at the time of HCT, karyotype at time of HCT, cytogenetic risk group at time of AML diagnosis, or presence of secondary AML) in addition to MRD status into various regression models for this outcome. The resultant adjusted HRs ranged from 5.04 to 5.72 (*P* values ranged from .002 to .005).

Finally, we performed similar uni- and multivariate models restricting the MRD-negative cohort to those 65 patients who met criteria for cytogenetic CR according to the response criteria proposed by the International Working Group.[12] We found similar HRs of MRD positive versus MRD negative after adjustment for the same covariates (overall mortality, 4.60 [range, 1.76 to 12.00], $P < .001$; relapse, 11.03 [range, 4.31 to 28.21], $P < .001$; failure for DFS, 7.90 [3.65 to 17.10], $P < .001$; various NRM models, between 5.24 and 6.20 [*P* ranging from .003 to .013]). The findings were similar when uni- and multivariate models were fit restricting both the MRD-negative and MRD-positive cohorts to those 80 patients (MRD-negative: n = 65; MRD-positive: n = 15) with recovered blood counts and normal karyotype analysis at the time of HCT. This was true for overall mortality (HR for MRD positive *v* MRD negative, 5.82; 95% CI, 2.05 to 16.53; $P = .001$), relapse (16.42; 95% CI, 5.68-47.47; $P < .001$), and failure for DFS (10.64; 95% CI, 4.35 to 26.04; $P < .001$), whereas the various NRM models showed HRs between 1.56 and 1.94 (*P* between .58 and .69).

DISCUSSION

The data presented in this retrospective analysis support three major conclusions. First, patients with AML who are in first CR without flow cytometric evidence of MRD and who underwent myeloablative allogeneic HCT had favorable outcomes, with 2-year DFS and OS rates that approximate 75% and a 2-year cumulative incidence of relapse of less than 20%. Second, relative to MRD-negative patients, MRD-positive patients had significantly worse outcomes, with a 2-year cumulative incidence of relapse that exceeded 60%, which resulted in poor DFS and high mortality. And lastly, although MRD-positive and MRD-negative patients differed in many factors that predict poor outcome in AML, our multivariate models suggested that pre-HCT MRD is an adverse risk factor for HCT outcome, even after adjusting for these other factors.

Determination of MRD levels during aplasia, either early after induction and/or after consolidation chemotherapy, has proven useful to predict relapse and poor outcome after autologous HCT and may help in identifying patients with AML who require allogeneic HCT for treatment intensification.[19-28] Similarly, MRD levels early after transplantation are predictive for outcome and may help as a tool for decision making after allogeneic HCT.[29] Our data now indicate that detection of MRD at the time of HCT defines a population of patients with AML that is at higher risk for relapse, reduced OS and DFS, and even increased NRM compared with MRD-negative patients. With only 24 MRD-positive patients, 10 of whom had an MRD level $\leq 0.1\%$, it is difficult to draw any firm conclusions regarding the association between level of MRD and outcome (ie, whether patients with higher MRD levels have a worse outcome than those with lower levels). On the basis of our limited data, however, the risk of relapse among MRD-positive patients who had a level $\leq 0.1\%$ did not appear to be lower than that among MRD-positive patients whose level was greater than 0.1% (HRs relative to MRD-negative patients, 10.26 and 7.20, respectively).

Of note, MRD was somewhat correlated with other adverse risk factors in our study population. In fact, patients according to the MRD status differed both with respect to disease-specific and treatment-specific factors. Specifically, besides being slightly older, MRD-positive patients more often had secondary disease and tended to more often have AML with poor-risk cytogenetics. The presence of secondary AML is an adverse predictor for outcome after myeloablative allogeneic HCT for patients with AML in first CR, as we found in a recent analysis of a larger cohort of patients with AML (data not shown). The cytogenetic risk at initial disease presentation is predictive for outcome after myeloablative allogeneic HCT for patients with AML in first CR[30]; this study revealed a similar finding (according to multivariate analysis; Table 4), although the smaller numbers of patients limited the power to detect a statistically significant effect. It is certainly not surprising that MRD-positive patients more often had poor-risk features, as such diseases are intuitively more likely to persist with MRD after induction and consolidation therapy than more favorable AML. Moreover, MRD-positive patients also received less

Table 4. Adjusted Multivariate Regression Models for Risk of Overall Mortality, Failure for DFS, Relapse, and NRM

Factor	Overall Mortality			Failure for DFS			Relapse			NRM*		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
MRD status												
Negative (n = 75)	1			1			1			1		
Positive (n = 24)	4.05	1.90 to 8.62	< .001	7.06	3.54 to 14.07	< .001	8.49	3.67 to 19.65	< .001	5.04	1.52 to 16.71	.008
Pre–HCT blood counts												
CR (n = 88)	1			Not used in model			Not used in model			Not used in model		
CRi (n = 11)	1.45	0.54 to 3.84	.459									
Pre–HCT karyotype												
Normal (n = 85)	1			1			1			1		
Abnormal (n = 11)	4.55	1.78 to 11.64	.002	2.92	1.24 to 6.87	.014	1.34	0.37 to 4.81	.653	8.35	2.41 to 28.89	.001
Cytogenetic risk group												
Unfavorable (n = 53)	1			1			1			Not used in model		
Favor/intermediate (n = 37)	0.83	0.35 to 1.98	.680	0.52	0.23 to 1.14	.102	0.35	0.12 to 1.04	.060			
Secondary AML												
No (n = 61)	1			Not used in model			Not used in model			Not used in model		
Yes (n = 38)	1.48	0.70 to 3.14	.305									

NOTE. Alternative models that included age at time of HCT, pretransplantation assessment and/or comorbidity index scores, and covariates not used in the models shown in this table revealed qualitatively similar results for all four outcomes.

Abbreviations: NRM, nonrelapse mortality; DFS, disease-free survival; HR, hazard ratio; MRD, minimal residual disease; HCT, hematopoietic cell transplantation; CR, complete remission; CRi, CR with incomplete blood count recovery; AML, acute myeloid leukemia.

*Because of the small number of events, only one factor was added to MRD status in various adjusted models. The table shows the model with adjustment for pre-HCT karyotype. Similar models were built adjusting for pre-HCT blood counts, cytogenetic risk group, and presence of secondary disease. These models revealed hazards of being MRD positive of 5.25 (range, 1.63-16.88; $P = .005$) after adjustment for pre-HCT blood counts; 5.72 (range, 1.80-18.15; $P = .003$) after adjustment for cytogenetic risk group; and 5.26 (range, 1.59-17.36; $P = .002$) after adjustment for presence of secondary disease.

consolidation therapy and, in particular, less HIDAC-containing consolidation therapy before HCT and had a shorter duration of remission before HCT. Given the retrospective nature of this analysis and that our center serves as a referral center for HCT, we can only speculate about the reasons for this difference. Previous studies did not find a benefit of postremission chemotherapy before allogeneic HCT for patients with AML in first CR.[31-33] Of note, however, these studies did not include analyses of MRD status, and it is unknown whether this lack of benefit extends equally to MRD-negative and MRD-positive patients. An important, testable question for future studies is whether additional pre-HCT consolidation chemotherapy could revert an MRD-positive state into an MRD-negative state and whether achievement of MRD negativity before HCT improves the outcome in these patients.

MRD-negative and MRD-positive patients differed additionally with regard to other pre-HCT characteristics. Relative to MRD-negative patients, MRD-positive patients more frequently had abnormal findings from routine karyotype analyses at the time of HCT, whereas the proportion of patients presenting with incomplete blood count recovery was comparable. Our analyses show that the presence of an abnormal karyotype in routine cytogenetic studies before HCT is a strong predictor for adverse outcome after HCT in our patient cohort, both in univariate and multivariate models. The presence of incomplete blood count recovery was similarly associated with an increased hazard of relapse and death, although the relatively small number of patients (and hence, events) limited the power to detect a statistically significant impact.

As expected, our data support the notion that the factors discussed in this paper are not independent from each other. Despite an imbalance in these adverse factors between MRD-positive and MRD-negative patients, our multivariate models suggest that evidence of MRD before HCT remains a significant risk factor for post-transplantation relapse and death. This negative impact on outcome extended, somewhat surprisingly, also to NRM in our patient cohort (unless the analysis is restricted to patients with recovered blood counts and normal cytogenetic analysis at the time of HCT), and additional studies will be necessary to fully understand this observation.

Together, our findings suggest that detection of any MRD by MFC at the time of HCT defines a population of patients with AML who are at higher risk for adverse outcome, even after adjusting for other factors that influence post-HCT outcome. Although these findings should be confirmed in a larger patient cohort, they support the routine use of pre-HCT MRD assessment for risk stratification of post-HCT outcome. Furthermore, they provide the rationale for future studies to test whether the outcome of MRD-positive patients could be improved through MRD-stratified interventions, for example additional pretransplantation chemotherapy, modified HCT conditioning, or additional pre-emptive treatment after HCT.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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