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Prognostic Utility of Routine Chimerism Testing at 2 to 6 Months after Allogeneic Hematopoietic Cell Transplantation

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The utility of routine chimerism analysis as a prognostic indicator of subsequent outcomes after allogeneic hematopoietic cell transplantation (HCT) with myeloablative conditioning regimens remains controversial. To address this controversy, routine chimerism test results at 2 to 6 months after HCT with myeloablative conditioning regimens were evaluated for association with subsequent risk of chronic graft-versus-host disease (GVHD), nonrelapse mortality (NRM), relapse, and overall mortality. Only 70 of 1304 patients (5%) had $< 95\%$ donor-derived cells in the marrow. Low donor chimerism in the marrow occurred more often in patients with low-risk diseases compared with those with higher-risk diseases and was significantly associated with a reduced risk of chronic GVHD. Among 673 patients evaluated, 164 (24%) had $< 85\%$ donor-derived T cells in the blood. Low donor T cell chimerism was more frequent in patients with low-risk diseases compared with those with higher-risk diseases, in those who received conditioning with busulfan compared with those who received conditioning with total body irradiation, and in those with lower-grade acute GVHD. Low donor T cell chimerism in the blood was significantly associated with a reduced risk of chronic GVHD but not with a reduced risk of relapse, NRM, or overall mortality. Routine testing of chimerism in the marrow and blood at 2 to 6 months after HCT with myeloablative conditioning regimens may be helpful in documenting engraftment in clinical trials, but provides only limited prognostic information in clinical practice.

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KEY WORDS: Chimerism analysis, Mixed chimerism, Allogeneic hematopoietic cell transplantation

INTRODUCTION

Chimerism testing can be used to document engraftment after allogeneic hematopoietic cell transplantation (HCT) and can be highly useful in the diagnosis of rejection and recurrent malignancy. Lineage-specific analysis increases the sensitivity of the method and may provide more specific information [1,2]. Chimerism tests demonstrating the persistence of recipient cells after HCT with nonmyeloablative regimens also can predict an increased risk of rejection or recurrent malignancy; however, the role of chimerism analysis as a prognostic indicator of subsequent transplantation outcomes after HCT with myeloablative conditioning regimens remains controversial. Whereas Lamba et al. [3] reported higher relapse rates and lower overall survival (OS) in patients with mixed chimerism (MC) on day 90 after HCT, Doney et al. [4] found no correlation between persistence of recipient cells at 2 to 3 months after HCT and subsequent outcomes in patients who received a myeloablative conditioning regimen. The results of Doney et al. are consistent with consensus recommendations that documentation of chimerism is not essential after HCT with myeloablative conditioning regimens and conventional graft-versus-host disease (GVHD) prophylaxis [1].

To address this controversy, we reviewed results of routine chimerism tests at 2 to 6 months after HCT with myeloablative conditioning regimens in a large cohort of patients, to evaluate whether test results were associated with subsequent risks of chronic GVHD, nonrelapse mortality (NRM), recurrent malignancy, and survival.

METHODS

The study cohort included all patients who had undergone a first allogeneic HCT with the use of

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a myeloablative conditioning regimen at the Fred Hutchinson Cancer Research Center and had routine chimerism testing of the marrow (n = 1304) (Table 1) or of both granulocytes and T cells in the blood (n = 673) (Table 2) with the use of sex markers or molecular markers as part of the departure evaluation before their care was transferred from the transplantation center to the referring physician. Routine testing was done on a single occasion, and the choice of samples for testing was dictated by institutional practices, which changed over time. Transplantations for the patients who had marrow testing were done between July

Table 1. Characteristics of Patients Who Underwent Marrow Cell Testing

Characteristic	Entire Cohort	< 95% Donor Marrow Cells
Number of patients (%)	1304	70 (5)
Patient age, years, median (range)	37 (0 to 66)	41 (0 to 63)
Disease at transplantation, n (%)		
Myelodysplastic syndrome	195	10 (5)
Acute myeloid leukemia	300	3 (1)
CML	443	40 (9)
ALL	182	4 (2)
Non-Hodgkin lymphoma or Hodgkin disease	68	5 (7)
Other	116	8 (7)
Pretransplantation risk category, n (%)		
Low	415	37 (9)
Intermediate	628	25 (4)
High	261	8 (3)
Donor age, years, median (range)	37 (0 to 81)	40 (1 to 65)
Donor/recipient sex, n (%)*		
Male/male	286	10 (4)
Male/female	411	28 (7)
Female/male	457	20 (4)
Female/female	148	12 (8)
Donor type, n (%)		
HLA-identical related	649	38 (6)
HLA-mismatched related	108	8 (7)
HLA-matched unrelated	293	13 (4)
HLA-mismatched unrelated	254	11 (4)
Conditioning regimen, n (%)		
Cyclophosphamide and TBI	676	26 (4)
Busulfan and cyclophosphamide	375	27 (7)
Busulfan and TBI	43	1 (2)
Busulfan, cyclophosphamide, and TBI	59	1 (2)
Busulfan, cyclophosphamide, and ATG	27	4 (15)
Cyclophosphamide, TBI, and ATG	41	3 (7)
Other containing ATG	55	4 (7)
Other	28	4 (14)
Stem cell source, n (%)		
Bone marrow	1012	57 (6)
Mobilized blood	279	10 (4)
Cord blood	13	3 (23)
GVHD prophylaxis, n (%)		
Cyclosporine and methotrexate	1061	63 (6)
Tacrolimus and methotrexate	92	4 (4)
Methotrexate	31	0
Calcineurin inhibitor	92	3 (3)
Calcineurin inhibitor and MMF	21	0
Other	7	0
Previous acute GVHD, n (%)†		
Grade 0-I	292	20 (7)
Grade II	742	36 (5)
Grade III-IV	269	14 (5)

ATG, antithymocyte globulin; MMF, mycophenolate mofetil.
*Two patients received cord blood transplants from 2 donors (1 male and 1 female).
†GVHD could not be graded in 1 patient because of severe regimen-related toxicity.

Table 2. Characteristics of Patients Who Underwent Blood Cell Testing

Characteristic	Entire Cohort	< 95% Donor Granulocytes	< 85% Donor T Cells
Number of patients (%)	673	14 (2)	164 (24)
Patient age, years, median (range)	39 (0 to 66)	34 (1 to 57)	42 (1 to 64)
Disease at transplantation, n (%)			
Myelodysplastic syndrome	164	3 (2)	57 (35)
Acute myeloid leukemia	216	4 (2)	36 (17)
CML	118	3 (3)	52 (44)
ALL	111	0	3 (3)
Other	64	4 (6)	16 (25)
Pretransplantation risk category, n (%)			
Low	144	4 (3)	55 (38)
Intermediate	434	8 (2)	98 (23)
High	95	2 (2)	11 (12)
Donor age, years, median (range)	38 (1 to 76)	30 (1 to 60)	38 (5 to 65)
Donor/recipient sex, n (%)*			
Male/male	204	5 (2)	50 (25)
Male/female	155	3 (2)	42 (27)
Female/male	160	2 (1)	39 (24)
Female/female	153	4 (3)	33 (22)
Donor type, n (%)			
HLA-identical related	288	10 (3)	79 (27)
HLA-mismatched related	15	0	2 (13)
HLA-matched unrelated	225	3 (1)	63 (28)
HLA-mismatched unrelated	145	1 (1)	20 (14)
Conditioning regimen, n (%)			
Cyclophosphamide and TBI	250	2 (1)	13 (5)
Busulfan and cyclophosphamide	287	6 (2)	106 (37)
Busulfan, cyclophosphamide, and ATG	42	2 (5)	6 (14)
Cyclophosphamide, TBI, and ATG	25	0	3 (12)
Other containing ATG	35	2 (6)	20 (57)
Other	34	1 (3)	16 (47)
Source of stem cells, n (%)			
Bone marrow	195	6 (3)	62 (32)
Mobilized blood	460	7 (2)	101 (22)
Cord blood	18	1 (6)	1 (6)
GVHD prophylaxis, n (%)			
Cyclosporine plus methotrexate	437	10 (2)	120 (27)
Tacrolimus plus methotrexate	157	3 (2)	37 (24)
Methotrexate	3	0	0
Calcineurin inhibitor	43	1 (2)	6 (14)
Calcineurin inhibitor and MMF	33	0	1 (3)
Previous acute GVHD, n (%)			
Grade 0-I	157	5 (3)	56 (36)
Grade II	426	8 (2)	96 (23)
Grade III-IV	90	1 (1)	12 (13)

*One patient received a cord blood transplant from 2 donors (1 male and 1 female).

1988 and September 2006, and transplantations for the patients who had blood cell testing were done between September 2000 and September 2006. Testing was done at a median of 79 days (range, 53 to 188 days) after HCT. A patient was excluded from further analysis if the departure testing showed evidence of recurrent malignancy. Every patient included for consideration signed an Institutional Review Board–approved informed consent document granting permission to review medical information for research purposes, and the Institutional Review Board approved the use of medical information for this retrospective study.

Blood cells were sorted according to CD33 and CD3 expression by flow cytometry before chimerism testing. Nonfractionated aspirated marrow and fractionated blood cells from patients with sex-mismatched donors were tested by fluorescent in situ hybridization with Y-chromosome–specific probes or with both Y- and X-chromosome–specific probes [5]. Starting in 1998, DNA samples from patients with same-sex donors were tested by amplification and semiquantitative analysis of variable-number tandem repeat loci with informative polymorphisms. Amplified products were analyzed semiquantitatively after electrophoresis and silver staining in polyacrylamide gels [6]. As assessed by mixing experiments, this method has a sensitivity of 0.1% to 5%, depending on the relative size of the informative markers. Starting in 2004, samples from patients with same-sex donors were tested by multiplex amplification of short tandem repeat loci with informative polymorphisms (PowerPlex 16; Promega, Madison, WI). Amplified products were analyzed quantitatively by capillary electrophoresis (ABI 3130x1; Applied Biosystems, Foster City, CA). This method has at least 0.5% sensitivity, as assessed by mixing experiments [7]. Pretransplantation samples from the donor and recipient were routinely included as controls.

Survival probabilities were estimated according to the Kaplan-Meier method. Probabilities of recurrent malignancy, NRM, and chronic GVHD were estimated by the cumulative incidence method. Follow-up for survival and recurrent malignancy was censored at the date of last contact or death. Follow-up for NRM and chronic GVHD was censored at the onset of recurrent malignancy or date of last contact, whichever occurred first. The χ^2 test or Fisher's exact test was used to estimate the statistical significance of categorical differences. Cox proportional hazards analysis was used to compare estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for mortality, recurrent malignancy, NRM, and chronic GVHD in patients with and without low donor chimerism. Adjustment factors considered were disease risk (low risk: chronic myeloid leukemia [CML] in the chronic phase and refractory anemia; high risk: malignant disease in relapse, refractory anemia with excess blasts in transformation, and CML in blast crisis; intermediate risk: all other diagnoses), conditioning (busulfan and cyclophosphamide, cyclophosphamide and total body irradiation [TBI], and all other regimens), grafting with growth factor–mobilized blood cells, and grade II-IV acute GVHD with onset before chimerism testing.

RESULTS

Two factors were considered when defining a threshold for low donor chimerism. First, the threshold percentage of recipient cells had to be above the lower limit of sensitivity in reliably detecting these cells by the assays used for testing (5%), to avoid confounding due to false-negative results. Second, a sufficient number of patients with low donor chimerism was needed to allow reasonable statistical power for observing associations with clinical outcomes, if possible. Routine testing of aspirated marrow at 2 to 6 months after HCT found > 5% recipient cells (< 95% donor cells) in only 70 of the 1304 patients tested (5%) (Table 1). Although this threshold does not provide optimal statistical power, using a less stringent definition of low donor chimerism was not feasible, because of limits in the sensitivity of chimerism tests.

Low donor chimerism (< 95% donor cells) in the marrow occurred more frequently in patients with low-risk diseases (predominantly CML) compared with those with higher-risk diseases (predominantly acute leukemia) ($P < .001$). The persistence of > 5% recipient cells was not significantly associated with donor–recipient sex combination, donor–recipient relationship, conditioning regimen, use of growth factor–mobilized blood versus marrow, posttransplantation immunosuppressive regimen, or severity of acute GVHD (Table 1). Correlation with clinical outcome showed a statistically significant association of low donor chimerism in the marrow with decreased subsequent risk of chronic GVHD (HR = 0.65; 95% CI = 0.5 to 0.9; $P = .02$) but not with subsequent risk of recurrent malignancy, NRM, or overall mortality (Table 3; Figure 1).

Based on the same considerations used to define low donor chimerism in the marrow, the presence of < 95% donor-derived cells was used to define low donor chimerism in blood granulocytes. Only 14 of 673 patients (2%) had < 95% donor-derived granulocytes in the blood. Low donor chimerism in blood granulocytes occurred too infrequently to enable meaningful analysis of correlation with transplant characteristics or clinical outcome (Tables 2 and 3). The percentile distribution of T cell chimerism suggests that the presence of < 85% donor-derived cells could be used to define low donor chimerism (Figure 2). Based on this definition, low donor chimerism (< 85%) was found

Table 3. Association of Low Donor Chimerism with Subsequent Outcome after HCT*

	Overall Mortality		Relapse		NRM		Chronic GVHD	
Comparison	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Donor chimerism in marrow								
≥ 95% (n = 1234)	1.0	.87	1.0	.17	1.0	.90	1.0	.02
< 95% (n = 70)	0.97 (0.7 to 1.4)		1.35 (0.9 to 2.1)		0.97 (0.6 to 1.7)		0.65 (0.5 to 0.9)	
Donor chimerism in blood granulocytes								
≥ 95% (n = 659)	1.0	.38	1.0	.45	1.0	.88	1.0	.30
< 95% (n = 14)	0.60 (0.2 to 1.9)		0.59 (0.1 to 2.4)		0.90 (0.2 to 3.6)		0.67 (0.3 to 1.4)	
Donor chimerism in blood T cells								
≥ 85% (n = 509)	1.0	.02	1.0	.38	1.0	.07	1.0	.02
< 85% (n = 164)	0.66 (0.5 to 0.9)		0.84 (0.6 to 1.2)		0.62 (0.4 to 1.0)		0.76 (0.6 to 1.0)	

*Results do not reflect adjustment for other risk factors.

in 164 of the 673 patients (24%) (Table 2). The prevalence of low T cell chimerism was greater in in patients with CML or myelodysplastic syndrome compared with those with acute lymphoblastic leukemia (ALL) ($P < .001$), in patients with low-risk diseases compared with those with high-risk diseases ($P < .001$), in patients who had conditioning with busulfan compared with those who had conditioning with TBI ($P < .001$), and in patients with grade 0-1 acute GVHD compared with those with grade III-IV GVHD ($P < .001$). Low T cell chimerism occurred less frequently when the donor and recipient were HLA-mismatched ($P < .001$), and in patients who received a combination of calcineurin inhibitor and mycophenolate mofetil compared with methotrexate after transplantation ($P = .003$). Low T cell chimerism was not significantly associated with donor–recipient sex combination, donor–recipient relationship, or stem cell source (Table 2).

Correlation with clinical outcome demonstrated a statistically significant association between low T cell chimerism and subsequent reduced risk of chronic GVHD (HR = 0.76; 95% CI = 0.6 to 1.0; $P = .02$) (Table 3; Figure 3). This association was slightly attenuated after adjustment for differences in

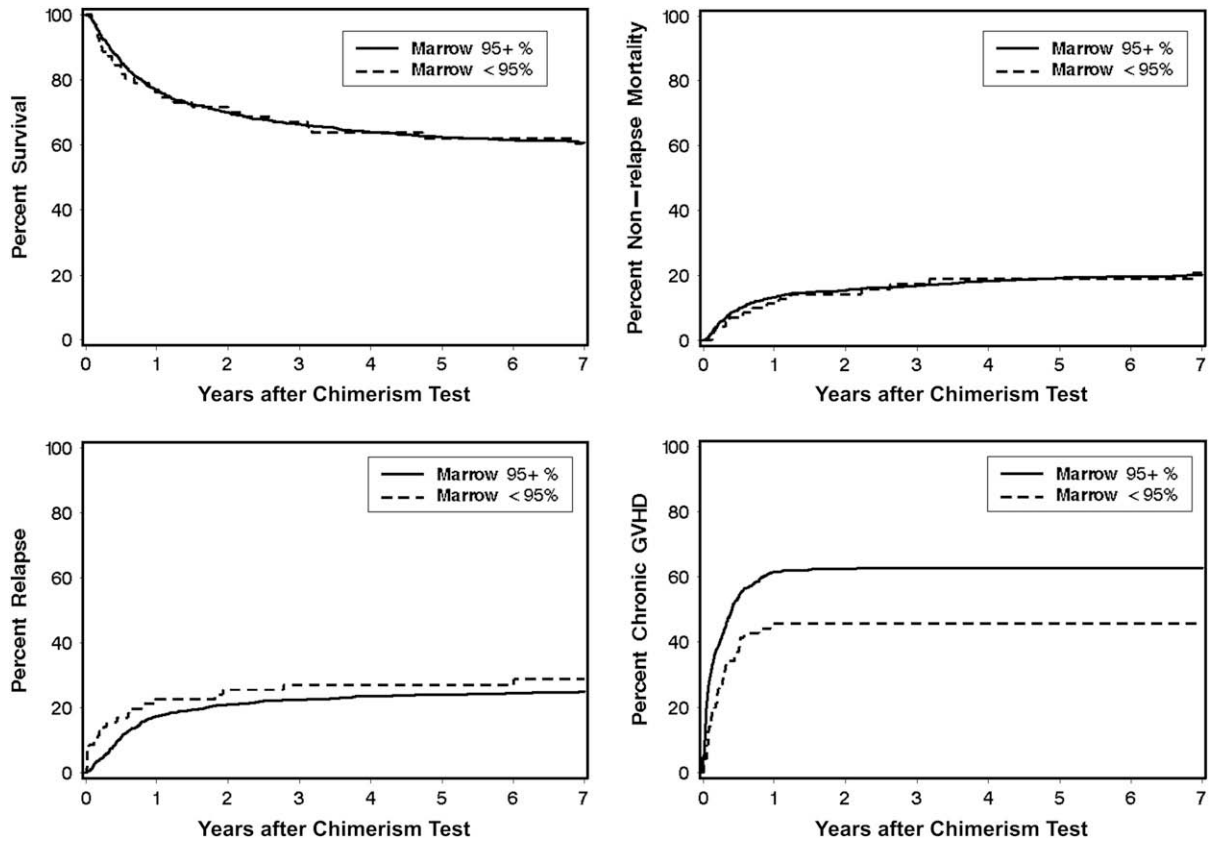

Figure 1. Clinical outcomes according to level of donor chimerism in the marrow.

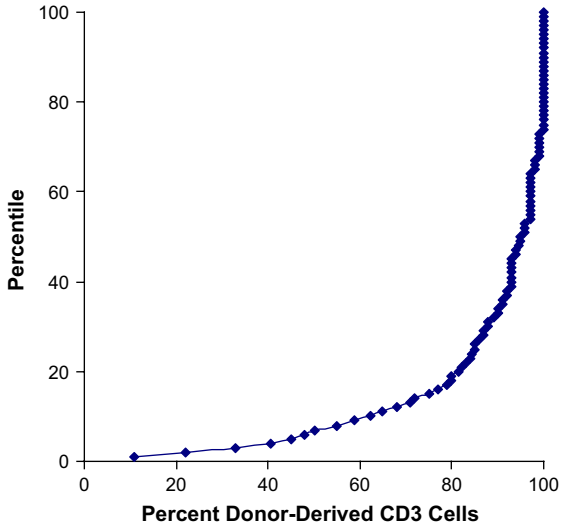

Figure 2. Percentile distribution of donor T cell chimerism in the blood.

pretransplantation risk category, conditioning regimen, use of mobilized blood cells, and previous grade II-IV GVHD (HR = 0.80; 95% CI = 0.6 to 1.0; P = .09). In univariate analysis, low T cell chimerism was significantly associated with lower overall mortality (HR = 0.66; 95% CI = 0.5 to 0.9; P = .02) (Table 3; Figure 3), but after adjustment for differences in disease risk group and conditioning regimen, this association was no longer statistically significant (HR = 0.77; 95% CI = 0.5 to 1.1; P = .16). Low T cell chimerism showed a trend for association with a lower risk of NRM (HR = 0.62; 95% CI = 0.4 to 1.0; P = .07), but not with an increased risk of recurrent malignancy (Table 3).

To some extent, the association of low donor chimerism in the marrow with a reduced risk of chronic GVHD could reflect low donor T cell chimerism in the blood, because aspirated marrow contains an appreciable number of T cells from the blood. To test this hypothesis, we evaluated the correlation between donor chimerism levels in the marrow and the blood in 173 patients who underwent both tests. Low donor marrow chimerism was seen in 6 of 47 patients (12.8%) with low T cell chimerism and in 3 of 126 patients (2.4%) with high donor T cell chimerism (P = .01, Fisher's exact test). In these patients, low donor T cell chimerism was significantly associated with a reduced risk of chronic GVHD (HR = 0.51; 95% CI = 0.3 to 0.9; P = .02), but low donor marrow chimerism was not (HR = 0.97; 95% CI = 0.4 to 2.7; P = .96).

None of the 509 patients with ≥ 85% donor T cell chimerism underwent a second transplantation for treatment of graft failure, and only 2 of the 164 patients

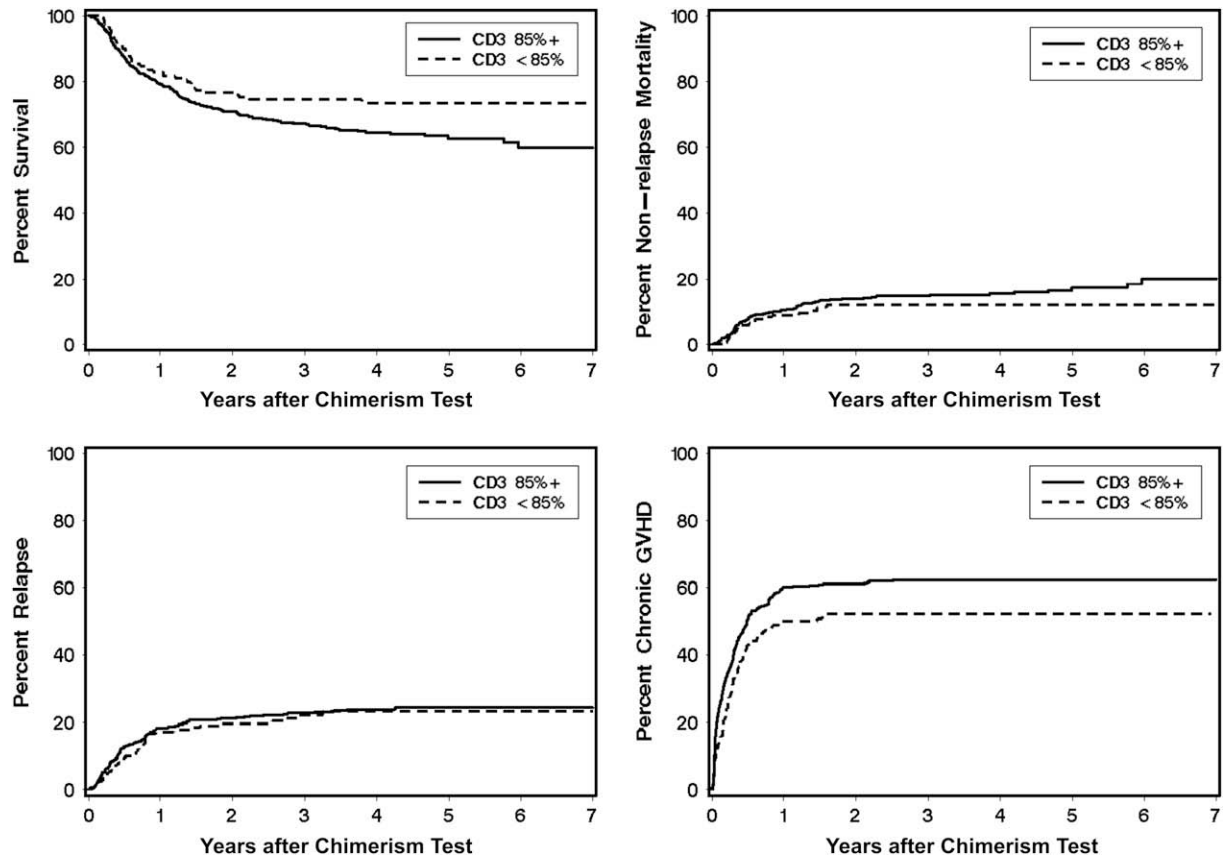

Figure 3. Clinical outcomes according to level of donor T cell (CD3) chimerism in the blood.

(1.2%) with low donor T cell chimerism underwent a second transplantation due to poor graft function. In 1 of these cases, test results on day 81 after the first transplantation showed 5% to 15% donor-derived T cells in the blood with 95% to 99% donor-derived granulocytes, and results on day 160 showed 1% to 5% donor-derived T cells in the blood, again with 95% to 99% donor-derived granulocytes. In the other case, the results on day 81 after the first transplantation showed 60% donor-derived T cells in the blood with 77% donor-derived granulocytes, with similar results seen on day 111.

DISCUSSION

In this study, we found that only a small minority of patients had < 95% donor-derived cells in the marrow and blood granulocytes at 2 to 6 months after HCT with a myeloablative conditioning regimen. Low donor chimerism in the marrow occurred predominantly in patients with low-risk diseases, such as chronic-phase CML, and was associated with a reduced risk of chronic GVHD. Approximately 25% of the patients in this study had < 85% donor-derived T cells in the blood at 2 to 6 months after HCT with myeloablative conditioning regimens. Low donor chimerism in blood T cells occurred predominantly in patients with low-risk diseases and was associated with the absence of TBI in the conditioning regimen and the absence of acute GVHD. Low donor chimerism in the marrow and in blood T cells at 2 to 6 months after HCT with myeloablative conditioning regimens was significantly associated with a reduced risk of chronic GVHD but not with reduced risk of recurrent malignancy, NRM, overall mortality, or graft failure necessitating a second transplantation. Routine testing of chimerism in the marrow and blood at 2 to 6 months after HCT with myeloablative conditioning regimens may be helpful in documenting engraftment in clinical trials but provides only limited prognostic information in clinical practice.

The threshold of < 85% donor-derived T cells used to define low donor chimerism in our study was selected empirically and may differ from the values used in other studies. The percentile distribution of T cell chimerism in our study showed a smooth progression from low values to high values, with no obvious break point. The 85% threshold approximates the inflection of the curve and had no a priori biological significance.

The absence of previous myelosuppressive treatment before referral for HCT most likely accounts for the associations of low-risk diseases with low donor marrow chimerism and of low donor T cell chimerism at 2 to 6 months after HCT with a myeloablative conditioning regimen. In this study, low-risk diseases included CML in chronic-phase and refractory anemia, which are almost never treated with high-dose myelosuppressive treatment. High-risk diseases included malignant disease in relapse, refractory anemia with excess blasts in transformation, and CML in blast crisis, and intermediate-risk diseases included all other diagnoses. We acknowledge that some patients in the intermediate- and high-risk categories did not receive previous myelosuppressive treatment. We used the low- and higher-risk categories as surrogates for the respective absence or presence of previous myelosuppressive treatment, although we do not have direct data demonstrating this correlation. A similar explanation has been invoked for the observation that the incidence of graft rejection after HCT with nonmyeloablative conditioning regimens is higher in patients with low-risk diseases than in those with higher-risk diseases [8]. These results support the hypothesis that previous exposure to multiple cycles of myelosuppressive chemotherapy increases the susceptibility of recipient hematopoietic cells and T cells to the effects of both myeloablative and nonmyeloablative pretransplantation conditioning regimens.

The finding that low donor T cell chimerism occurred more frequently than low donor myeloid chimerism suggests that previous myelosuppressive treatment, the pretransplantation conditioning regimen, and the effects of acute GVHD have a greater cumulative effect on myeloid cells than on T cells in the recipient. Mattsson et al. [9] reported similar lower levels of T cell chimerism compared with myeloid chimerism. Chimerism testing at 2 to 6 months after HCT suggests that the fractionated TBI exposures and doses of busulfan used in this study had equivalent cumulative effects on recipient myeloid cell but different effects on recipient T cells. These results indicate that fractionated TBI has a more potent immunosuppressive effect than busulfan, as demonstrated by other studies [9]. The association of HLA-mismatching and acute GVHD with decreased proportions of persisting recipient T cells and higher levels of donor T cell chimerism is consistent with the hypothesis that the targets of acute GVHD include recipient T cells as well as basal epithelial cells in the skin, bile duct epithelial cells in the liver, and crypt epithelial cells in the gastrointestinal tract.

Our results demonstrating that persistence of recipient cells in the marrow or blood T cells beyond 2 months after HCT is associated with a reduced risk of chronic GVHD after allogeneic HCT with myeloablative conditioning regimens are consistent with the findings of other studies. McCann et al. [10] reported that patients with MC were at decreased risk for chronic GVHD after HCT for treatment of aplastic anemia, whereas Balon et al. [11] reported that the development of complete chimerism within the first 3 months after HCT was associated with an increased

risk of chronic GVHD. To some extent, low donor chimerism in aspirated marrow specimens can be explained by low T cell chimerism in the blood. The reduced risk of chronic GVHD associated with low donor chimerism in the blood but not with low donor chimerism in the marrow in patients who underwent both tests suggests that low donor T cell chimerism (or persistence of recipient T cells) represents the dominant association. Recipient T cells could have suppressive or veto effects on donor cells that cause chronic GVHD, thereby inducing a state of tolerance. Alternatively, the association of high donor T cell chimerism with an increased risk of chronic GVHD could reflect the activity of some other factor that simultaneously eliminates recipient T cells and also induces chronic GVHD.

The absence of a correlation between low donor T cell chimerism and the subsequent risk of recurrent malignancy is somewhat unexpected, because the persistence of recipient T cells is associated with a decreased risk of chronic GVHD, and the development of chronic GVHD is associated with a decreased risk of recurrent malignancy. Any increment in risk of recurrent malignancy associated with the observed decrease in risk of chronic GVHD in patients with persisting recipient T cells could have been too small to measure in a cohort of patients with a variety of malignant diseases. The absence of a correlation between low donor marrow chimerism or low donor T cell chimerism and the subsequent risk of recurrent malignancy in our study is consistent with results of several previous studies [4,12-14]. Other studies, however, have shown an association between the persistence of recipient myeloid cells [15,16] or T cells [17] after unmanipulated [15,16] or T cell–depleted [16,17] HCT and an increased risk of recurrent malignancy in patients with CML. Associations between persistence of recipient cells and increased risk of recurrent malignancy also have been reported in patients with acute leukemias [3,18]. Chimerism assays certainly can be used as a diagnostic indicator of recurrent malignancy, but their utility as a prognostic indicator of recurrent malignancy depends on the nature of the malignancy, the intensity of the conditioning regimen [8], and the methods used to prevent GVHD.

Contrary to results reported by Lamba et al. [3], our data do not support an association between survival and low donor T cell chimerism at 2 to 6 months after HCT with myeloablative conditioning. On the other hand, we found a trend for an association between low donor T cell chimerism and reduced risk of subsequent NRM. This association can be explained, at least in part, by the reduced risk of chronic GVHD associated with persistence of recipient T cells, because chronic GVHD is the primary cause of late NRM after allogeneic HCT.

Our findings do not support an association between low donor T cell chimerism and a subsequent risk of graft failure after T cell–replete HCT with myeloablative conditioning regimens, although other studies have found such associations [2]. Although graft rejection can be caused by recipient T cells that survive the conditioning regimen, the mere presence of a particular cell population does not allow us to direct inferences concerning its functional capabilities [19]. Thus, our findings emphasize that the persistence of recipient T cells after HCT does not necessarily indicate that rejection is likely to occur. Chimerism assays certainly can be used as a diagnostic indicator of rejection, but their utility as a prognostic indicator of rejection depends on other factors that affect the risk of rejection, such as the intensity of the conditioning regimen and the use of T cell depletion to prevent GVHD.

To some extent, the association between donor T cell chimerism and the subsequent risk of chronic GVHD can be considered when making decisions about the withdrawal of immunosuppressive treatment or enrollment of a patient in a clinical trial evaluating new approaches to preventing chronic GVHD. Routine chimerism testing at 2 and 6 months after HCT with myeloablative conditioning regimens also can be used to document engraftment in clinical trials. Our results demonstrating the absence of strong correlations with risks of recurrent malignancy, NRM, and OS apply only for chimerism testing in patients who have undergone HCT with a myeloablative conditioning regimen, and should not be extrapolated to patients who have received a nonmyeloablative conditioning regimen [8]. Although our findings apply to the use of routine chimerism testing for prognostic purposes, they have no relevance to the use of chimerism testing for diagnostic purposes.

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