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Clinical Research

Donor Chimerism Early after Reduced-Intensity Conditioning Hematopoietic Stem Cell Transplantation Predicts Relapse and Survival

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The impact of early donor cell chimerism on outcomes of T cell–replete reduced-intensity conditioning (RIC) hematopoietic stem cell transplantation (HSCT) is ill defined. We evaluated day 30 (D30) and 100 (D100) total donor cell chimerism after RIC HSCT undertaken between 2002 and 2010 at our institution, excluding patients who died or relapsed before D30. When available, donor T cell chimerism was also assessed. The primary outcome was overall survival (OS). Secondary outcomes included progression-free survival (PFS), relapse, and nonrelapse mortality (NRM). We evaluated 688 patients with hematologic malignancies (48% myeloid and 52% lymphoid) and a median age of 57 years (range, 18 to 74) undergoing RIC HSCT with T cell–replete donor grafts (97% peripheral blood; 92% HLA-matched), with a median follow-up of 58.2 months (range, 12.6 to 120.7). In multivariable analysis, total donor cell and T cell chimerism at D30 and D100 each predicted RIC HSCT outcomes, with D100 total donor cell chimerism most predictive. D100 total donor cell chimerism <90% was associated with increased relapse (hazard ratio [HR], 2.54; 95% confidence interval [CI], 1.83 to 3.51; $P < .0001$), impaired PFS (HR, 2.01; 95% CI, 1.53 to 2.65; $P < .0001$), and worse OS (HR, 1.50; 95% CI, 1.11 to 2.04, $P = .009$), but not with NRM (HR, .76; 95% CI, .44 to 2.27; $P = .33$). There was no additional utility of incorporating sustained D30 to D100 total donor cell chimerism or T cell chimerism. Low donor chimerism early after RIC HSCT is an independent risk factor for relapse and impaired survival. Donor chimerism assessment early after RIC HSCT can prognosticate for long-term outcomes and help identify high-risk patient cohorts who may benefit from additional therapeutic interventions.

INTRODUCTION

Reduced-intensity conditioning (RIC) allogeneic hematopoietic stem cell transplantation (HSCT) is critically dependent on the immunologic graft-versus-tumor response for its curative potential. In RIC HSCT, T cell depletion (TCD) with in vivo antithymocyte globulin–based conditioning appears associated with increased relapse and impaired survival [1]. In T cell–replete RIC HSCT, early donor engraftment and immune reconstitution kinetics remain variable and may predict the likelihood of graft-versus-tumor response and long-term survival.

The prognostic impact of donor chimerism early after T cell–replete RIC HSCT is ill defined. Data on total donor cell chimerism are especially scanty and discordant, as most studies have focused on donor T cell chimerism. Two small studies found no association of low total donor cell chimerism with outcomes [2,3], whereas other small studies, with varying chimerism thresholds and assessment time points, described an association with 4-week relapse risk [4] or impaired survival [5,6]. Several small studies have also described an association of donor T cell chimerism with impaired RIC HSCT outcomes [7-10]. However, in the largest study (n = 322), lack of full donor T cell chimerism was a predictor of relapse but not of progression-free survival (PFS) [11]. Additional limitations are that studies often included recipients of TCD (where increased mixed chimerism has been reported) and did not fully adjust for disease relapse likelihood (eg, Disease Risk Index [DRI] [12]) or other

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measures of early engraftment (eg, absolute lymphocyte count [ALC] [13,14] or WBC risk score [15]), which can also affect HSCT outcomes. The comparative utility of total donor cell versus T cell chimerism is also ill defined. Early donor cell chimerism has, therefore, not been incorporated into RIC HSCT prognostic models.

We undertook a retrospective analysis of adult T cell–replete RIC HSCT performed at our institution between 2002 and 2010 to comprehensively assess the impact of early donor chimerism on overall survival (OS). We sought to evaluate the following: (1) the impact of day 30 (D30) and day 100 (D100) total donor cell chimerism, (2) the additional utility of "sustained" total donor cell chimerism between D30 and D100, and (3) the additional utility of D30 and D100 donor T cell chimerism.

MATERIALS AND METHODS

Patients

At our institution, RIC HSCT is offered to adults with advanced and/or aggressive hematologic malignancies unsuitable for myeloablative transplantation because of disease, age, or comorbidities. Pretransplantation eligibility includes Eastern Cooperative Oncology Group performance status $\leq$2, adequate organ function (pulmonary, cardiac, renal, hepatic), and no active uncontrolled infections. Recipients are routinely consented to institutional review board–approved data collection.

T cell–replete RIC HSCT in 688 adult hematologic malignancy patients between 2002 and 2010 are included in the analysis. Patients who relapsed or died before the dates of D30 or D100 chimerism were excluded from the respective D30 or D100 analysis.

HSCT Regimen

Recipients received RIC fludarabine (30 mg/m^2 $\times$ 4 days) and busulfan (1.6 mg/kg i.v. once or twice daily $\times$ 4 days) followed by T cell–replete bone marrow or peripheral blood stem cell (PBSC) grafts. Graft-versus-host disease (GVHD) prophylaxis routinely comprised methotrexate on days 1, 3, 6, $\pm$11, with tacrolimus $\pm$ sirolimus starting day $-$3 and tapered through weeks 9 to 26 in the absence of GVHD. All patients received 12 months of infection prophylaxis, surveillance, and preemptive therapy. Donor lymphocyte infusions were at the discretion of the treating physician, but in the absence of relapse, they were used only in 2 patients to treat falling donor chimerism.

Chimerism Analysis

Post-transplantation D30 (range, 20 to 50) and D100 (range, 80 to 120) chimerism were determined using recipient peripheral blood (PB) or bone marrow samples collected in EDTA. Total donor cell chimerism was performed on buffy coat leukocytes. T cell chimerism was on Ficoll Hypaque–separated lymphocytes from which purified CD3$^+$ T cells were isolated using immunomagnetic beads (Stem Cell Technologies Inc., Vancouver, Canada). Pretransplantation PB samples were used to determine the donor and recipient genotypes based on 9 CODIS short tandem repeat loci using the Applied Biosystems Profiler Plus kit with the ABI 3130 capillary genetic analyzer (Thermo Fisher Scientific Inc., Waltham, MA) to determine the alleles at each locus. Informative alleles unique to either donor or recipient were used to calculate percent donor chimerism at each locus, using the median peak intensity of amplicons attributable to donor divided by the sum of all amplicons at that locus. In cases where there were only unique recipient amplicons, the percent donor chimerism was calculated as 100 less the percent recipient chimerism. The 95% confidence interval for chimerism was $\pm$5%.

Statistical Methods

Patient baseline characteristics were reported descriptively. OS and PFS were calculated with the Kaplan-Meier method. OS, PFS, cumulative incidence of relapse, and nonrelapse mortality (NRM) were defined previously [12,15]. The log-rank test was used for comparisons of Kaplan-Meier curves. The difference between cumulative incidence curves in the presence of a competing risk was tested with the Gray method [16]. The cumulative incidence of GVHD was also calculated in the competing risks framework, reflecting death or relapse without developing GVHD as a competing event. Potential prognostic factors for OS, PFS, relapse, and NRM were examined in the proportional hazards model, as well as in the competing risks regression model [17]. The models included pretransplantation factors (age, diagnosis, DRI, donor-recipient HLA-match, sex-match, ABO-match, cytomegalovirus serostatus, prior allotransplantation), transplantation factors (year, conditioning intensity, sirolimus use), and post-transplantation factors (WBC risk score based on 1- and 3-month WBC values, 1- and 3-month ALC). (Supplementary Table 1). The proportional hazards assumption for each variable of interest was tested and interaction terms were examined. The linearity assumption for continuous variables was examined by the use of restricted cubic spline estimates of the relationship between the continuous variable and log relative hazard [18], and the cutoff points of these variables were determined by the change of the log relative hazards. For total donor cell and T cell chimerism, this approach is supplemented with calculation of hazard ratios of PFS by intervals of 10 and 20, along with the consideration of the number of events in each interval. From these approaches, the proposed cutoff value for total donor cell chimerism was $<$90% (low) versus $\geq$90%, and $<$70% (low) versus $\geq$70% for donor T cell chimerism (Figure 1C,D). Although the linearity assumption for total donor cell and T cell chimerism values was not violated, we propose these cutoff values to facilitate the practical use of chimerism data. We compared predictive capacities via the concordance index (C-index) [19,20]. All P values are 2-sided with a significance level of .05. All calculations were performed with SAS 9.3 (SAS Institute, Cary, NC), and R 2.14.1 (the Comprehensive R Archive Network project).

RESULTS

Patients

Characteristics of the 688 patients are shown in Table 1. Diagnoses were 52% lymphoid and 48% myeloid malignancies. Seventeen percent had undergone prior allogeneic HSCT. Donors were 64% unrelated, 92% 8/8 HLA-matched, and 97% provided PBSC grafts. Median recipient age was 57 years (range, 18 to 74), with median donor age of 39 years (range, 12 to 73). Median follow-up in survivors was 58.2 months (range, 12.6 to 120.7).

Total Donor Cell Chimerism

D30 and D100 total donor chimerism data were available for 688 and 530 patients, respectively, as 146 patients died or relapsed before D100 and 12 had missing D100 chimerism. Low ($<$90%) total donor cell chimerism occurred in 26% of the cohort at D30 and in 21% at D100 (Figure 1A). Fourteen percent had persistent low total donor cell chimerism at both D30 and D100, identical for lymphoid and myeloid disease.

Total Donor Cell Chimerism and HSCT Outcomes

In univariable analyses, total donor cell chimerism was associated with better outcomes (Table 2). Low D30 total donor cell chimerism had higher 2- and 5-year relapse rates of 60% and 67%, compared with 44% and 47%, respectively ($P < .0001$) and worse 2- and 5-year PFS of 31% and 26%, compared with 47% and 36%, respectively ($P = .0001$). Five-year NRM was 10% compared with 17% ($P = .047$), and 5-year OS was 41% compared with 45% ($P = .046$).

Low D100 total donor cell chimerism had higher 2- and 5-year relapse rates of 53% and 60%, compared with 30% and 33%, respectively ($P < .0001$), worse 2- and 5-year PFS of 37% and 27%, compared with 59% and 48%, respectively ($P < .0001$), and worse 5-year OS of 44%, compared with 56% ($P = .0007$) (Figure 2A-C). The 1-year incidence of chronic GVHD was lower at 46%, compared with 58% ($P = .005$). The 5-year NRM did not differ (Figure 2C). The impact on lymphoid and myeloid disease was similar (Table 2, Supplementary Figure 1A-D).

In multivariable models, total donor chimerism was independently associated with better outcomes. Low D30 total donor cell chimerism was associated with higher relapse (HR, 1.66; $P < .0001$) and impaired PFS (HR, 1.41; $P = .0013$) but not with OS (HR, 1.08; $P = .50$) (Table 3). Low D100 total donor cell chimerism was associated with higher relapse ($P < .0001$) and impaired PFS (HR, 2.01; $P < .0001$) and OS (HR, 1.50; $P = .009$) but not with NRM (HR, .76; $P = .33$) (Table 3, Supplementary Table 1). Myeloid or lymphoid diagnosis did not affect the results of the analysis.