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Effect of Recipient Age and Stem Cell Source on the Association between Donor Telomere Length and Survival after Allogeneic Unrelated Hematopoietic Cell Transplantation for Severe Aplastic Anemia

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A B S T R A C T

We previously showed an association between donor leukocyte relative telomere length (RTL) and post-hematopoietic cell transplantation (HCT) survival in patients with severe aplastic anemia (SAA) who received bone marrow grafts at ages <40 years. Here, we tested the generalizability of the prior findings in an independent validation cohort and by recipient age and stem cell source in the combined discovery and validation cohorts. We used monoplex quantitative real-time PCR to measure RTL in: (1) a new SAA validation cohort of 428 patients (age range, .2 to 77 years) with available pretransplantation donor blood samples in the Center for International Blood and Marrow Transplant Research repository, and (2) 278 patients from the original cohort who had sufficient DNA to repeat RTL testing. We used Cox proportional hazard models to calculate hazard ratios (HRs), and 95% confidence intervals (CIs) across categories of donor RTL. Data from the validation cohort showed no association between donor RTL and patient survival, but further analysis identified differences by recipient age and stem cell source as the likely explanation. In patients <40 years, the HR comparing longest with shortest and middle RTL tertiles = .75; 95% CI, .44 to 1.30 versus HR = 1.05; 95% CI, .59 to 1.89 for patients ≥40 years, *P* interaction = .37. In bone marrow recipients, the HR = .68; 95% CI, .72 to 1.10 versus HR = 1.29; 95% CI, .64 to 2.62 for peripheral blood stem cell grafts; *P* interaction = .88. Analyses using data from the 2 cohorts showed a statistically significant survival benefit only in <40-year-old patients receiving bone marrow graft (HR comparing longest and middle RTL tertiles with shortest = .69; 95% CI, .50 to .95, *P* = .02). The study suggested that the association between donor RTL and post-HCT outcomes in recipients with SAA may vary by recipient age and stem cell source. A larger study is needed to account for multiple comparisons and to further test the generalizability of our findings.

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INTRODUCTION

Severe aplastic anemia (SAA) is a multifactorial life-threatening bone marrow (BM) failure disease. Most cases of SAA are thought to be acquired and due to an immune-mediated defect [1]. The disease affects all ages with a biphasic incidence distribution in adolescence and young adulthood (ages 10 to 25 years) and in the elderly (>60 years of age) [2]. Hematopoietic cell transplantation (HCT) is the first line of therapy for young SAA patients who have matched sibling donors, with a nearly 90% success rate [3]. Patients who lack a matched sibling donor or those older than 40 years typically receive at least 1 round of immunosuppression therapy before considering alternative donor HCT [4]. Despite

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advances in HLA-typing techniques and therapeutic strategies for HCT, long-term survival after unrelated donor HCT for SAA is still unsatisfactory [5-8]. Recipient age, stem cell source, and time between SAA diagnosis and HCT are the strongest risk factors for poor survival in both matched sibling and unrelated donor HCT for SAA [9-12]. We recently showed that among 330 patients who underwent HCT for SAA at age <40 years, receiving a graft from donors with long telomeres was associated with an approximately 40% reduction in mortality (hazard ratio [HR], .61; 95% confidence interval [CI], .44 to .86; $P = .006$), independent of donor age [13].

Telomeres, the tandem nucleotide repeats at chromosome ends, are markers of cellular replicative capacity, senescence, and biological aging [14]. Telomere length (TL) of newly engrafted cells shortens early after HCT as a consequence of the high degree of cellular replication needed to achieve engraftment [15-17]. Telomere shortening after HCT has been observed in HCT survivors for years after transplantation and has been associated with older donors, female gender, and graft-versus-host disease [18].

In the current study, we evaluated a new cohort of patients with SAA who received HCT in more recent years to validate our previous finding of a survival benefit associated with longer donor TL. We then used data from both the original and validation cohorts to test the effect of donor TL on patient survival by patient age and stem cell source.

METHODS

Data Source and Patient Eligibility

Participants in the validation cohort were recipients of allogeneic unrelated donor HCT for SAA at 1 of the HCT centers reporting to the Center for International Blood and Marrow Transplant Research (CIBMTR) database. The CIBMTR is a research collaboration between the National Marrow Donor Program (NMDP) "Be The Match Registry" and the Medical College of Wisconsin. It collects longitudinal clinical and HCT-related outcome data from a voluntary working group of more than 450 transplantation centers around the world.

The independent validation cohort included patients of all ages who received a first HCT for SAA between 1996 and 2013 and who had an available pretransplantation donor blood sample in the NMDP/CIBMTR research sample repository. In a combined analysis, we included a subset from the original cohort (n = 287) [13] who had sufficient DNA available for TL retesting (of them, 243 patients were <40 years of age and included in our previous study); patients who received umbilical cord transplantations were excluded from the analysis (n = 9). Supplementary Table S1 provides a comparison between demographics and clinical characteristics of the original and validation cohorts included in this study. Most notably, patients in the independent validation cohort were older and had more peripheral blood stem cell (PBSC) grafts than patients in the previous discovery cohort.

All patients and donors provided informed consent, and the study was approved by the NMDP institutional review board.

Telomere Length Measurement

We used QIAamp Maxi Kit procedure (Qiagen Inc., Valencia, CA) to extract DNA from donors' peripheral blood mononuclear cells or whole blood samples. We measured relative telomere length (RTL) using a monoplex quantitative real-time PCR (qPCR) assay adapted from methods described elsewhere [19,20]. Briefly, PCR was performed using 5-uL reaction volumes consisting of: 2.5 uL of 2X Rotor-Gene SYBR Green PCR Master Mix (Qiagen, Germantown, MD), 2.0 uL of molecular biology grade water, and .5 uL of 1 μM assay-specific mix of primers. Primers for the telomeric PCR were *Telo_FP* [5′-CGGTTT(GTTTGG)$_5$GTT-3′] and *Telo_RP* [5′-GGCTTG (CCTTAC)$_5$CCT-3′] [20]. Primers for the single-copy gene (36B4) PCR were *36B4_FP* [5′-CAGCAAGTGGGAAGGTGTAATCC-3′] and *36B4_RP* [5′-CCCATT CTATCATCAACGGGTACAA-3′] [19]. The LightCycler software (Release 1.5.0, Roche, Indianapolis, IN, USA) was used for initial analysis of raw data. Utilizing absolute quantification analysis with the second-derivative maximum method and high-sensitivity detection algorithm, single-target sequences were quantified and expressed as an absolute value (ng/uL) based on the internal standard curve of known concentrations. The concentration of telomere (T) signal was divided by the concentration of 36B4 (S) signal to yield a T/S ratio. This raw T/S ratio was then divided by the average T/S ratio of the internal QC calibrator samples, within the same plate, to yield the final standardized T/S ratio. For quality control, all telomeric and 36B4 reactions were measured in triplicate, and the average was used for final calculations. The mean coefficient of variations for the standardized T/S measure from replicate samples were 5.6% for the original cohort and 6.3% for the validation cohort.

Statistical Analysis

We used the Kaplan-Meier estimator to calculate the probability of overall survival (OS) and 95% CIs at 1 and 5 years after HCT. Follow-up time started at the date of HCT and ended at death or was censored at date of last follow-up or end of study in May 2015. The log-rank test was used to compare the survival distribution across categories of donor leukocyte RTL.

For multivariable analyses, we used Cox proportional hazard models to calculate the HRs and the 95% CI of death, comparing leukocyte RTL categories. The proportional hazard assumption was tested for all variables included in the model, and stratification was used if the proportionality assumption was not met. Variables included in the final model were selected based on a P threshold of .05 in a stepwise forward-backward procedure. We adjusted for donor age in all models to account for the association of RTL with age. Interactions between RTL and included clinical variables were tested and no significant interactions were detected.

To correct for differences between the discovery and validation cohorts (patients in the validation cohort were older and received more PBSC grafts than patients in the previous discovery cohort), we further stratified the analyses on patient age (<or ≥40 years) and stem cell source (BM or PBSC). Dichotomization of the age variable was based on a concern about the linearity of age effect in the Cox model—a statistical test shows that the linearity of age does not hold in the Cox model. From the model-fitting perspective, we identified age 37.5 as the optimal cut-off point for survival differences. Given that it is very similar to that used in clinical care setting (40 years), we elected to use age 40 as our cut-off point.

RTL was categorized in tertiles based on RTL measurement distribution in each batch to minimize the potential batch effect when combining data from multiple batches. SAS Versions 9.3 (SAS institute, Cary, NC) was used for all analyses.

RESULTS

Characteristics of Study Participants

The validation cohort included 428 patients who received HCT for acquired SAA at a median age of 22 years (range, .2 to 77 years); 71.4% were white, 53.5% were males, and 77.3% underwent HCT after 2007. BM grafts (75.2%), 8/8 HLA matching (65.7%), and nonmyeloablative or reduced-intensity regimens (55.4%) were the most commonly used. The median follow-up of survivors was 3 years (range, 9 months to 15 years). Table 1 summarizes patient demographics and clinical characteristics by age at HCT (<40, and ≥40 years). Briefly, younger patients were more likely to receive BM grafts (82% versus 59%, $P < .001$) and cyclosporine-based graft-versus-host disease prophylaxis (43% versus 30%, $P = .007$). However, there was no statistically significant difference in donor age, degree of HLA matching, or conditioning regimens (Table 1).

Donor RTL and Recipient Outcomes in the Validation Cohort

Overall, data from the validation cohort showed no statistically significant association between donor RTL and patient post-HCT survival (log-rank $P = .18$). The probabilities of 1-year post-HCT OS were 76% (95% CI , 69% to 83%) for the longest tertile, 70% (95% CI, 62% to 77%) for the middle, and 75% (95% CI, 67% to 81%) for the shortest. The 5-year OS were 71% (95% CI, 63% to 78%), 62% (95% CI, 53% to 70%), and 70% (95% CI, 62% to 77%), respectively.

In analyses stratified by recipient age, the data suggested a possible survival improvement associated with long donor RTL in patients <40 years (log-rank $P = .06$). At 1 year, OS in the longest tertile of donor RTL versus middle and shortest combined was 85% versus 76%, $P = .06$, and at 5 years was 81% versus 71%, $P = .06$. For patients ≥40 years, no association was noticed (log-rank $P = .51$); the OS probabilities