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Biomarkers

Relative Telomere Length before Hematopoietic Cell Transplantation and Outcome after Unrelated Donor Hematopoietic Cell Transplantation for Acute Leukemia

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Telomeres are tandem nucleotide repeats and a protein complex located at the end of the chromosomes maintaining genomic stability. Their potential as a predictive biomarker for outcomes after allogeneic hematopoietic cell transplant (HCT) in hematologic malignancies is still unclear. From the Center for International Blood and Marrow Transplant Research we randomly selected 536 acute leukemia patients from those who underwent myeloablative 8/8 HLA-matched unrelated donor HCT between 2005 and 2012 and who had an available pre-HCT blood sample in the repository. Relative telomere length (RTL) was measured by real-time quantitative PCR. We used Kaplan-Meier and competing risk estimators to calculate survival probability and cumulative incidence, respectively, across patient RTL tertiles. Cox proportional hazard regression was used for adjusted analyses. The study included 396 acute myeloid leukemia (AML) and 140 acute lymphoblastic leukemia (ALL) patients. Median age at HCT was 41 years (range, .5 to 66), and median follow-up for survivors was 5.1 years (range, .4 to 8.3). Significant inverse correlations between age and RTL were observed in patients with AML ($r = -.44$, $P < .0001$) and ALL ($r = -.48$, $P < .0001$). Patients with ALL had longer RTL than those with AML (.48 versus .43, respectively); the difference was not statistically significant after adjusting for patient age ($P = .96$). Pre-HCT RTL in acute leukemia patients was not statistically significantly associated with overall survival (HR for longest RTL compared with shortest, .91; 95% CI, .65 to 1.28), disease-free survival (HR, .90; 95% CI, .64 to 1.25), transplant-related mortality (HR, .97; 95% CI, .60 to 1.59), incidence of relapse (HR, .89; 95% CI, .56 to 1.40), neutrophil engraftment (HR, 1.06; 95% CI, .85 to 1.32), or grades II to IV acute graft-versus-host disease (HR, 1.11; 95% CI, .81 to 1.53), grades III-IV acute graft-versus-host disease (HR, .92; 95% CI, .54 to 1.59), and chronic graft-versus-host disease (HR, 1.10; 95% CI, .81 to 1.50). In this study, recipient pre-HCT RTL had no prognostic role in post-transplant outcomes in acute leukemia patients.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment option for patients with acute leukemia [1,2]. Despite recent advances in HLA matching, conditioning regimens, and supportive care, post-HCT mortality and morbidity risks are still high [3]. Leukemia relapse remains the main cause of death in most patients, followed by graft-versus-host disease (GVHD) and infections [3]. Identifying biomarkers for pre-HCT patient risk stratification is needed.

Telomeres are tandem hexanucleotide $(TTAGGG)_n$ repeats and a protein complex located at the end of the chromosomes. They shorten with each cell division and therefore are a marker for cellular aging [4]. Critically short telomeres trigger cellular senescence [5], p53-dependent apoptosis [6], and genomic instability [7]. Patients with hematologic ma-

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lignancies have been reported to have shorter telomeres than healthy control subjects [8,9]. Shorter telomeres in patients with leukemia were associated with the presence of aberrant karyotypes [8] and possibly unfavorable outcomes [10]. Telomere length was associated with leukemia outcomes in several small studies. For example, in a study of 97 pediatric and young adults with acute myeloid leukemia (AML), shorter telomere length after chemotherapy induction was associated with a delay in neutrophil recovery in later chemotherapy courses [11]. In a single-center study of 178 patients who underwent myeloablative matched-sibling HCT, mainly for hematologic malignancy, longer recipient pre-HCT telomere length was associated with a lower risk of treatment-related mortality (hazard ratio [HR], .4; 95% confidence interval [CI], .2 to .8) but no other outcome [12]. In the current study we evaluated the association between recipient pretransplant leukocyte relative telomere length (RTL) and post-transplant outcomes focusing on patients who received an 8/8 HLA-matched unrelated donor HCT for acute leukemia using myeloablative regimens.

METHODS

Data Source and Population Selection

Clinical and outcome data and pre-HCT blood samples for acute leukemia patients in this study were obtained from the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a research collaboration between the National Marrow Donor Program/Be The Match and the Medical College of Wisconsin. It was established in 2004 and includes a large network of more than 500 transplant centers worldwide that report baseline and longitudinal data on clinical parameters and transplant outcome. Reported data go through a rigorous validation and verification process to ensure high data quality.

This study included 536 patients with acute leukemia, randomly selected from patients with the following criteria: underwent an 8/8 HLA-matched unrelated donor HCT, transplanted between 2005 and 2012, received myeloablative conditioning, and had an available pre-HCT blood sample in the repository. The study was approved by the National Marrow Donor Program Institutional Review Board and the National Institutes of Health Office of Human Subjects Research Protections, and all patients provided informed consent.

Telomere Length Measurement

Genomic DNA were extracted from recipient peripheral blood mononuclear cells or whole blood samples using QIAamp Maxi Kit procedure (Qiagen Inc., Valencia, CA). Pretransplant leukocyte RTL was measured using a monoplex quantitative real-time PCR assay adopting the methods described previously [13-15]. In brief, the ratio between telomere signal concentration (T) to that of a single-copy gene (S; *36B4*) (T/S) was calculated for each sample and then divided by the average T/S ratio obtained from the internal QC calibrator samples in the same plate. Final T/S was exponentiated to ensure normal distribution. All telomere and 36B4 reactions were run in triplicate, and the average of the measurements was used for all calculations. Details are described elsewhere [16]. The mean coefficient of variation for the standardized T/S measure from replicate samples was 5%.

Outcome Definitions and Endpoints

Death from any cause was considered an event for overall survival (OS) analysis. Disease-free survival (DFS) was defined as survival without disease relapse. Transplant-related mortality (TRM) was defined as death during continuous complete leukemia remission. Neutrophil engraftment was defined as achieving an absolute neutrophil count $\geq .5 \times 10^9$/L for 3 consecutive days. Acute and chronic GVHD were diagnosed and graded according to previously described criteria [17,18].

Statistical Analysis

RTL was categorized into tertiles based on the distribution in the entire study cohort (short, RTL < .37; intermediate, RTL .37 to < .47; long, RTL ≥ .47). We calculated the probabilities of OS and DFS at 1, 3, and 5 years post-transplant using the Kaplan-Meier estimator. The log-rank test was used to compare probabilities across RTL categories.

For relapse and TRM we used cumulative incidence functions to account for the presence of competing risks. TRM was treated as a competing risk in the analysis of relapse, and relapse was used as a competing risk in the TRM analysis.

We used Cox proportional hazard regression for multivariate adjusted analysis and calculated the HRs and 95% CIs of post-HCT outcomes comparing long and intermediate RTL with short RTL. Follow-up started at the date of HCT and ended at the date of event, death, date of last follow-up, or end of study in October 14, 2014.

We used a stepwise forward-backward conditional procedure to select clinical variables to enter and stay in the model with a threshold of $P = .05$. Variables that violated the proportional hazard assumption were adjusted for through stratification. All P values were 2-sided, and $P < .05$ was considered to be statistically significant. All statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics

The study included 396 patients with AML and 140 patients with acute lymphoblastic leukemia (ALL). The median age at HCT for all patients was 41 years (range, .5 to 66), for AML patients was 45 years (range, .6 to 66), and for ALL patients was 30 years (range, .5 to 62). Most patients in this study received HCT in first complete remission (n = 359, 67%) and received peripheral blood stem cells (n = 382, 71%). The median follow-up for survivors was 5.1 years (range, .4 to 8.3).

RTL negatively correlated with age in all patients (r = –.47, $P < .0001$) and in patients with AML (r = –.44, $P < .0001$) and ALL (r = –.48, $P < .0001$) (Figure 1). Patients with ALL had longer RTL than those with AML (mean T/S, .48 versus .43, respectively; $P < .001$). This RTL difference was not statistically significant after adjusting for patient age ($P = .96$). Table 1 summarizes patient demographics and clinical factors by recipient RTL.

Association between Recipient Pre-HCT RTL and Survival Outcomes after HCT

Post-HCT OS and TRM were not associated with patient pre-HCT RTL. For DFS, only 5-year probabilities showed statistically significant differences by pre-HCT RTL (39%, 48%, and 54% for the short, intermediate, and long RTL, respectively; $P = .03$) (Figure 2 and Table 2).

In multivariable analyses, no significant association was observed with any survival outcome. The HRs comparing the longest with shortest RTL tertile were .91 for OS (95% CI, .65 to 1.28), .97 for TRM (95% CI, .60 to 1.59), and .90 for DFS (95% CI, .64 to 1.25) (Table 3).

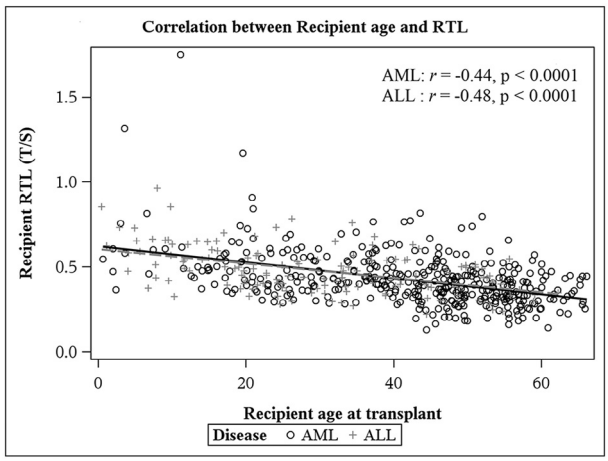

Figure 1. Scatter plots of the correlation between RTL and age in patients with AML and ALL.