Original Investigation

Association Between Donor Leukocyte Telomere Length and Survival After Unrelated Allogeneic Hematopoietic Cell Transplantation for Severe Aplastic Anemia

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IMPORTANCE Telomeres protect chromosome ends and are markers of cellular aging and replicative capacity.

OBJECTIVE To evaluate the association between recipient and donor pretransplant leukocyte telomere length with outcomes after unrelated donor allogeneic hematopoietic cell transplantation (HCT) for patients with severe aplastic anemia.

DESIGN, PARTICIPANTS, AND SETTING The study included 330 patients (235 acquired, 85 Fanconi anemia, and 10 Diamond-Blackfan anemia) and their unrelated donors who had pre-HCT blood samples and clinical and outcome data available at the Center for International Blood and Marrow Transplant Research. Patients underwent HCT between 1989 and 2007 in 84 centers and were followed-up to March 2013.

EXPOSURES Recipient and donor pre-HCT leukocyte telomere length classified into long (third tertile) and short (first and second tertiles combined) based on donor telomere length distribution.

MAIN OUTCOMES AND MEASURES Overall survival, neutrophil recovery, and acute and chronic graft-vs-host disease, as ascertained by transplant centers through regular patient follow-up.

RESULTS Longer donor leukocyte telomere length was associated with higher survival probability (5-year overall survival, 56%; number at risk, 57; cumulative deaths, 50) than shorter donor leukocyte telomere length (5-year overall survival, 40%; number at risk, 71; cumulative deaths, 128; $P = .009$). The association remained statistically significant after adjusting for donor age, disease subtype, Karnofsky performance score, graft type, HLA matching, prior aplastic anemia therapy, race/ethnicity, and calendar year of transplant (hazard ratio [HR], 0.61; 95% CI, 0.44-0.86). Similar results were noted in analyses stratified on severe aplastic anemia subtype, recipient age, HLA matching, calendar year of transplant, and conditioning regimen. There was no association between donor telomere length and neutrophil engraftment at 28 days (cumulative incidence, 86% vs 85%; HR, 0.94; 95% CI, 0.73-1.22), acute graft-vs-host disease grades III-IV at 100 days (cumulative incidence, 22% vs 28%; HR, 0.77; 95% CI, 0.48-1.23), or chronic graft-vs-host disease at 1-year (cumulative incidence, 28% vs 30%; HR, 0.81; 95% CI, 0.53-1.24) for long vs short, respectively. Pretransplant leukocyte telomere length in the recipients was not associated with posttransplant survival (HR, 0.91; 95% CI, 0.64-1.30).

CONCLUSIONS AND RELEVANCE Longer donor leukocyte telomere length was associated with increased 5-year survival in patients who received HCT for severe aplastic anemia. Patient leukocyte telomere length was not associated with survival. The results of this observational study suggest that donor leukocyte telomere length may have a role in long-term posttransplant survival.

JAMA. 2015;313(6):594-602. doi:10.1001/jama.2015.7

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Telomeres are long $(TTAGGG)_n$ tandem nucleotide repeats and an associated protein complex located at chromosome ends that are essential for maintaining chromosomal stability. Telomeres shorten with each cell division due to the inability of DNA polymerases to completely replicate the chromosome ends. Consequently, telomeres are markers of cellular replicative capacity, cellular senescence, and aging.[1]

Aplastic anemia is a heterogeneous bone marrow failure disorder of multifactorial etiology. Inherited bone marrow failure is caused by germline defects in telomere biology (eg, dyskeratosis congenita), ribosomal function (eg, Diamond-Blackfan anemia or Shwachman Diamond syndrome), or DNA repair (eg, Fanconi anemia).[2] The acquired form of aplastic anemia is typically immune-mediated[3] and may also be related to environmental exposures.[4] Allogeneic hematopoietic cell transplantation (HCT) is recommended as initial therapy for young patients with acquired severe aplastic anemia when a matched sibling donor is available. Older severe aplastic anemia patients or those who do not have a sibling donor typically receive at least one course of immunosuppressive therapy before considering unrelated donor HCT.[5] HCT requires extensive replication and rapid expansion of transplanted donor cells to achieve engraftment.[6,7] Several studies have demonstrated accelerated posttransplant telomere shortening in the transplanted hematopoietic cells when compared with age-matched controls or matched donors.[7-10] Older donors,[8] female sex, and chronic graft-vs-host disease were associated with shorter telomeres in transplanted hematopoietic cells after allogeneic HCT.[7] Short telomeres in patients with acquired severe aplastic anemia are associated with relapse, clonal evolution, and poor survival after immunosuppressive therapy.[11]

In this study, we evaluated the association between pre-HCT recipient and donor relative leukocyte telomere length and outcomes after HCT in patients with severe aplastic anemia.

Methods

Data Source

We identified patients who received an allogeneic HCT for severe aplastic anemia facilitated by the National Marrow Donor Program (NMDP) and who met study eligibility listed below. The NMDP is a nonprofit organization that manages the Be The Match Registry to facilitate unrelated HCT for those who lack a matched related donor.

Clinical and outcome data from study participants were obtained from the Center for International Blood and Marrow Transplant Research (CIBMTR) database. The CIBMTR is a research collaboration between the NMDP and the Medical College of Wisconsin and collects data from a voluntary working group of more than 450 transplant centers around the world. Participating centers contribute comprehensive baseline and longitudinal follow-up data including patient and donor demographics, patient diagnosis, pretransplant therapy, and clinical parameters, other transplant-related information, and patient outcomes.

Study Participants and Patient Eligibility Criteria

Patients were eligible for the study if they received a first HCT for severe aplastic anemia prior to age 40 years, between the years 1989 and 2007, and from an unrelated donor and if there were an available pretransplant blood sample from both the patient and the donor in the NMDP-CIBMTR Research Sample Repository.

Of the 518 patients who underwent unrelated donor HCT with a high-resolution HLA typing for severe aplastic anemia during the study period at 97 centers, 342 met study eligibility criteria. Fifty-nine patients were excluded because they were older than 40 years and 117 patients because of the unavailability of pre-HCT blood sample for either the recipient or the donor. We excluded 12 patients from the final analysis because of failed DNA extraction or telomere length assay. The final analyses included 330 patients from 84 centers.

All patients and donors provided informed consent, and the study was approved by the NMDP Institutional Review Board. Cord blood transplants were excluded from this study.

Definitions of Study End Points and Selected Clinical and Demographic Variables

The study outcomes included overall survival, neutrophil and platelet recovery, and acute and chronic graft-vs-host disease. Neutrophil recovery was defined as an absolute neutrophil count of 0.5×10^9/L or higher for 3 consecutive days. Platelet recovery was defined as a platelet count of 20×10^9/L or higher independent of transfusions for 7 consecutive days. Acute and chronic graft-vs-host disease were defined according to standard criteria.[12,13]

Conditioning regimens were classified as myeloablative, reduced intensity, or nonmyeloablative according to standard CIBMTR definitions.[14]

Patient race/ethnicity was reported by the transplant center using standardized CIBMTR forms while donor race/ethnicity was self-identified at the time of registration to the NMDP registry. Recipient and donor race for this study were classified as white, African American, or other if Asian, Pacific Islander, Hispanic, Native American, or other/unknown race.

Telomere Length Measurement

We used samples of peripheral blood mononuclear cells or whole blood collected, processed, and stored in liquid nitrogen or at −80°C at the NMDP-CIBMTR repository using standard operating procedures. Samples were transferred to the DNA extraction facility on dry ice at which time QIAamp Maxi Kit procedure (QIAGEN Inc) was used to extract DNA from all samples. While at the DNA extraction facility, all samples went through a tightly regulated, automated DNA staging procedure; DNA volume was quantified using PicoGreen, normalized to 50 ng/μL, plated, and stored in a robotic automated cold storage unit until transferred (shipped overnight on dry ice) to the telomere assay laboratory. We measured relative leukocyte telomere length in extracted DNA using monoplex quantitative real-time polymerase chain reaction (qPCR), as previously described.[15] Briefly, the qPCR assay calculates the ratio between telomeric repeat copy number (T) and that of a single-reference gene (β-globin gene; 36B4) (S). Relative T/S is calcu-

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lated in relation to a reference curve and final measurements are exponentiated to ensure normality. Details are available elsewhere.[16] For quality control, all telomeric and 36B4 reactions were measured in triplicate, and the average was used for final calculations. The mean coefficient of variation among all samples was 0.6% for the telomere assay, 0.34% for the 36B4 assay. The mean coefficient of variation for the exponentiated T/S measure from quality control samples was 13.2%. Laboratory personnel were blinded to the recipient and donor status, participant age, and recipient outcome.

Statistical Analysis

We used the Kaplan-Meier estimator to calculate the probability of overall survival and 95% CIs at 1, 3 and 5 years post-HCT. Follow-up time started at the date of HCT, and ended at death or censored at date of last follow-up (only 3 patients were lost to follow-up) or at the end of study on March 22, 2013. The log-rank test was used to compare the survival distribution across categories of leukocyte telomere length. The cumulative incidence estimator was used to calculate the probabilities of neutrophil recovery, platelet recovery, and acute and chronic graft-vs-host disease; death was treated as a competing event for those analyses.

For multivariable analyses, we calculated hazard ratios (HRs) and the 95% CI of the outcome of interest, comparing leukocyte telomere length categories using Cox regression models. The proportional hazard assumption was tested for all variables included in the model, and stratification was used if the proportionality assumption was not met. The associations between recipient or donor leukocyte telomere length and outcomes of interest have been tested in separate models. Variables included in the model were selected by a stepwise forward-backward procedure with a *P* threshold of .05 for both entry and retention in the model. All variables presented in **Table 1** were tested for their eligibility to stay in the final model. Final models for both recipient and donor leukocyte telomere length analyses for survival outcome included: corresponding leukocyte telomere length and age, severe aplastic anemia subtype, Karnofsky performance score, HLA matching, prior therapy for aplastic anemia, race/ethnicity, and calendar year of transplant. Final models for neutrophil recovery were adjusted for patient age, disease subtype, donor age, HLA matching, graft type, and graft-vs-host disease prophylaxis. Final models for acute graft-vs-host disease grades III-IV were adjusted for patient age, HLA matching, T-cell depletion, and calendar year of transplant. Final models for chronic graft-vs-host disease were adjusted for patient age, donor-recipient cytomegalovirus matching status, cyclophosphamide, and total body irradiation given.

Interactions between leukocyte telomere length and clinical covariates were tested, and none were statistically significant. Wald test was used to compare stratum-specific HRs. We also tested for the association between patient survival and leukocyte telomere length in the recipient and donor simultaneously by including them in 1 model.

Pearson correlation coefficient was used to evaluate the strength of association between leukocyte telomere length and age. Leukocyte telomere length for both recipient and donor analyses was categorized into 3 categories based on the leukocyte telomere length tertiles in the donors (T/S <0.62, 0.62-<0.81, or ≥0.81). In final analyses, leukocyte telomere length was categorized into short if less than 0.81, or long if 0.81 or higher. Combining the 2 shortest telomere length categories was a post hoc decision based on the similarity of the calculated HRs between the shortest and intermediate categories in the final model.

For all statistical tests, 2-sided $P < .05$ was considered statistically significant. We used SAS versions 9.2 and 9.3 (SAS Institute Inc) for all analyses.

Results

Characteristics of Study Participants

Patients in this study were predominantly white (71%) with a nearly equal sex distribution (53% men). They underwent transplant at a median age of 15 years (range, 0.5-39 years). Two hundred thirty-five patients (71%) had acquired severe aplastic anemia, 85 (26%) had Fanconi anemia, and 10 (3%) had Diamond-Blackfan anemia. Donors were older than recipients (donor median age, 36 years; range, 19-57 years), and 55% were sex-matched to the recipients. Eighty-eight percent of patients received bone marrow grafts, 45% received a myeloablative-conditioning regimen, and 46% had an 8/8 HLA-matched donor graft. The most commonly myeloablative regimens used—95 of 121 patients (78.5%)—were cyclophosphamide and total-body irradiation of more than 500 cGy for a single dose or more than 800 cGy fractionated with or without etoposide (VP16). The most commonly used reduced intensity regimen—95 of 108 patients (88%)—total body irradiation of more than 200 cGy but not exceeding 500 cGy in a single dose or more than 200 cGy and not exceeding 800 cGy in fractionated doses. The most commonly used nonmyeloablative regimen—34 of 74 patients (45.9%)—was 200 cGy total-body irradiation. Post-HCT follow-up for survivors ranged from 5 months to 21 years (median, 6 years). Table 1 summarizes patient demographics and clinical and HCT characteristics by leukocyte telomere length categories.

Leukocyte telomere length was inversely correlated with age in both donors (r, −0.20; $P < .001$) and recipients (r, −0.31; $P < .001$). Even though donors were older, patients with severe aplastic anemia had significantly mean (SD) shorter leukocyte telomere lengths (0.66 [0.27]), than their donors (0.77 [0.22]), even after adjusting for age (adjusted mean leukocyte telomere length difference comparing recipients with donors, −0.18; $P < .001$).

Recipient Pretransplant Leukocyte Telomere Length and Post-HCT Outcome

Longer pretransplant leukocyte telomere length in the recipients was not associated with better survival after HCT. The overall survival probability of recipients with long vs short pre-HCT leukocyte telomere length were 52% vs 55% at 1 year ($P = .65$), 50% vs 50% at 3 years ($P = .99$), and 47% vs 46% at 5 years ($P = .87$). Multivariable models adjusting for age, disease subtype, Karnofsky performance score, HLA matching, prior therapy for aplastic anemia, graft type, race/ethnicity, and

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Table 1. Characteristics of Hematopoietic Cell Transplant Recipients for Severe Aplastic Anemia by Donor Leukocyte Telomere Length Categories

	No. (%) by Tertile of Donor Telomere Length			
	First, T/S<0.62 (n = 106)	Second, T/S 0.62-<0.81 (n = 111)	Third, T/S≥0.81 (n = 113)	*P* Value
Age at HCT, median (range), y	14 (<1-39)	17 (<1-39)	11 (<1-39)	.02
Age at HCT in decades				
0-9	29 (27)	23 (21)	42 (37)	.09
10-19	40 (38)	47 (42)	42 (37)	
20-29	26 (25)	22 (20)	16 (14)	
30-<40	11 (10)	19 (17)	13 (12)	
Race				
White	77 (73)	83 (75)	75 (66)	.09
Black	6 (6)	11 (10)	13 (12)	
Others	23 (21)	17 (15)	25 (22)	
Men	52 (49)	65 (59)	59 (52)	.36
Pre-HCT Karnofsky score ≥90[a]	82 (80)	79 (75)	92 (86)	.11
HLA matching				
8/8	51 (48)	53 (48)	48 (42)	.23
7/8	26 (25)	33 (30)	32 (28)	
6/8	19 (18)	15 (14)	22 (19)	
≤5/8	10 (9)	10 (8)	11 (10)	
Graft type				
Bone marrow	98 (92)	95 (86)	99 (88)	.27
Peripheral blood stem cell	8 (8)	16 (14)	14 (12)	
Disease Subtype				
Acquired aplastic anemia	80 (75)	85 (77)	70 (62)	.09
Fanconi anemia	22 (21)	24 (22)	39 (34)	
Diamond-Blackfan anemia	4 (4)	2 (2)	4 (4)	
Conditioning regimen				
Myeloablative	48 (45)	37 (33)	36 (32)	.24
Reduced intensity	29 (27)	37 (33)	42 (37)	
Nonmyeloablative	18 (17)	29 (26)	27 (24)	
Other	11 (10)	8 (7)	8 (7)	
Donor/recipient sex match				
Male/male	35 (33)	39 (35)	40 (35)	.67
Male/female	29 (27)	29 (26)	30 (27)	
Female/male	17 (16)	26 (23)	19 (17)	
Female/female	25 (24)	17 (15)	24 (21)	
Prior severe aplastic anemia therapy				
None	9 (8)	11 (10)	17 (15)	.76
Androgens but no corticosteroids	10 (9)	6 (5)	11 (10)	
Corticosteroids but no androgens	53 (50)	52 (47)	47 (42)	
Both androgens and corticosteroids	11 (10)	11 (10)	13 (12)	
Others	16 (15)	21 (19)	15 (13)	
Missing/unknown	7 (7)	10 (9)	10 (9)	
Recipient age at severe aplastic anemia diagnosis, y				
<20	80 (75)	75 (68)	86 (76)	.31
≥20	25 (24)	30 (27)	21 (19)	
Missing	1 (1)	6 (5)	6 (5)	
Aplastic anemia diagnosis to HCT, median (range), mo	20 (<1-318)	24 (1-340)	19 (2-186)	.66

(continued)

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Table 1. Characteristics of Hematopoietic Cell Transplant Recipients for Severe Aplastic Anemia by Donor Leukocyte Telomere Length Categories (continued)

	No. (%) by Tertile of Donor Telomere Length			
	First, T/S<0.62 (n = 106)	Second, T/S 0.62-<0.81 (n = 111)	Third, T/S≥0.81 (n = 113)	*P* Value
Graft-vs-host disease prophylaxis				
Tacrolimus + methotrexate or mycophenolate mofetil or steroids ± other	17 (16)	30 (27)	15 (13)	
Tacrolimus or mycophenolate mofetil ± other	2 (2)	2 (2)	2 (2)	
Cyclosporine + methotrexate ± other	46 (43)	41 (37)	41 (36)	.21
Cyclosporine ± other or no methotrexate	13 (12)	11 (10)	17 (15)	
T-cell depletion	28 (26)	27 (24)	36 (32)	
Others[b]	0	0	2 (2)	
Donor/recipient cytomegalovirus match				
Negative/negative	32 (30)	39 (35)	36 (32)	
Negative/positive	28 (26)	32 (29)	36 (32)	
Positive/negative	19 (18)	11 (10)	14 (12)	.66
Positive/positive	27 (25)	27 (24)	25 (22)	
Unknown	0	2 (2)	2 (2)	
Donor age, median (range), y	39 (21-57)	36 (19-57)	33 (19-53)	.003
18-29	20 (19)	35 (32)	44 (39)	
30-39	36 (34)	37 (33)	33 (29)	.04
40-49	43 (41)	32 (29)	33 (29)	
≥50	7 (7)	7 (6)	3 (3)	
Years of transplant				.27
1988-1995	27 (25)	25 (23)	21 (19)	
1996-1999	33 (31)	27 (24)	26 (23)	
2000-2007	46 (43)	59 (53)	66 (58)	
Follow-up of survivors, median (range), mo	84 (17-248)	74 (5-221)	75 (11-217)	.62

Abbreviation: HCT, hematopoietic cell transplantation.

[a] The Karnofsky score is a performance scale index to quantify patients' functional abilities and general well-being. It ranges from 0 (dead) to 100 (normal). Scores of 90 or more indicate that the patient is able to carry on normal activities.

[b] Others include tacrolimus, mycophenolate mofetil, and methotrexate with or without other immunosuppressive medication (no cyclosporine).

calendar year of transplant showed similar results (number of events in the long telomere length, 51; short telomere length, 113; HR, 0.91; 95% CI, 0.64-1.30; *P* = .59; **Table 2**).

Additionally, there was no association between recipient pre-HCT leukocyte telomere length and the cumulative incidence of neutrophil recovery 28 days after HCT (86% vs 86%, *P* = .82; number of events in the long telomere length, 86; in the short telomere length, 174; HR, 1.03; 95% CI, 0.78-1.37; *P* = .83), acute graft-vs-host disease grades III-IV at 100 days (25% vs 24%, *P* = .88; number of events in the long telomere length, 24; short telomere length, 48; HR, 1.21; 95% CI, 0.74-2.00; *P* = .45), or chronic graft-vs-host disease at 1 year (29% vs 30%, *P* = .88; number of events in the long telomere length, 28; short telomere length, 59; HR, 1.00; 95% CI, 0.62-1.60; *P* = .98) in the long vs short pre-HCT recipient leukocyte telomere length. Adjusted hazard ratios are summarized in Table 2.

Donor Leukocyte Telomere Length and Recipient Post-HCT Outcomes

Longer donor leukocyte telomere length was associated with a significantly higher probability of post-HCT overall survival in patients with severe aplastic anemia. Patient overall survival probabilities at 1 year were 46% (95% CI, 37%-56%) for the shortest, 53% (95% CI, 43%-62%) for the intermediate, and 61% (95% CI, 52%-70%) for the longest tertiles (*P* = .09); at 2 years they were 41% (95% CI, 32%-51%) for the shortest, 50% (95% CI, 41%-59%) for the intermediate, and 59% (95% CI, 50%-68%) for the longest tertiles (*P* = .03); and at 3 years were 38% (95% CI, 29%-47%) for the shortest, 49% (95% CI, 40%-58%) for the intermediate, and 59% (95% CI, 50%-68%) for the longest tertiles (*P* = .006). Multivariable analysis comparing the second or third tertile of donor telomere length with the first tertile showed statistically significant results only in patients who received their transplants from donors with telomere length in the longest tertile (HR comparing second to first tertile, 0.92; 95% CI, 0.65-1.29; *P* = .61; HR comparing third to first tertile, 0.59; 95% CI, 0.41-0.86; *P* = .006).

The survival probabilities for patients who received HCT from donors with long (longest tertile) vs short telomeres (first and second tertiles combined) were 60% vs 50% at 1 year (*P* = .07), 58% vs 44% at 3 years (*P* = .01), and 56% vs 40% at 5 years (*P* = .009; **Figure 1**). Multivariable analysis showed a lower probability of post-HCT death for recipients who received their transplants from a donor with long leukocyte telomere length (HR, 0.61; 95% CI, 0.44-0.85; *P* = .006). This finding was independent of donor age (Table 2), and results were not altered by severe aplastic anemia subtype (acquired vs inherited, *P* for interaction = .71). When including recipient leukocyte telomere length in the model, neither it nor its interaction with donor measures were statistically significant (recipient telomere length, *P* = .73; *P* for interaction = .48).

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Table 2. Hematopoietic Cell Transplantation Outcomes in Patients With Severe Aplastic Anemia Comparing Long to Short Leukocyte Telomere Length

	Recipient Leukocyte Telomere Length				Donor Leukocyte Telomere Length			
	No. Events/ Sample Size		Adjusted HR (95% CI)[a]	*P* Value	No. Events/ Sample Size		Adjusted HR (95% CI)[a]	*P* Value
	Long	Short			Long	Short		
Overall survival	51/92	113/193	0.91 (0.64-1.30)	.59	53/113	134/215	0.61 (0.44-0.86)	.006
Neutrophil recovery	86/92	174/192	1.03 (0.78-1.37)	.83	102/111	195/215	0.94 (0.73-1.22)	.64
Acute graft-vs-host disease grade III-IV	24/92	48/195	1.21 (0.74-2.00)	.45	25/113	61/217	0.77 (0.48-1.23)	.27
Chronic graft-vs-host disease	28/89	59/191	1.00 (0.62-1.60)	.98	33/111	66/212	0.81 (0.53-1.24)	.34

Abbreviation: HR, hazard ratio.

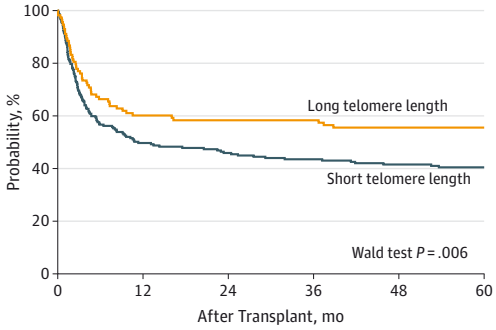
[a] All models compared long (T/S≥0.81) with short leukocyte telomere length (T/S<0.81). The overall survival model is adjusted for patient age in the recipient leukocyte telomere length model or donor age in the donor leukocyte telomere length model, disease subtype, Karnofsky performance score, HLA matching, prior therapy for aplastic anemia, race/ethnicity, graft type, and calendar year of transplant. The neutrophil recovery model is adjusted for patient age, disease subtype, donor age, HLA matching, graft type, and graft-vs-host disease prophylaxis. Acute graft-vs-host disease grades III-IV model is adjusted for patient age, HLA matching, T-cell depletion, and calendar year of transplant. The chronic graft-vs-host disease model is adjusted for patient age, cytomegalovirus matching status, cyclophosphamide and total-body irradiation given.

Longer donor leukocyte telomere length was associated with a similar survival advantage (*P* = .94) in young (HR, 0.61; 95% CI, 0.40-0.93; *P* = .02) and relatively older (HR, 0.61; 95% CI, 0.29-1.25; *P* = .18) recipients in this study when multivariable models were stratified by recipient age (≤19 years and >19 years). Stratified analyses of survival outcome in patients with severe aplastic anemia by HCT preparative regimen or HLA matching revealed a similar pattern. Similarly, analysis stratified on the combined effect of recipient age and HLA matching was consistent with previous observation except for an attenuated inverse association in relatively older patients with HLA matching at 7/8 or less. No statistical significant difference (*P* = .88) was observed when stratifying the analysis by calendar year of transplant. The HR for patients who received a transplant between 1988-1999 was 0.60 (95% CI, 0.38-0.96) and for 2000-2007 was 0.69 (95% CI, 0.39-1.21; **Table 3**).

Donor leukocyte telomere length was not associated with neutrophil recovery at 28 days post-HCT (86% vs 85%, *P* = .90) or at 100 days (95% and 91%, *P* = .13) or with platelet engraftment at 100 days (67% and 59%, *P* = .19), acute graft-vs-host disease grades III-IV at 100 days (22% vs 28%, *P* = .20), or chronic graft-vs-host disease at 1 year (28% vs 30%, *P* = .62) and at 2 years (32% and 34%, *P* = .72) for the long vs short donor leukocyte telomere length, respectively (**Figure 2**). Table 2 summarizes results from multivariable models.

Review of causes of death reported by centers did not identify a statistically significant difference in reported mortality causes by donor leukocyte telomere length (See the eTable in the Supplement for cause of death by tertiles of donor leukocyte telomere length.)

Figure 1. Probability of Overall Survival After Hematopoietic Cell Transplant for Patients With Severe Aplastic Anemia by Donor Leukocyte Telomere Length



No. at risk

Telomere length						
Short (T/S <0.81)	216	106	95	89	80	70
Long (T/S ≥0.81)	113	67	64	63	57	57

Discussion

Although survival after HCT has improved over the last decade, the survival outcomes of unrelated donor transplants for severe aplastic anemia are still unsatisfactory with a 5-year survival probability of 39% to 62%.[17-20] Previous studies suggest that optimal donor selection is a key parameter for improving survival in those patients.[21,22] In our study, longer donor leukocyte telomere length was associated with a higher overall survival probability in patients with severe aplastic anemia who underwent allogeneic unrelated HCT (5-year overall survival was 56% vs 40% in the long vs short donor leukocyte telomere length group, respectively). After adjusting for donor age and clinical factors associated with survival following HCT in severe aplastic anemia, the risk of post-HCT all-cause mortality remained approximately 40% lower in patients receiving HCT from donors with long vs short leukocyte telomere length. We observed similar patterns in patients with acquired severe aplastic anemia (5-year overall survival, 61% vs 46%), Fanconi anemia and Diamond-Blackfan anemia combined (5-year overall survival, 47% vs 23%), and by disease subtype (5-year overall survival in Fanconi anemia, 46% vs 22%; in Diamond-Blackfan anemia, 50% vs 33%).

Telomere length is a biological marker for cellular aging and replicative capacity and could explain, in part, the previously reported association between donor age and outcomes after

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Table 3. Donor Leukocyte Telomere Length and Hematopoietic Cell Transplant Overall Survival in Patients With Severe Aplastic Anemia Stratified by Disease Subtype and Selected Transplant Covariables

Variables	No. of Events/Sample Size in Leukocyte Telomere Length		Adjusted HR (95% CI)[a]	*P* Value
	Long	Short		
Disease Subtype				
Acquired aplastic anemia	28/70	93/164	0.64 (0.41-1.0)	.05
Inherited aplastic anemia, Fanconi anemia and Diamond-Blackfan anemia combined	25/43	41/51	0.61 (0.36-1.03)	.06
Conditioning Regimen				
Myeloablative	21/36	61/84	0.56 (0.31-1.0)	.05
Reduced intensity or non-myeloablative	28/69	66/113	0.62 (0.38-1.0)	.05
HLA matching				
8/8	15/48	51/103	0.51 (0.26-0.98)	.04
≤7/8	38/65	83/112	0.61 (0.40-0.94)	.02
Recipient age, y				
≤19	39/84	85/138	0.61 (0.40-0.93)	.02
>19	14/29	49/77	0.61 (0.29-1.25)	.18
Combined effect of recipient age and HLA matching, y				
≤19 y and 8/8	11/36	27/62	0.59 (0.26,1.37)	.22
≤19 y and ≤7/8	28/48	58/76	0.58 (0.33,1.01)	.06
>19 y and 8/8	4/12	24/41	0.25 (0.04,1.42)	.12
>19 y and ≤7/8	10/17	25/36	0.94 (0.34,2.57)	.90
Calendar year at transplant				
1988-1999	32/47	84/111	0.60 (0.38-0.96)	.03
2000-2007	21/66	50/104	0.69 (0.39-1.21)	.19

[a] All models compared long (T/S≥0.81) to short leukocyte telomere length (T/S<0.81); hazard ratio and 95% confidence interval adjusted for donor age, Karnofsky performance score, prior therapy for aplastic anemia, race/ethnicity, graft type, calendar year of transplant, disease subtype (except for model stratified on it), and HLA matching (except for model stratified on it).

HCT. For example, in a large study involving 6978 unrelated HCT recipients, a significant decline in survival was noted with higher donor age.[23] Donor age has also been reported to predict recipient risk of post-HCT obstructive lung disease,[24] secondary graft failure,[25] and B-cell lymphoproliferative disorder.[26] Of note, no association between donor leukocyte telomere length and incidence of secondary graft failure was present in our study at 42 days after HCT in the long and short leukocyte telomere length, respectively (8% vs 7%, *P* = .88). In solid organ transplantation, donor age is one of the main variables guiding graft selection.[27] Biological markers of graft aging including telomere length or *POT1* (protection of telomeres 1) gene expression have been associated with recipient's outcomes after both liver[28] and kidney transplant.[29,30]

Our study showed no statistically significant association between donor leukocyte telomere length and post-HCT primary engraftment. These results are concordant with previously reported data[31] on 47 transplant recipients that found a positive correlation between donors' hematopoietic stem cell telomere length and cellular regenerative capacity in vitro but found no association with recipient hematopoietic recovery. On the other hand, donor telomere length correlated with time to granulocyte recovery in a small study including 19 children.[32]

Our data show that patients with severe aplastic anemia have shorter pretransplant age-adjusted leukocyte telomere length than their donors but there was no association between their pretransplant leukocyte telomere length and post-HCT outcomes. Our results are in agreement with a previous report of 178 patients (86% of them transplanted for a hematological malignancy)[33] that found no statistically significant association between recipient pre-HCT leukocyte telomere length and overall survival, engraftment, acute or chronic graft-vs-host disease. However, a statistically significant association was observed with transplant-related mortality in the same study (17% vs 33% in the long vs short recipient leukocyte telomere length, respectively, *P* = .02). Transplant-related mortality is an important HCT outcome parameter in hematological malignancies in which relapse is treated as a competing risk event, but this is not a concern in severe aplastic anemia.

Strengths of this study include the relatively large sample size, the prospective assessment of leukocyte telomere length with blood samples collected prior to HCT and the availability of detailed covariate data known to influence transplant outcome.

The study was limited by restricting participants to young patients with severe aplastic anemia, and therefore might not be applicable to patients older than 40 years or those with different indications for HCT. Validating our findings in a different HCT patient population, particularly in patients with hematologic malignancies, is warranted. Our study did not have sufficient statistical power to identify cause-specific deaths associated with short donor leukocyte telomere length; a larger study would be necessary to answer this question. The statistically significant association between donor leukocyte telomere length and patient survival after HCT in this study was limited to the longest tertile, another reason for the need of a larger sample size to allow for the reevaluation of this association in more categories of telomere length. The use of qPCR in this study provides an average measure of telomere length

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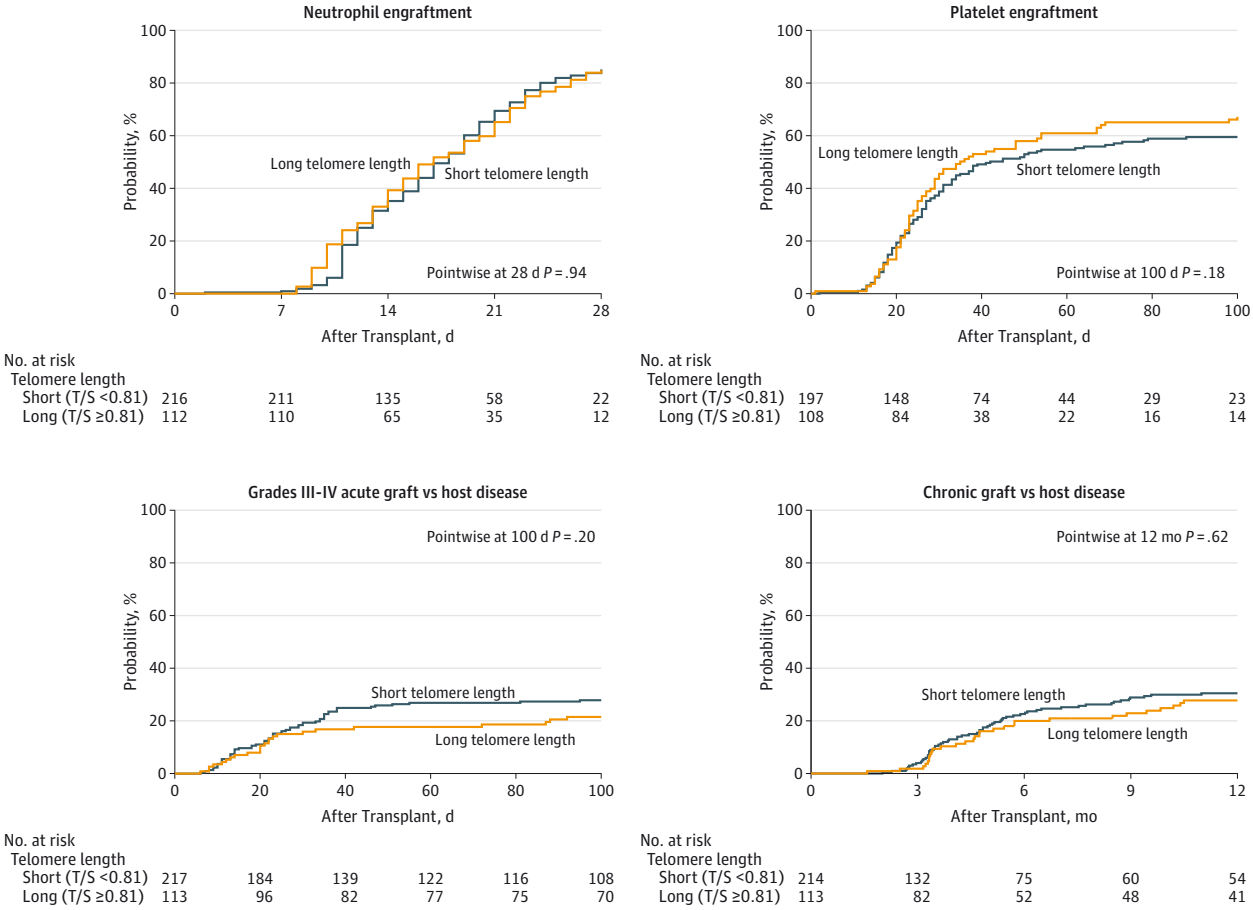
Figure 2. Hematopoietic Cell Transplant Outcomes for Patients With Severe Aplastic Anemia by Donor Leukocyte Telomere Length



in all white blood cell subsets. Because leukocyte telomere length varies by leukocyte subset,[34] the average value across cell subtypes could be affected by their differential leukocyte proportions in the sample at blood collection. A future study with a cell-specific method, such as flow cytometry with fluorescence in situ hybridization, would avoid this limitation. Additionally, qPCR telomere length assay may be limited by possible sensitivity to DNA quality.[35] We have not compared results obtained from qPCR assay in this study to other know standard techniques such as Southern blots; however, high correlation between the 2 methods has been reported earlier (R^2 = 0.68[15]; R^2 = 0.84[36]).

Conclusions

Among patients with severe aplastic anemia who received unrelated donor allogeneic HCT, longer donor leukocyte telomere length was associated with increased overall survival at 3 and 5 years. There was no association between donor leukocyte telomere length and engraftment or graft-vs-host disease. However, recipient telomere length was not associated with patient overall survival. This observational study suggests that donor leukocyte telomere length may have a role in long-term posttransplant survival.

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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: The study was supported by Public Health Service grant U24-CA76518 from the National Cancer Institute, the National Heart, Lung, and Blood Institute and the National Institute of Allergy and Infectious Diseases, Health Resources (Drs Wang and Lee and Messrs Haagenson and Spellman), and grant HHSH234200637015C from the Services Administration, grants N00014-13-1-0039 and N00014-14-1-0028 from the Office of Naval Research (Dr Wang and Messrs Haagenson and Spellman), grant 2R01 CA082838 from the National Institutes of Health (Dr De Vivo), and the Intramural Research Program of the National Cancer Institute (Drs Gadalla and Savage).

Role of the Sponsors: The above-mentioned funding agencies were not responsible for the design and conduct of the study, for the collection, management, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Other Contributions: We thank Heather Severance, MS, of the Center for International Blood and Marrow Transplant Research for assistance in manuscript preparation, who received no compensation for her assistance.

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