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ARTICLE

Change in Peripheral Blood Leukocyte Telomere Length and Mortality in Breast Cancer Survivors

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Background Progressive telomere shortening with cell division is a hallmark of aging. Short telomeres are associated with increased cancer risk, but there are conflicting reports about telomere length and mortality in breast cancer survivors.

Methods We measured peripheral blood leukocyte telomere length at two time points in women enrolled in a multiethnic, prospective cohort of stage I to stage IIIA breast cancer survivors diagnosed between 1995 and 1999 with a median follow-up of 11.2 years. We evaluated associations between telomere length measured at mean 6 (baseline; LTL_0; n = 611) and 30 months (LTL_{30}; n = 478) after diagnosis and the change between those time points (n = 478), with breast cancer–specific and all-cause mortality using Cox proportional hazards models adjusted for possible confounders. Statistical tests were two-sided.

Results There were 135 deaths, of which 74 were due to breast cancer. Neither baseline nor 30-month telomere length was associated with either all-cause or breast cancer–specific mortality (LTL_0: hazard ratio [HR] = 0.83, 95% confidence interval [CI] = 0.67 to 1.02; HR = 0.88; 95% CI = 0.67 to 1.15; LTL_{30}: HR = 0.78, 95% CI = 0.59 to 1.05; HR = 0.86; 95% = CI = 0.58 to 1.26, respectively). However, participants whose telomeres shortened between baseline and 30 months were at a statistically significantly increased risk of breast cancer–specific (HR = 3.03; 95% CI = 1.11 to 8.18) and all-cause mortality (HR = 2.38; 95% CI = 1.28 to 4.39) compared with participants whose telomeres lengthened. When follow-up was censored at 5-years after diagnosis, LTL_0 (HR = 0.66; 95% CI = 0.45 to 0.96), LTL_{30} (HR = 0.51; 95% CI = 0.29 to 0.92), and change in telomere length (HR = 3.45; 95% CI = 1.11 to 10.75) were statistically significantly associated with all-cause mortality.

Conclusions Telomere shortening was associated with increased risk of breast cancer–specific and all-cause mortality, suggesting that change in blood telomere length over time could be a biomarker of prognosis. Research on determinants of telomere length and change is needed.

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Telomeres are protective structures that cap the end of eukaryotic chromosomes (1), comprising multiple 5′-TTAGGG-3′ repeats, ending in a single-stranded overhang of the G-rich sequence (2). Telomeres protect chromosome ends from end-to-end fusion, nucleolytic decay, degradation, and atypical recombination (3).

Quantitative polymerase chain reaction (Q-PCR) offers a fast, high-throughput, and reproducible way to measure relative leukocyte telomere length (LTL), which correlates well with Southern blot measurements of absolute LTL (4). DNA from peripheral blood leukocytes is amplified for telomeric repeats and a single copy control gene, allowing calculation of the ratio of telomere copy number to single gene copy number (T/S ratio) (5). A lower T/S ratio reflects shorter LTL. Several studies (6–10), but not all (11–13), have reported associations between shorter LTL and increased risk for cancer, including breast cancer, and meta-analyses suggested a 1.4- to threefold increased risk of cancer in those with shortest vs longest telomeres (14,15). However, associations between shorter telomeres in patients with cancers compared with control subjects appear to be weaker in prospective than in retrospective studies (16). Few studies have examined associations between LTL and mortality in breast cancer survivors (17–19), and results have been conflicting. One study found no association between LTL and outcome (19); in another, longer LTL statistically significantly correlated with increased risk of all-cause mortality in a subgroup of patients with HER-2/neu–negative tumors (18). A case–control study reported that patients with node-positive tumors and shorter telomeres had increased survival compared with patients with longer telomeres (17). Finally, a recent population-based prospective study of 47 102 individuals found increased hazard ratios (HRs) of early death after a diagnosis

of any cancer (HR = 1.42; 95% confidence interval [CI] = 1.13 to 1.80) or breast cancer (HR = 1.20; 95% CI = 0.99 to 1.46) for shortest telomeres compared with longest (20).

Longitudinal changes in LTL might be more informative than cross-sectional measurements because they reflect characteristics affecting rate of attrition in specific individuals: shortening of LTL over 2.5 years was related to greater cardiovascular mortality in men followed for 12 years (21).

Here, we examined the association between longitudinal change of LTL and breast cancer–specific and all-cause mortality over a median of 11.2 years of follow-up in the Health Eating Activity and Lifestyle (HEAL) study, a cohort of breast cancer survivors diagnosed with stage I to stage IIIa breast cancer (22). We investigated these associations for baseline (mean = 6 months after diagnosis; n = 611), 30 months of follow-up (mean = 30 months after diagnosis; n = 478), and changes in LTL between those time points.

Methods

Study Setting, Participants, and Recruitment

The HEAL study is a multicenter, multiethnic, prospective cohort study that enrolled 1183 women diagnosed with breast cancer. Aims, study design, and recruitment procedures have been published previously (22).

Women were recruited through Surveillance, Epidemiology, and End Results (SEER) registries in New Mexico (n = 615; aged >18 years; with in situ stage IIIA breast cancer diagnosed 1996–1999); Los Angeles County, California (n = 366; aged 35–64 years; with stage 0–IIIA breast cancer diagnosed 1995–1998), and western Washington (n = 202; aged 40–64 years; with stage 0–IIIA breast cancer diagnosed 1997–1998). The study was approved by institutional review boards of participating centers, in accordance with an assurance filed with and approved by the US Department of Health and Human Services. Written informed consent was obtained from each subject.

Baseline surveys were conducted on average 6 months after diagnosis. Of 1183 participants, 944 women completed the 30-month data collection. We excluded 336 participants with in situ disease given the low likelihood of mortality in these women, 24 participants with nonfatal breast cancer events less than 9 months before their 30-month interview to avoid potential confounding from possible recent treatment, and 106 participants with 30-month LTL measurements only; leaving 611 participants with complete data on baseline LTL and 478 participants with complete data for LTL at both time points.

Data Collection and Covariables

Assays. Thirty milliliter fasting blood samples were collected from patients at baseline and at 30 months, processed within 3 hours, and stored at –80° C until analysis. DNA was extracted using Qiagen Midi-Prep (Germantown, MD) columns from buffy coat preparations. LTL from baseline (LTL_0) and 30-month samples (LTL_{30}) was measured by Q-PCR (5). Samples were run in triplicate, and the median was used for calculations. The amount of telomeric DNA (T) was divided by the amount of single-copy control gene DNA (S), producing a relative, unit-less measurement of telomere length (T/S ratio). Two control samples were run in each experiment to allow for normalization between experiments, and periodic reproducibility experiments were performed. Intra- and interassay coefficients of variation (CV) were 6% and 7%, respectively.

We calculated change in LTL as difference between LTL_0 and LTL_{30}, divided by elapsed time between the two measurements. For baseline and 30-month LTL we rescaled the variables to have a unit standard deviation, calculated as (telomere length – mean)/standard deviation. Negative values imply telomere shortening, and positive values imply telomere lengthening. We categorized the rate of change as 1 = any telomere lengthening or 2 = any telomere shortening.

Covariables. Standardized questionnaire information, including medical history, demographic, and physical activity assessments (23), was collected at baseline and at 30 months. Participants completed a quality-of-life assessment between 24 and 59 months after diagnosis (24), which contained a four-item scale to measure perceived stress (25). Response categories ranged from one to five, with higher scores associated with more stress. Scores were summed, and the total was divided into low stress (<12) and high stress groups (>12), where 12 was the median value. Dietary intake over the previous month (Washington and California) or year (New Mexico) was assessed at 30 months using self-administered food frequency questionnaires (26); nutrient data were converted using Nutrition Data Systems for Research (University of Minnesota).

Body mass index (BMI; components measured in Washington and New Mexico; self-report in California) was calculated as kilograms per meter squared. A race/ethnicity/study site four-category variable was created to adjust for race and site-associated confounding because these were highly correlated. The four categories were non-Hispanic whites (New Mexico), non-Hispanic whites (Washington), Hispanics, and blacks.

Stage of Disease and Cancer Treatment. Disease stage was obtained from local SEER registries before participant recruitment and classified as stage I (localized) or Stage II to IIIA (regional) breast cancer based on American Joint Committee on Cancer stage of disease classification (27). Treatment received and estrogen receptor (ER) and progesterone receptor (PR) status were abstracted from medical records; the latter were categorized as 1 = positive, 2 = negative, or 3 = unknown/borderline. Breast cancer treatment was categorized into three groups: surgery only; surgery and chemotherapy; surgery and radiation. Chemotherapy was categorized as 5-fluorouracil, cyclophosphamide, taxanes, doxorubicin, other, and combination therapy.

Outcome Assessment. Individuals were followed until their last follow-up assessment or SEER vital status update, whichever was most recent. All-cause mortality was defined as time from either baseline or 30-month follow-up interview to death from any cause, or patients were censored at December 31, 2009. Median follow-up was 11.2 years. Breast cancer mortality was defined as death from breast cancer or end of follow-up, with the same intervals as for all-cause mortality. Because 5-year survival rates are a common statistic for breast cancer survival (28), we censored data at 5 years after diagnosis to investigate more proximal associations of LTL with outcome.

Statistical Analysis

Analyses were performed using STATA 11 (StataCorp, College Station, TX). Correlations between continuous variables were estimated using Pearson correlation coefficients. Differences in distribution of LTL by participant characteristics were estimated using either the χ^2 test or analysis of covariance, adjusting for age. Covariables with missing data were assigned to a "missing" category. These categories were omitted in analysis of covariance and χ^2 analyses but included when testing inclusion in Cox models. We used Pearson correlations to calculate associations between LTL_{30} and dietary nutrients, correcting for multiple testing.

Hazard ratios for all-cause or breast cancer–specific mortality and 95% confidence intervals were based on the partial likelihood for Cox's proportional hazards model. Tests of the proportional hazard assumption were carried out using Schoenfeld residuals and held for all covariables tested, with the exception of age. We thus stratified the model by age at entry (either age at baseline or at 30 months, as appropriate; ie, stratified Cox regression estimates), allowing different strata to have different baseline hazard functions. This corresponds to adjusting for age using categorical indicators for age. Age was used as the underlying time metric, with entry and exit time defined as the participant's age at baseline (for LTL_0), and at 30-month follow-up interview for change in length and age at death or end of follow-up, respectively.

We adjusted for race/study site to adjust for the multisite design of the study and included age because it correlates strongly with telomere length. We examined the effect of the following variables on the Cox models testing associations between LTL and all-cause or breast cancer–specificmortality by sequentially adding the variable of interest and using the likelihood ratio test: physical activity, cigarette smoking (ever/never), stress (low vs high), BMI, tumor stage, ER and PR status, micronutrient intake, tamoxifen, and treatment. Of these, only tumor stage at diagnosis had a statistically significant effect; treatment had a similar effect as tumor stage but was not included because of the high degree of correlation between the two variables. Inclusion of other variables did not substantially alter the models.

We estimated the relationship between LTL_0, LTL_{30}, and the difference between these measures and breast cancer–specific and all-cause mortality, adjusted for tumor stage and ethnicity/site, and stratified by age. *P* values were estimated using the Wald test for trend. When estimating the association between change in telomere length and mortality, we also adjusted by LTL_0, to account for regression to the mean.

We determined whether associations of telomere length with outcome were the same across subgroup categories using a test of homogeneity and trend across groups—specifically stage, ER status, BMI less than 25 kg/m^2 and greater than 25 kg/m^2 (events were too few to investigate additional BMI subgroups), and breast cancer treatment. Adjusting for multiple comparisons for each endpoint, statistical significance was set at $P = .01$ (ie, 0.05/4). Small numbers of deaths in premenopausal participants prevented comparisons between pre- and postmenopausal subgroups. All tests of statistical significance were two-sided.

Results

Characteristics of HEAL participants are shown in Table 1. Median follow-up time from baseline interview was 11.2 years; 135 participants died, of whom 74 died from breast cancer. Fifteen participants died between their baseline and 30-month interview. There were no differences in telomere length between these two groups (data not shown).

Associations Between Demographic and Clinical Variables and Telomere Length

Telomere data were normally distributed. Mean telomere lengths at baseline and 30 months were 0.82 and 0.77, respectively. LTL_0 correlated statistically significantly with LTL_{30} ($r = 0.77$; $P < .001$). Age negatively correlated with LTL_0 ($r = -0.33$; $P < .001$) and LTL_{30} ($r = -0.39$; $P < .001$). Telomeres were longer at both baseline and 30-month time points in black women compared with other racial/ethnic groups, adjusted for age ($P < .001$) (Table 1). Participants with higher levels of stress had shorter telomeres at baseline ($P = .05$) and 30 months ($P = .008$). At 6 months after diagnosis, postmenopausal women had shorter telomeres than premenopausal women ($P = .02$). Change in telomere length was not associated with any patient characteristics examined, either as a continuous (data not shown) or categorical variable (any shortening/any lengthening). Micronutrient intake (eg, folate, vitamin B12, vitamin D) was not associated with LTL_{30} (data not shown).

Telomere Lengthening

One hundred fifty-four (32.2%) participants had telomeres that lengthened between baseline and 30 months, whereas 324 (67.8%) participants had telomeres that shortened between these two time points.

Telomere Length and Survival

With full follow-up, neither baseline nor 30-month telomere length was statistically significantly associated with either all-cause or breast cancer–specific mortality (LTL_0: HR = 0.83, 95% CI = 0.67 to 1.02; HR = 0.88, 95% CI = 0.67 to 1.15; LTL_{30}: HR = 0.78, 95% CI = 0.59 to 1.05; HR = 0.86, 95% CI = 0.58 to 1.26, respectively) (Table 2). In comparison, participants whose telomeres shortened between baseline and 30 months had statistically significantly worse outcome (all-cause mortality: HR = 2.38; 95% CI = 1.28 to 4.39; breast cancer–specific mortality: HR = 3.03; 95% CI = 1.11 to 8.18) compared with women whose telomeres lengthened. Confidence intervals were wide for the latter because of small numbers of events. We observed similar results when we restricted the analysis to postmenopausal women.

When we censored follow-up at 5 years, LTL_0 and LTL_{30} had a statistically significant inverse association with all-cause mortality (LTL_0: HR = 0.66, 95% CI = 0.45 to 0.96; LTL_{30}: HR = 0.51, 95% CI = 0.29 to 0.92) but not with breast cancer–specific mortality (LTL_0: HR = 0.69, 95% CI = 0.46 to 1.04; LTL_{30}: HR = 0.57, 95% CI = 0.28 to 1.13), although effect sizes were similar.

Change in telomere length was associated with all-cause mortality (HR = 3.45; 95% CI = 1.11 to 10.75); however, confidence

Table 1. Associations of telomere length with characteristics of the Health, Eating, Activity and Lifestyle cohort*

Patient characteristics	No. at baseline (n = 611)	Mean (SD) telomere length at 6 months after diagnosis (T_0)	*P*† (ANCOVA)	No. at 30 months (n = 478)	Mean (SD) telomere length at 30 months after diagnosis (T_{30})	*P*† (ANCOVA)	ΔTelomere		*P*‡
							No. <0, shortened	No. >0, lengthened	
All	611	0.82 (0.18)		478	0.76 (0.17)		324	154	
Age									
≤50 y	235	0.87 (0.18)	<.001	135	0.83 (0.18)	<.001	106	59	.22
>50 y	376	0.78 (0.17)		343	0.74 (0.17)		218	95	
Menopausal status									
Pre	216	0.87 (0.17)	.02	77	0.83 (0.17)	.27	214	94	.38
Post	358	0.78 (0.17)		371	0.75 (0.17)		97	55	
Unknown§	37	0.87 (0.26)		30	0.85 (0.18)		13	5	
Physical activity, MET hr/wk‖									
None	97	0.75 (0.15)	.21†	87	0.74 (0.18)	.18†	57	30	.88
>0 to <9	153	0.81 (0.16)		189	0.76 (0.17)		129	60	
≥9	199	0.79 (0.17)		202	0.78 (0.18)		138	64	
Unknown§	162	0.90 (0.19)							
Cigarette use									
Ever	322	0.80 (0.18)	.11	253	0.75 (0.16)	.06	179	78	.34
Never	289	0.83 (0.18)		225	0.78 (0.18)		145	76	
Stress scale									
Low stress, ≤12	275	0.83 (0.20)	.05	311	0.78 (0.18)	.008	158	83	.14
High stress, >12	216	0.79 (0.14)		115	0.73 (0.15)		134	51	
Unknown§	120	0.83 (0.17)		52	0.79 (0.42)		32	20	
BMI at time of blood draw									
<18.5 kg/m^2	11	0.77 (0.14)	.18	8	0.70 (0.17)	.89	6	3	.94
≥18.5 to <25 kg/m^2	210	0.79 (0.16)		189	0.77 (0.16)		121	62	
≥25 to <40 kg/m^2	375	0.83 (0.19)		262	0.76 (0.18)		190	86	
≥40 kg/m^2	15	0.76 (0.16)		19	0.81 (0.14)		7	3	
Race/ethnicity									
White	359	0.78 (0.16)	<.001	306	0.72 (0.15)	<.001	217	89	.15
Hispanic-white	75	0.83 (0.16)		61	0.77 (0.18)		41	20	
Black	160	0.90 (0.20)		96	0.89 (0.17)		58	38	
Other#	17	0.85 (0.14)		15	0.85 (0.13)		8	7	
Tumor stage									
Local, stage I	427	0.80 (0.18)	.15	343	0.75 (0.17)	.54	230	113	.59
Regional, stage II–IIIA	183	0.84 (0.18)		135	0.79 (0.17)		94	41	
Unknown#	1	1.17 (—)							
ER status									
ER negative	134	0.85 (0.18)	.39	93	0.79 (0.18)	.68	64	29	.89
ER positive	411	0.81 (0.18)		342	0.76 (0.17)		233	109	
Unknown#	66	0.82 (0.16)		43	0.77 (0.14)		27	16	
PR status									
PR negative	176	0.82 (0.17) 0.80	.46	129	0.77 (0.18)		87	42	
PR positive	332	(0.18)		280	0.76 (0.17)		191	89	
Unknown#	103	0.84 (0.18)		69	0.78 (0.15)	.61	46	23	.87

(Table continues)

Table 1 (Continued).

Patient characteristics	No. at baseline (n = 611)	Mean (SD) telomere length at 6 months after diagnosis (T_0)	*P*† (ANCOVA)	No. at 30 months (n = 478)	Mean (SD) telomere length at 30 months after diagnosis (T_{30})	*P*† (ANCOVA)	ΔTelomere		*P*‡
							No. <0, shortened	No. >0, lengthened	
Tamoxifen use at time of blood draw									
No	246	0.79 (0.16)	.68	228	0.76 (0.17)	.17	154	74	.93
Yes	202	0.78 (0.16)		249	0.76 (0.18)		169	80	
Unknown#	163	0.90 (0.20)		1	0.80 (-)		1	—	
Treatment received									
Surgery	148	0.83 (0.19)	.10	114	0.78 (0.20)	.06	74	40	.30
Radiotherapy+ surgery	220	0.78 (0.17)		185	0.73 (0.16)		121	64	
Chemotherapy +surgery	242	0.84 (0.18)		179	0.79 (0.17)		129	50	
Unknown#	1	1.17 (—)		—			—		
Chemotherapy**									
5-Fluorouracil	21	0.88 (0.16)	.25	11	0.81 (0.11)	.08	9	2	.58
Cyclophosphamide	93	0.87 (16)	(.13)¶	74	0.80 (0.15)	(.10)¶	53	21	(.69)¶
Doxorubicin	18	0.77 (0.13)		12	0.70 (0.12)		10	2	
Taxanes, any	2	0.68 (0.10)		1	0.44 (1)		1	0	
Other chemo	17	0.88 (0.27)		13	0.87 (0.21)		7	6	
Combination therapy	91	0.82 (0.17)		68	0.77 (0.18)		49	19	

* ANCOVA = analysis of covariance; BMI = body mass index; ER = estrogen receptor; MET = metabolic equivalents of energy expenditure; PR = progesterone receptor; SD = standard deviation.

† Differences among categorical groups (ANCOVA) adjusted by age. Associations between baseline telomere length were calculated for baseline age, baseline menopausal status, moderate/vigorous physical activity in the year before diagnosis, baseline cigarette use and baseline tamoxifen use. Similarly, associations between 24-month telomere lengths are calculated for age at 24 months, menopausal status at 24 months, physical activity in the year before 30-month interview, cigarette use at 24 months, and tamoxifen use at 24 months.

‡ Two-sided Pearson χ^2 test.

§ *P* value in parentheses excludes other chemotherapy and combination therapies. Taxanes also excluded for telomere length at 30 months (n = 1).

|| Total moderate/vigorous physical activity in the year before diagnosis and in the year before 30-month blood collection in MET hours per week.

¶ Stress scale based on a four-item scale to measure perceived stress (26).

Omitted from analysis.

** Subset n = 242 at baseline; n = 179 at 30 months.

Table 2. Associations between baseline and 30-month telomere length and telomere length change with breast cancer and all-cause mortality adjusted for race/ethnicity/study site and tumor stage at diagnosis and stratified by age at time of blood draw*

	5-year censored follow-up		Full follow-up		Postmenopausal women only full follow-up†	
Outcomes	**No. events / No. total**	**HR (95% CI)**	**No. events / No. total**	**HR (95% CI)**	**No. events / No. total**	**HR (95% CI)**
Breast cancer–specific mortality						
Baseline age‡	41/611	1.23 (1.10 to 1.39)	74/611	0.97 (0.91 to 1.04)	34/358	0.95 (0.85 to 1.05)
P§		<.001		.49		.34
Baseline telomere length‖	41/611	0.69 (0.46 to 1.04)	74/611	0.88 (0.67 to 1.15)	34/358	1.06 (0.68 to 1.64)
P§		.08		.34		.79
Baseline telomere length median split						
0.40–0.81	21/306	1.00 (referent)	36/306	1.00 (referent)	19/208	1.00 (referent)
0.81–1.66	20/305	0.59 (0.29 to 1.22)	38/305	0.75 (0.44 to 1.26)	15/150	0.81 (0.35 to 1.88)
P§		.16		.27		.64
30-month telomere length‖	15/478	0.57 (0.28 to 1.13)	36/478	0.86 (0.58 to 1.26)	29/371	0.82 (0.53 to 1.25)
P§		.11		.44		.36
30-month telomere length median split						
0.33–0.76	8/249	1.00 (referent)	15/239	1.00 (referent)	13/211	1.00 (referent)
0.76–1.38	7/229	0.54 (0.11 to 2.59)	21/239	1.15 (0.41 to 3.19)	16/160	0.77 (0.23–2.51)
P§		.44		.79		.66
Change in telomere length¶						
Lengthened	4/154	1.00 (referent)	7/154	1.00 (referent)	5/116	1.00 (referent)
Shortened	11/324	2.46 (0.64 to 9.43)	29/324	3.03 (1.11 to 8.18)	24/255	3.90 (1.22 to 12.39)
P§		.19		.03		.02
All-cause mortality						
Baseline age‡	53/611	1.69 (1.40 to 2.04)	135/611	0.95 (0.90 to 1.00)	84/358	0.93 (0.86 to 0.99)
P§		<.001		.04		.04
Baseline telomere length‖	53/611	0.66 (0.45 to 0.96)	135/611	0.83 (0.67 to 1.02)	84/358	0.88 (0.64 to 1.19)
P§		.03		.08		.42
Baseline telomere length median split						
0.40–0.81	30/306	1.00 (referent)	77/306	1.00 (referent)	57/208	1.00 (referent)
0.81–1.66	23/305	0.55 (0.28 to 1.06)	58/305	0.75 (0.50 to 1.11)	27/150	0.62 (0.36 to 1.07)
P§		0.08		0.14		.09
30-month telomere length‖	25/478	0.51 (0.29 to 0.92)	89/478	0.78 (0.59 to 1.05)	78/371	0.75 (0.54 to 1.02)
P§		.03		.10		.06
30-month telomere length median split						
0.33–0.76	16/239	1.00 (referent)	50/239	1.00 (referent)	48/201	1.00 (referent)
0.76–1.38	9/239	0.36 (0.11 to 1.22)	39/239	0.79 (0.40 to 1.52)	30/170	0.52 (0.24 to 1.09)
P§		.10		.48		.09

(Table continues)

Table 2 (Continued).

Outcomes	5-year censored follow-up		Full follow-up		Postmenopausal women only full follow-up†	
	No. events / No. total	HR (95% CI)	No. events / No. total	HR (95% CI)	No. events / No. total	HR (95% CI)
Change in telomere length¶						
Lengthened	5/154	1.00 (referent)	20/154	1.00 (referent)	18/116	1.00 (referent)
Shortened	20/324	3.45 (1.11 to 10.75)	69/324	2.38 (1.28 to 4.39)	60/255	2.45 (1.28 to 4.69)
P§		.03		.006		.007

* CI, confidence interval; HR, hazard ratio.

† Three hundred fifty-eight women were postmenopausal at baseline with complete data on leukocyte telomere length (LTL); 371 were postmenopausal at 30 months with complete data on both baseline and 30-month LTL.

‡ Adjusted for race/ethnicity/study site and tumor stage at diagnosis.

§ Two-sided Wald test for trend.

‖ Rescaled such that baseline and 30-month LTL have a unit standard deviation, calculated as (telomere length – mean)/standard deviation.

¶ $LTL_{30}-LTL_0$/(date of 30 month interview – date of baseline interview). A negative value implies telomere shortening, and a positive value implies telomere lengthening. Change in telomere length was also adjusted for baseline telomere length.

intervals were wide. There was no statistically significant association between change in length and 5-year breast cancer–specific mortality, but again, effect sizes suggested an association between telomere shortening and poor outcome.

Finally, we examined the modifying effect of subgroups on associations between telomere length and mortality. For participants with ER-negative tumors, longer telomeres at 30 months were associated with decreased risk of both breast cancer–specific and all-cause mortality (HR = 0.01, 95% CI = 0.01 to 1.01; HR = 0.02, 95% CI = 0.01 to 0.71, respectively) compared with those with ER-positive tumors (HR = 3.09, 95% CI = 0.16 to 17.99; HR = 0.74, 95% CI = 0.11 to 5.01; respectively) using fully adjusted models. However, after adjustment for multiple testing (statistical significance set at $P = .01$) the differences in effects of telomere length at 30 months between ER-negative and ER-positive tumors were not statistically significant ($P_{trend} = .03$; $P_{trend} = .05$, respectively) (Table 3). There was no evidence of effect modification for other subgroups examined.

Discussion

Herein we describe associations between LTL and change (any shortening/any lengthening) between 6 and 30 months after diagnosis and mortality in a multiethnic cohort of breast cancer survivors followed for a median of 11.2 years. LTL was inversely associated with age and menopausal status, which may act as a proxy for age. Participants with higher levels of stress had shorter telomeres at both time points, which supports findings in female caregivers (29) and more modest associations in healthy women aged 35 to 75 years (30).

LTL at either time point was not statistically significantly associated with either breast cancer–specific or all-cause mortality. Within the initial 5 years of follow-up, the association between shorter telomeres at baseline and mortality was statistically significant. Telomere shortening from baseline to 30 months was associated with increased risk of both all-cause and breast cancer–specific mortality.

A recent study of 47 102 healthy participants followed for 20 years reported that decreasing quartiles of LTL were associated with reduced survival after a diagnosis of cancer (log-rank $P < .001$) (20). In a cohort of 787 healthy individuals followed for 10 years, short telomeres at baseline were associated with risk of cancer mortality (HR = 2.13; 95% CI = 1.58 to 2.86) (31,32). Low telomere DNA content, measured by slot-blot titration, in archival breast tumor tissues from a subset of 530 HEAL cohort participants was associated with increased risk of recurrence, death from breast cancer, or new primaries (relative hazard = 2.88; 95% CI = 1.16 to 7.15) (33). However, another study examining LTL and telomerase expression in tumor samples of 348 breast cancer patients found no association with outcome (19). A case–control study of 265 breast cancer patients and 466 control subjects found a statistically significant association between increasing LTL and worse outcome (HR = 2.92; 95% CI = 1.33 to 6.95) (17), as did a population-based cohort study of women with HER-2/*neu*–negative breast tumors (HR = 1.90; 95% CI = 1.12 to 3.22) (18). Differences in population studied (eg, tumor stage) and techniques used to measure telomere length might account for these differences.

Table 3. Hazard ratios (HRs) and 95% confidence intervals (CIs) of all-cause and breast cancer–specific mortality in subgroups of Health, Eating, Activity and Lifestyle participants using a multifactorially adjusted model*

	Baseline telomere length		30-month telomere length		Change in telomere length	
Subgroup	**HR (95% CI)**	**P_{trend}†**	**HR (95% CI)**	**P_{trend}†**	**HR (95% CI)**	**P_{trend}†**
Breast cancer–specific mortality						
BMI ≤25 kg/m²	1.22 (0.08 to 17.34)	.88	0.54 (0.01 to 29.01)	.83	3.87 (0.81 to 18.34)	.40
BMI >25 kg/m²	0.31 (0.05 to 1.78)		0.32 (0.02 to 4.81)		1.73 (0.58 to 5.14)	
SEER stage I, local	0.57 (0.07 to 4.52)	.86	0.66 (0.03 to 13.69)	.64	2.96 (0.80 to 10.87)	.54
SEER stage II–IIIA, regional	0.44 (0.06 to 3.10)		0.25 (0.02 to 5.87)		1.71 (0.51 to 5.74)	
ER negative‡	0.26 (0.03 to 2.97)	.31	0.01 (0.01 to 1.01)	.03	1.07 (0.24 to 4.67)	.15
ER positive	1.22 (0.19 to 7.99)		3.09 (0.16 to 17.99)		4.18 (1.17 to 14.93)	
Treatment§						
Surgery only	3.69 (0.19 to 77.87)	.07	0.92 (0.02 to 53.40)	.42	3.12 (0.07 to 13.92)	.21
Chemotherapy + surgery	0.15 (0.02 to 0.98)		0.12 (0.01 to 2.26)		0.17 (0.01 to 3.18)	
Surgery only	3.69 (0.19 to 77.87)	.69	0.92 (0.02 to 53.40)	.17	3.12 (0.07 to 13.92)	.22
Radiotherapy + surgery	8.23 (0.32 to 90.41)		12.51 (0.23 to 65.50)		20.71 (0.54 to 79.27)	
All-cause mortality						
BMI ≤25 kg/m²	0.50 (0.08 to 3.28)	.60	0.23 (0.02 to 2.55)	.97	2.40 (0.94 to 6.08)	.61
BMI >25 kg/m²	0.27 (0.07 to 1.12)		0.25 (0.03 to 1.91)		1.76 (0.85 to 3.64)	
Unknown						
SEER stage I, local	0.34 (0.07 to 1.48)	.83	0.31 (0.05 to 2.04)	.67	1.80 (0.90 to 3.59)	.65
SEER stage II–IIIA, regional	0.41 (0.08 to 2.23)		0.16 (0.01 to 2.24)		2.40 (0.85 to 6.73)	
Unknown						
ER negative‡	0.29 (0.04 to 2.26)	.58	0.02 (0.01 to 0.71)	.05	1.06 (0.36 to 3.02)	.28
ER positive	0.58 (0.13–2.49)		0.74 (0.11 to 5.01)		2.12 (1.01 to 4.46)	
Unknown						
Treatment§						
Surgery only	0.39 (0.05 to 3.17)	.69	0.19 (0.01 to 2.78)	.97	0.36 (0.03 to 4.54)	.94
Chemotherapy + surgery	0.23 (0.05 to 1.12)		0.20 (0.02 to 1.93		0.31 (0.04 to 2.73)	
Surgery only	0.39 (0.05 to 3.17)	.45	.19 (0.01 to 2.78)	.46	0.36 (0.03 to 4.54)	.67
Radiotherapy + surgery	1.25 (0.11 to 13.93)		.77 (0.04 to 13.61)		0.79 (0.04 to 13.89)	

* Multifactorially adjusted model: telomere length, race/ethnicity/study site; tumor stage at diagnosis; stratified by age at blood draw. BMI = body mass index; ER = estrogen receptor; SEER = Surveillance, Epidemiology, and End Results.

† Test for homogeneity of trends significant at $P = .01$ after correction for multiple testing.

‡ Patients with unknown ER status were excluded.

§ Comparing chemotherapy + surgery vs surgery only; and radiotherapy + surgery vs surgery only.

To our knowledge there are no studies examining changes in LTL over time in breast cancer survivors. Longitudinal studies of LTL have indicated that both lengthening and shortening are observed over time. A study in 959 healthy individuals found stable or increased LTL over 10 years in approximately one-third of participants, and individual telomere attrition rate inversely correlated with initial LTL ($r = -0.752$; $P = .001$); however, they found no association with rate of individual telomere attrition or prediagnostic telomere length and later tumor development (34). Although dependency of LTL attrition on baseline LTL has been attributed to regression to the mean, a study correcting for this statistical artifact found that a modest but statistically significant effect remained, indicating that high baseline LTL is associated with higher LTL attrition even when correcting for the regression to the mean effect (35). Similarly, telomere shortening and lengthening was observed in 787 healthy individuals followed for 10 years (31). Finally 10 men diagnosed with prostate cancer underwent a lifestyle intervention; after 5 years, participants' LTL increased by a median of 0.06 units, compared with a decrease of –0.03 units in control subjects ($P = .03$) (36).

Recent reports have suggested that telomere lengthening is a measurement artifact (37,38). However, models were adjusted for baseline LTL, and we show that, regardless of initial telomere length, patients whose telomeres shortened were more likely to die of breast cancer or any cause. Increased mortality became more apparent as follow-up was extended, compared with results censored at 5 years. Conversely, long telomeres at baseline were associated with overall survival only in the short term (5-year censored), but not the longer term (full follow-up). This may suggest that, although shorter telomeres at time of diagnosis increase risk of mortality in the short term, long-term risks might be modifiable, because risk of mortality is not related to baseline LTL, but rather to rate of change over time, which may be amenable to intervention (39).

Telomere shortening may play different roles in cellular transformation. At critically short telomere lengths, cells enter p53- or Rb-dependent replicative cellular senescence (40–42). However, in the absence of appropriate checkpoint mechanisms, telomere shortening may contribute to neoplastic transformation through chromosomal instability, subsequent to breakage–fusion–bridge cycles (43–45). The basis for the associations between improved outcome and telomere lengthening are not yet well understood. It is possible that lengthening in healthy cancer survivors may protect cells from entering into breakage–fusion–bridge cycles, especially if those cells tend to lack some of the checkpoints necessary to trigger cellular senescence.

Limitations of our study include relatively small number of events and the lack of statistical power to effectively test associations between LTL in clinically important subpopulations: although we observed differential effects in ER-positive and ER-negative tumors, these results should be interpreted with caution. Although we did not see effects by other subgroups examined, we cannot discount inadequate power. These issues could be addressed in future larger cohort studies or pooled analyses of existing breast cancer cohort studies. There was also a possible selection bias in this study: because we excluded participants receiving treatment for recurrence within 9 months of their 30-month interview, associations with early breast cancer mortality would not be observed. Lack of available data on complete blood counts limits investigation into the effect of treatment on leukocyte subsets (46,47), which may alter observed LTL. Finally, although we did not observe associations between chemotherapy received and LTL, we were not able to evaluate combinations, dosage of specific chemotherapeutic agents, or duration of treatment in detail

This is the first study to link telomere lengthening in peripheral blood leukocytes over time to survival in a well-characterized, multiethnic cohort of breast cancer survivors. These findings should be confirmed in a larger cohort and point to the need to determine causes of telomere change and to test interventions that may slow the rate of shortening of telomeres in this population.

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