Review

Cancer Epidemiology, Biomarkers & Prevention

Telomere Length and Breast Cancer Prognosis: A Systematic Review

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Abstract

Telomeres ensure genome integrity during replication. Loss of telomeric function leads to cell immortalization and accumulation of genetic alterations. The association of telomere length (TL) with breast cancer prognosis is examined through a systematic review. Electronic databases (MEDLINE, EMBASE, CENTRAL), from inception to December 2015, and relevant reviews were searched. Studies that evaluated TL (blood and/or tumor) in association with breast cancer survival or prognostic factor were included. Thirty-six studies met inclusion criteria. Overall risk of bias was critical. Eight studies reported survival outcomes. Overall, there was a trend toward an association of longer telomeres with better outcomes (tumor, not blood). Of the 33 studies reporting associations with prognostic factors, nine adjusted for potential confounders. Among the latter, shorter telomeres were associated with older age (blood, not tumor), higher local recurrence rates (normal tissue), higher tumor grade (tumor), and lower physical activity (blood), which were reported in one study each. TL was not associated with molecular subtype (blood, one study), family history (tumor, one study), chemotherapy (blood, three of four studies), and stress reduction interventions (blood, two of two studies). Although major methodologic differences preclude from drawing conclusive results, TL could be a valuable breast cancer prognostic marker. *Cancer Epidemiol Biomarkers Prev; 26(1); 3–10. ©2016 AACR.*

Introduction

Telomeres are highly specialized structures capping the ends of linear chromosomes and consist of repeated DNA sequences, 5′-TTAGGG-3′ of 5- to 15-kb length in humans, bound by multiple telomeric-interacting proteins (1–5). Telomeres ensure the stability of chromosomes and genome integrity during replication. In somatic cells, telomeres shorten with each cell division, due to incomplete replication of the chromosome's end by DNA polymerase enzymes, called the end replication problem. In embryonic and adult stem cells, such as germline cells and some somatic tissues, including blood leukocytes, telomere length (TL) is kept within a cell type-specific narrow range by the telomerase enzyme complex (6), a specialized reverse transcriptase that extends the 3′ end of chromosomes by adding TTAGGG repeats (3, 6, 7). In the absence of telomerase, the gradual shortening of telomeres reaches a critical point that elicits DNA damage responses. Hence, telomere shortening leads to replicative senescence, which protects from chromosome end-to-end fusion, chromosomal rearrangements, and instability. Eventually, the cell undergoes apoptosis, known as programmed cell death. Telomeres shorten with increasing age. Inflammation and oxidative stress have been shown to result in accelerated telomere shortening, and some lifestyle factors seem to have an impact on telomeres (7–9).

Telomere shortening is thought to decrease tissue renewal capacity and increase the cancer susceptibility observed with aging (8). Loss of telomeric function leads to genomic instability, through cell immortalization and accumulation of genetic alterations, which is a main factor in the initiation and progression of cancers, particularly in cancer cells that lack normal DNA damage response mechanisms (10). Telomere dysfunction could therefore be of prognostic significance in tumors such as breast cancer (11). The objective of the current systematic review is to examine the association between TL and survival and/or known or potential breast cancer prognostic factors to evaluate the current state of knowledge concerning the value of TL as a prognostic factor.

Materials and Methods

A systematic review was conducted following a preestablished protocol and the general methods for Cochrane reviews (12). Considering the expected methodologic diversity and heterogeneity between eligible studies, the great susceptibility of observational designs to selection bias and the variability in methods used to control for confounding, no quantitative synthesis was planned (12).

Search methods for identification of studies

An electronic search of the following databases was performed, from inception to December 2015: MEDLINE (via PubMed), EMBASE, and CENTRAL (Cochrane Central Register of Controlled Trials). Search strategies were developed for each of these databases with text words and index terms referring to telomere, telomerase, and breast cancer (Supplementary Table S1). No language or publication date restrictions were applied. The reference lists of relevant reviews as well as the included studies were scanned for any additional relevant studies not otherwise identified.

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Criteria for considering studies for this review

Types of studies. Any observational or intervention study that evaluated the association between TL and breast cancer survival and/or a known or potential breast cancer prognostic factor, whatever the study design, was eligible for inclusion. No restrictions were applied regarding the language or type (articles, short reports, and abstracts) of publication.

Types of participants. Breast cancer patients, regardless of age, stage, treatment regimen, and menopausal status, were eligible. No participants were excluded on the basis of ethnicity.

TL measurement. Studies that measured TL in peripheral blood and/or in breast tumors tissue, whatever the method of measurement, were eligible.

Breast cancer prognosis. Survival outcomes, including overall survival (all-cause mortality), breast cancer–specific survival (breast cancer–specific mortality), and breast cancer–free survival (breast cancer recurrence), were the primary outcomes.

Studies that assessed the association of TL with traditional breast cancer prognostic factors [i.e., age, stage, tumor size, lymph node involvement, histologic type, grade, estrogen receptor (ER) and progesterone receptor (PR) status, HER2 status or molecular subtype, treatment received], as well as potential breast cancer prognostic factors, such as lifestyle factors (weight, body mass index, other measures of adiposity, diet, energy intake, smoking, alcohol consumption, and physical activity) and psychologic factors [mood disturbances (depression and/or anxiety), perceived stress, sleep disturbances, such as insomnia, fatigue] were also eligible, as were studies that assessed the effect of an intervention (therapeutic, lifestyle modification, or psychologic intervention) on TL.

Data collection and analysis

Selection of studies. The references identified by the search strategy were reviewed by one author (K. Ennour-Idrissi) in a 2-step process. First, the title and abstract of each study were screened to exclude the obviously noneligible studies, and second, the full text of retained articles was examined and subjected to evaluation using the predefined eligibility criteria. Whenever required, a second review author (C. Diorio) was consulted. When required, further information was sought from the authors by email.

Data extraction. Data extraction was performed using an exhaustive standardized form (Supplementary File S2) designed for this review. Information about the study design features (inclusion criteria, sample size, and methodology), participant characteristics (age, ethnicity, menopausal status, stage, tumor size, lymph node involvement, grade, ER, PR, and HER2 status, molecular subtype, and treatment received), TL measurement (DNA source, tissue processing, DNA extraction method, measurement method, and parameters measured), variables and potential confounders studied, as well as statistical analysis methods and study results, were collected. For observational studies, special attention was paid to distinguishing between adjusted and unadjusted results, and to the variable selection method used in multivariate analyses. The study's definition of each characteristic or variable retained was recorded. In the case of multiple publications related to the same study, the publication reporting the outcomes of interest to the current review or the one with the longest follow-up of these outcomes was considered as the reference, and information was supplemented by secondary publications as required. The data were extracted twice over the course of several days to ensure their consistency.

Assessment of risk of bias in retained studies. On the basis of the "Reporting Recommendations for Tumor Marker Prognostic Studies" criteria (13), and the rating approach of the "Cochrane Risk Of Bias Assessment Tool for Non-Randomized Studies of Interventions" (14), the following domains were evaluated for risk of bias of included studies: selection of participants into the study, TL measurement, measurement of prognostic variables, potential confounding accounted for, missing data, and selective reporting (Supplementary File S3).

The assessment of the risk of bias was performed twice by a review author (K. Ennour-Idrissi), both for the risk of bias in each study and for the overall risk of bias across studies. When required, a second reviewer (C. Diorio) was consulted.

Assessment of heterogeneity. Differences between studies, including TL measurement (DNA source, tissue processing, DNA extraction method, and measurement method), participant characteristics (age, ethnicity, menopausal status, stage, molecular subtype, and treatment received), study design, and different levels of risk of bias, were considered for exploring possible sources of heterogeneity. For survival outcomes, heterogeneity was explored using the I^2 test, obtained from forest plots of individual study results, with an I^2 of 50 % or greater indicating the presence of substantial heterogeneity (15).

Data synthesis

Given that high heterogeneity between studies was expected, quantitative synthesis of data was not considered appropriate. For survival outcomes, forest plots of individual study results were drawn, without pooling estimates, using RevMan 5.3 software (Cochrane Review Manager Version 5.3; Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark). Adjusted HRs for the comparison "long versus short telomeres" were used (short telomeres as the referent group), as the majority of studies reported this comparison. Reported HRs for the comparison "short versus long telomeres" (long telomeres as the referent group) were converted to their reciprocal (1/HR). Using additional tables (12), a systematic qualitative synthesis of study characteristics and results was performed for both survival outcomes and associations with prognostic factors.

Results

Results of the search

Of the 1,594 references retrieved by electronic search, 36 met eligibility criteria (Fig. 1) and included one ongoing randomized controlled trial (16), 13 longitudinal cohort studies, 16 cross-sectional studies, 2 case–control studies, and 4 randomized controlled trials.

Description of studies

The 36 included studies were published between 1994 and 2015 and involved between 30 and 1,026 participants (median = 104 participants).

Eight longitudinal cohort studies reported survival outcomes (17–24), with three measuring TL in peripheral blood cells (Supplementary Table S2; refs. 17–19), and five measuring it in breast tumor tissue (Supplementary Table S3; refs. 20–24). Participants had stage 0 to stage IV breast cancer, and follow-up times varied between 2.4 months and 23 years. TL was measured by qPCR in all of the three studies of peripheral blood cells, whereas two studies of breast tumor tissue used qPCR, two studies used a slot blot method, and one study used the single TL analysis (STELA) of chromosome X.

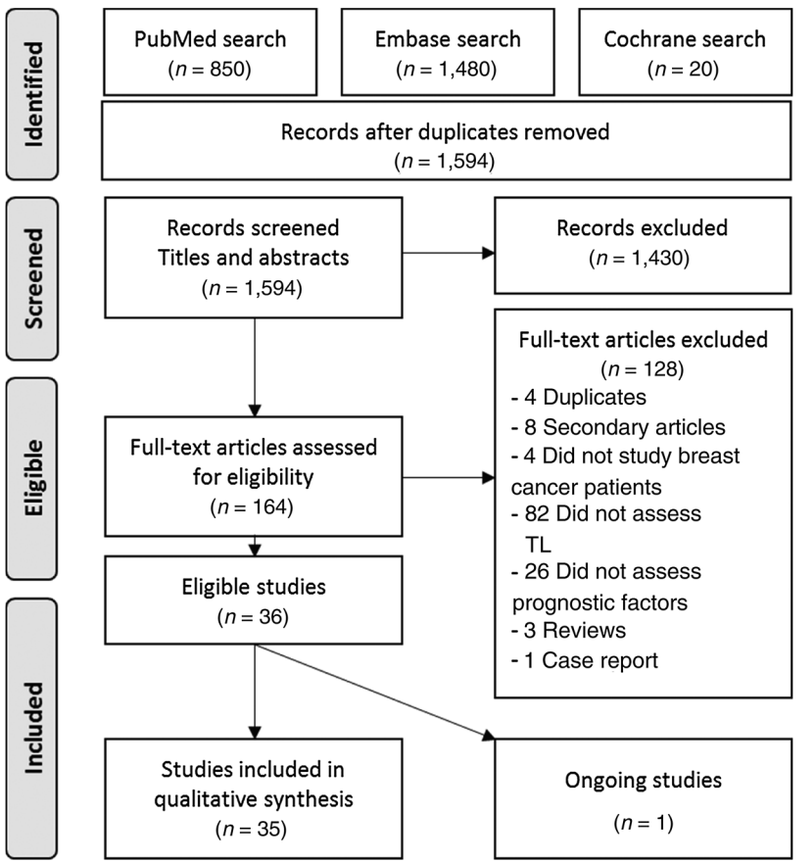

Figure 1.
Flow diagram according to PRISMA (preferred reporting items of systematic reviews and meta-analyses), with modifications.

Characteristics of the 33 studies reporting associations of TL with one or more prognostic factor are summarized in Table 1 and described in Supplementary Tables S4 and S5. Participants' mean age varied between 44 and 75 years, and they had stage 0 to stage IV disease. TL was measured in peripheral blood cells in 13 studies (17, 19, 25–35), of which eight studies used qPCR and four studies used Southern blot analysis [one study (33) did not report the measurement method]. Twenty studies measured TL in breast tumor tissue (21–24, 36–51), of which the majority used FISH (nine studies, five quantitative measures, and four qualitative measures) and Southern blot analysis (five studies); a slot blot method was used in four studies, qPCR in one study, and STELA in one study.

Risk of bias in retained studies

Overall, studies reporting TL in peripheral blood cells and survival outcomes were at moderate risk of bias (Supplementary Fig. S1), whereas studies reporting TL in breast tumor tissue and survival outcomes were at critical risk of bias (Supplementary Fig. S2).

Overall, studies reporting associations of TL with prognostic factors were at serious risk of bias (Supplementary Figs. S3 and S4).

Systematic data synthesis

TL and survival outcomes.

Overall survival: Of the three studies evaluating baseline TL in peripheral blood and survival outcomes, two reported overall survival (Fig. 2; Supplementary Table S2; refs. 17, 18). Compared with the group with shorter telomeres, patients with longer telomeres had nonsignificantly lower all-cause mortality in one study [$n = 611$; HR = 0.75; 95% confidence interval (CI), 0.50–1.13; ref. 17) and nonsignificantly higher all-cause mortality in the other study ($n = 1{,}026$; HR = 1.10; 95% CI, 0.83–1.46; ref. 18). The I^2 test for heterogeneity of these two studies' results was 57%. The first study involved patients with stage I to IIIA disease and had a median follow-up time of 11.2 years, whereas the second one included patients with *in situ* and invasive stages and a mean follow-up time of 8.0 years. Both of these studies used a qPCR method. The first one used the column-based method for DNA extraction and a rescaled telomere to single-copy gene ratio (T/S ratio) and obtained an intraassay and interassay coefficient of variation of 6% and 7%, respectively. The second study used the phenol–chloroform method for DNA extraction and a raw standard curve–derived T/S ratio, and the assay coefficient of variation ranged from 16% to 21%. In addition to baseline TL in peripheral blood, the first study evaluated overall survival after censoring follow-up at 5 years and assessed measures of TL at 30 months and TL change between baseline and 30 months (17). Using the 5-year censored follow-up, the all-cause mortality HR for baseline TL was significantly lower when increasing TL was considered as continuous variable but not when dichotomized into long versus short telomeres. Long 30-month TL was associated with nonstatistically significant lower all-cause mortality that became statistically significant when TL was considered as a continuous variable at 5-year censored follow-up. Shortening of telomeres between baseline and 30 months was associated with 2-fold higher all-cause mortality ($P = 0.006$) for full follow-up and 3-fold higher mortality at 5 years ($P = 0.03$).

Two studies evaluated baseline TL in breast tumor tissue and overall survival (Fig. 2; Supplementary Table S3; refs. 21, 22). The first one reported a significantly lower all-cause mortality with longer telomeres ($n = 120$; HR = 0.12; 95% CI, 0.08–0.19), and

Table 1. Summary characteristics of studies reporting associations of TL with prognostic factors ($N = 33$)

Design	Cross-sectional studies: $n = 16$
	Case–control studies: $n = 2$
	Longitudinal cohort studies: $n = 11$
	Randomized controlled trials: $n = 4$
Participants	Number of participants: 30–657
	Mean age: 44–75 years
	Stage: 0–IV
TL	DNA source:
	Peripheral blood: $n = 13$
	Breast tumor tissue: $n = 20$
	DNA extraction method:
	Columns: $n = 8$
	Phenol–chloroform: $n = 2$
	Salt extraction method: $n = 2$
	Magnetic glass particles: $n = 1$
	Not reported: $n = 12$
	Not applicable: $n = 8$
	Measurement method:
	Southern blot: $n = 9$
	Slot blot: $n = 4$
	qPCR: $n = 9$
	FISH (qualitative/semi-quantitative): $n = 4$
	Q-FISH: $n = 5$
	STELA: $n = 1$
	Not reported: $n = 1$
Potential prognostic factors	Ethnicity: $n = 5$
	Physical activity: $n = 3$
	BMI/weight: $n = 2$
	Smoking/nicotine intake: $n = 3$
	Alcohol intake: $n = 1$
	Psychologic factors: $n = 4$
	Mood disturbances: $n = 3$
	Perceived stress: $n = 2$
	Sleep: $n = 2$
	Fatigue: $n = 1$
	Psychosocial intervention: $n = 3$
Traditional prognostic factors	Age: $n = 16$
	Tumor size: $n = 10$
	Tumor grade: $n = 10$
	Histologic type: $n = 6$
	Nodal involvement: $n = 11$
	Stage: $n = 13$
	Metastasis: $n = 1$
	Local recurrence: $n = 1$
	Molecular subtype: $n = 16$
	Chemotherapy: $n = 6$
	Hormonal therapy: $n = 3$
	Treatment received: $n = 2$
	Comorbid conditions: $n = 1$

Abbreviation: *n*: number of studies.

the second one a nonsignificantly lower all-cause mortality with longer telomeres ($n = 302$; HR = 0.83; 95% CI, 0.49–1.41). The I^2 test for heterogeneity of these two studies' results was 97%. The first study included stage I to III patients for a median follow-up time of 4.6 years, whereas the second one included stage I to IV patients for a median follow-up time of 7.2 years. The first study used the column-based method for DNA extraction from frozen tumor samples with 20% to 100% tumor content, measured the X chromosome TL (XpYp telomere) using a PCR-derived method (STELA), and a threshold for low telomeres derived from the optimum fusion threshold identified in chronic lymphocytic leukaemia. The second study used the phenol–chloroform method for DNA extraction from snap-frozen fresh samples with 80% to 90% tumor content and qPCR measured T/S ratios with a coefficient of variation <15%.

Breast cancer–specific survival: Three studies evaluated baseline TL in peripheral blood and breast cancer–specific survival (Fig. 2; Supplementary Table S2; refs. 17–19). The first one reported nonstatistically significant lower breast cancer–specific mortality with longer telomeres ($n = 611$; HR = 0.75; 95% CI, 0.44–1.28; ref. 17), the second study reported no association ($n = 1{,}026$; HR = 1.01; 95% CI, 0.69–1.48; refs. 18), and the third study reported statistically significant higher breast cancer–specific mortality with longer telomeres ($n = 176$; HR = 2.92; 95% CI, 1.33–6.41; ref. 19). The I^2 test for heterogeneity of these three studies' results was 75%. In the third study (19), 35.9% of patients had tumors >16 mm in size (stage not reported), the follow-up lasted 7 years, and TL was measured by qPCR using a relative quantification of the telomere to single-copy gene (T/S) ratio (sample T/S ratio on a reference cell line T/S ratio), with an interassay coefficient of variation of 3.96% (DNA extraction method not reported; ref. 19). In addition, the first study (17), which evaluated breast cancer–specific survival using 5-year censored follow-up and TL measures at 30 months, reported nonsignificantly lower breast cancer–specific mortality for longer telomeres and significantly higher breast cancer-specific mortality for telomere shortening; HR = 3.03 (95% CI, 1.11–8.18) for full follow-up and HR = 2.46 (95% CI, 0.64–9.43) for 5-year censored follow-up.

No study reported on TL in breast tumor tissue and breast cancer–specific survival.

One study evaluated baseline TL in breast tumor tissue and breast cancer–related adverse event–free survival (Fig. 2; Supplementary Table S3; ref. 23). Breast cancer–related adverse events included death due to breast cancer, breast cancer recurrence, or development of a new primary breast tumor. Longer telomeres in breast tumor tissue were associated with a significantly lower rate of breast cancer–related adverse events ($n = 530$; HR = 0.35; 95% CI, 0.14–0.86). This study included stage 0 to IIIA patients for a mean follow-up time of 6.7 years, used the phenol–chloroform method for DNA extraction from formalin-fixed paraffin-embedded (FFPE) samples with 75% to 100% tumor cells and a slot blot measure of telomere DNA content (TC; percentage of standard DNA TC) with a coefficient of variation <10%.

Breast cancer–free survival: No study reported on TL in peripheral blood and breast cancer–free survival.

Two studies evaluating baseline TL in breast tumor tissue and breast cancer–free survival (Fig. 2; Supplementary Table S3; refs. 22, 24) reported lower breast cancer recurrence rates associated with longer telomeres, one nonsignificantly lower ($n = 302$; HR = 0.83; 95% CI, 0.53–1.30) and the other significantly lower ($n = 77$; HR = 0.23; 95% CI, 0.07–0.69). The I^2 test for heterogeneity of these two studies' results was 77%. Both of these studies included patients with stage I to IV disease, the first one for a median follow-up time of 7.2 years, and the second for up to 23 years of follow-up. The first one used the phenol–chloroform method for DNA extraction from snap-frozen fresh samples with 80% to 90% tumor content and qPCR-measured T/S ratios (coefficient of variation <15%), whereas the second one used the column-based method for DNA extraction from FFPE samples with 75% to 100% tumor cells and a slot blot measure of telomere DNA content (TC, percentage of standard DNA TC, coefficient of variation not reported).

TL and prognostic factors

The main results of the 33 studies reporting associations of TL and prognostic factors are summarized in Table 2. Sixteen studies were cross-sectional studies and nine reported adjustment for potential confounders. Adjustment was deemed appropriate

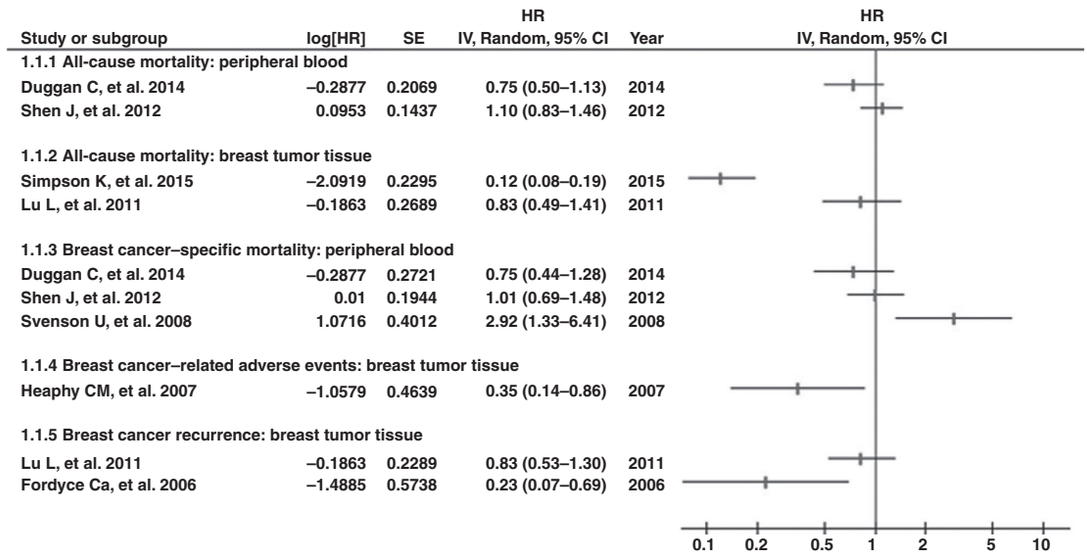

Figure 2.

Forest plot for comparison of "long telomeres" versus "short telomeres" with survival outcomes. IV, inverse variance; breast cancer–related adverse events: death due to breast cancer, breast cancer recurrence, or development of a new primary breast tumor.

when studies mentioned considering potential confounders, including age. Unadjusted results were considered appropriate (i.e., adjusted) when authors mentioned that adjustment for age and other potential confounders did not change their results.

TL in peripheral blood: Among the 13 studies evaluating the associations of TL measured in peripheral blood with prognostic factors, three were cross-sectional studies and seven reported adjustment for potential confounders (Supplementary Table S4; refs. 19, 26, 30–33, 35). In the studies that accounted for confounding, shorter telomeres were associated with older age, and longer telomeres with higher levels of physical activity in one study (26); telomeres were slightly longer in patients with positive tumor ER status in one study (19), but the difference between ER-positive and ER-negative patients was not statistically significant. Shorter telomeres were associated with getting chemotherapy in one study (30) but not in the three other studies (26, 31, 35). Randomization to mindfulness-based stress reduction intervention was not associated with TL in three studies (32–34).

TL in breast tumor tissue: Among the 20 studies evaluating the association of TL measured in breast tumor tissue with prognostic factors, 13 used a cross-sectional design and two reported adjustment for potential confounders (Supplementary Table S5; refs. 38, 49). In these latter two studies, there was no association of TL with age or breast cancer hereditary origin, while shorter telomeres were associated with higher tumor grade in one study (38), and shorter telomeres in normal breast tissue adjacent to tumor were associated with higher rates of local recurrence in the other study ($n = 152$; OR = 5.1; 95% CI, 1.2–22.2; ref. 49).

Discussion

With the exception of a single study reporting a negative association, the current systematic review of TL and breast cancer prognosis indicates an overall trend toward a positive association of longer telomeres with a better prognosis, when measured in breast tumor tissue, but not for TL measured in peripheral blood. Although reported in one study each, shorter telomeres in normal breast tissue adjacent to tumor seem to be associated with higher local recurrence rates. In breast tumor tissue, shorter telomeres seem to be associated with higher tumor grade, but not with the hereditary origin of the tumor. Telomeres seemed to be shorter with older age when measured in peripheral blood but not when measured in breast tumor. In peripheral blood, longer telomeres seem to be associated with higher levels of physical activity and were not associated with molecular subtype. Blood TL was not associated with chemotherapy (three of four studies) and stress reduction interventions (two of two studies).

Even though the overall strength of evidence is weak, as few studies contributed to estimations of associations and the overall risk of bias in these studies ranged from moderate to critical, with nearly the half of studies being cross-sectional, these results are consistent with a recent systematic review and meta-analysis of the association of TL with cancer risk (52). In that review related to cancer risk, findings suggest that shorter TL in surrogate tissue (mostly peripheral blood cells) is associated with higher risk of cancer incidence; for breast cancer risk, in eight studies, all but two suggested a trend toward an association of shorter telomeres with higher risk of breast cancer incidence.

The overall trend toward a positive association of longer telomeres in breast tumor tissue with a better prognosis is based on four studies of moderate-to-critical risk of bias and might be explained by these four studies being biased, and the true association is negative. Actually, studies with the highest risk of bias were more prone to reporting statistically significant associations despite having wider CIs and/or fewer participants. It is though difficult to predict the direction of potential biases when a mixture of selection, confounding, and misclassification biases are involved and all the necessary information for such a prediction is not always available. In particular, all of these four studies have considered age and disease severity (as reflected by stage or Nottingham prognostic index) as potential confounders, and thus, the remaining heterogeneity between these studies could be explained by the methods used for DNA extraction and TL

Table 2. Main results of studies reporting associations of TL and prognostic factors ($N = 33$)

Prognostic factors	Total number of studies	Number of studies reporting association of the factor with short telomeres		Number of studies reporting association of the factor with long telomeres		Number of studies reporting no association of the factor with TL	
		Without adjustment	With adjustment	Without adjustment	With adjustment	Without adjustment	With adjustment
Peripheral blood ($n = 13$)							
Age	5	3	1			1	
Ethnicity	2			1[a]		1	
Physical activity	3				1	2	
BMI	1					1	
Smoking/nicotine intake	3					3	
Alcohol intake	1					1	
Mood disturbances	3					3	
Perceived stress	2	1				1	
Sleep	2					2	
Fatigue	1					1	
Psychosocial intervention	3					1	2
Tumor size	1					1	
Nodal involvement	1					1	
Stage	3					3	
Histologic type	1					1	
Molecular subtype	4					3	1
Chemotherapy	4		1				3
Hormonal therapy	2					2	
Treatment received	2					2	
Comorbid conditions	1					1	
Breast tumor tissue ($n = 20$)							
Age	11	1				9	1
Ethnicity	3					3	
Menopausal status	2					2	
Weight	1					1	
Hereditary/family history	2					1	1
Metastasis	1	1					
Local recurrence	1		1[b]				
Tumor size	9	2				7	
Nodal involvement	10	2		1		7	
Stage	10	3				7	
Tumor grade	10	1	1			8	
Histologic type	5					5	
Molecular subtype	12	3		1[c]		8	
Chemotherapy	2					2	
Hormonal therapy	1					1	

NOTE: If study reported results before and after adjustment, only adjusted results presented.
Abbreviation: BMI, body mass index.
[a]Black versus white patients.
[b]In normal breast tissue adjacent to tumor.
[c]Alternative lengthening of telomeres phenotype.

measurement, which were different and not quite appropriate. However, it should be kept in mind that measurement errors lead to nondifferential misclassification, which results in an underestimation of the true associations. Thereby, breast tumor TL is likely to be more strongly positively associated with survival than it actually seems. The inconsistency of results between studies of blood TL and survival could also be explained by biases, although all the three of them were of moderate risk of bias and have considered age and disease severity as potential confounders. The single study reporting a negative association was the only one to report measuring blood TL before treatment, which may explain this finding. However, it had the widest CI and the smallest sample size, as well as the shortest follow-up period. The study reporting nearly a null association also reported a trend toward a negative association in the HER2-negative subgroup and had the highest variation coefficient for TL measurements. Thus, blood TL might be inversely associated with survival in some specific subgroups, and the observed heterogeneity would be explained by an effect modification by molecular subgroups, which needs to be confirmed by further studies. Nevertheless, at least some of the observed heterogeneity between studies of blood TL could also be explained by the DNA extraction methods, which would have led to a nondifferential misclassification bias.

In fact, in addition to traditional causes of heterogeneity inherent in observational designs, that is, study population selection (breast cancer stage), length of follow-up, loss of follow-up, and handling of confounders, the variability in the methods used for TL measurement and/or DNA extraction is of particular importance. The Southern blot method is historically the first technique used for TL measurement and is still considered the "gold standard" method (53). However, this method estimates the terminal restriction fragment (TRF) length, which includes variable lengths of subtelomeric sequences, depending on the cutting restriction enzymes used and the last restriction site at a given chromosome arm, thus precluding reliable comparisons between studies (54, 55). Subtelomeric fragments are dynamic structures that contribute to the normal polymorphism in the human genome (56, 57), and therefore, differences of TRF lengths

between individuals may not reflect the actual differences of their TLs. Furthermore, the Southern blot technique is known to be relatively insensitive to very short telomeres and thus underestimates the length and number of short telomeres (54, 55). The slot blot method is a variant of the Southern blot requiring less DNA material; it provides an indirect measure (ratio of telomere to centromere signals) and is relatively insensitive (54). In the qPCR assay, the telomeric repeats (T) are amplified using primers that hybridize to telomeres but have mismatches across their length that prevent formation of primer dimer-derived products (i.e., products of hybridization of two primers; ref. 58); amplification is measured quantitatively and compared with that of a single-copy gene (S) to adjust for the amount of DNA in the reaction, yielding a relative TL estimation, the T/S ratio. Thus, qPCR is not suitable for measurement of TL in nondiploid and karyotypically unstable samples, such as tumor tissue samples (54). Furthermore, tumor tissue samples are heterogeneous, especially breast cancer tumors, and can include variable amounts of benign and reactional cells, such as lymphocytes and fibroblasts, which have longer telomeres than cancer cells (43). It has been shown recently that the DNA extraction method used has an impact on TL. The column-based method yields the least accurate measurement and results in shorter TL when compared with phenol–chloroform and salting-out methods, which likely contributed to the observed discrepancies between studies that measured TL by qPCR (59, 60).

The strength of this systematic review is the use of the Cochrane reviews rigorous methodology, including the extensive and highly sensitive search strategy to retrieve as many relevant studies as possible, the use of preestablished protocol, the assessment of the risk of bias, and the systematic analysis of results, in addition to considering the variability in TL measurement methods between studies. Limitations include the small number of studies involved in survival outcome analyses, the lack of high-quality evidence inherent in observational study designs, and the overall critical risk of bias in studies reporting associations with prognostic factors due to confounding, in addition to specific aspects of TL measurement.

Finally, the cross-sectional design, used to date to explore the possible relation between TL and different prognostic factors, is relatively weak. TL seems to be a dynamic feature that responds to processes that can shorten telomeres and processes that can lengthen telomeres (61), especially in cells that express telomerase, such as tumor cells and blood leukocytes. As a result, its prognostic value might be improperly evaluated by a single measurement of TL. TL change between consecutive measurements may reflect both the ability of underlying dynamic processes to restore or maintain telomere homeostasis and the impact of modifiable environmental factors. Thus more longitudinal studies are needed where potentially changes in TL can be assessed as well.

Conclusions

Numerous observational studies of TL have been conducted among breast cancer patients in the last 20 years. Despite the major methodologic differences between studies, when considering all the studies (peripheral blood and tumor tissue) and all outcomes together, there was a trend toward an association of longer telomeres with a better prognosis. Further longitudinal studies with rigorous methodology, including proper control of confounding and an adequate TL measurement method, are still needed to determine the exact prognostic significance of TL for breast cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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