

# Breast Cancer Survival Is Associated with Telomere Length in Peripheral Blood Cells

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## Abstract

**Telomeres are essential for maintaining chromosomal stability. Previous studies have indicated that individuals with shorter blood telomeres may be at higher risk of developing various types of cancer, such as in lung, bladder, and kidney. We have analyzed relative telomere length (RTL) of peripheral blood cells in relation to breast cancer incidence and prognosis. The study included 265 newly diagnosed breast cancer patients and 446 female controls. RTL was measured by real-time PCR, and our results show that the patient group displayed significantly longer telomeres compared with controls ( $P < 0.001$ ). Age-adjusted odds ratios (OR) for breast cancer risk increased with increasing telomere length, with a maximal OR of 5.17 [95% confidence interval (95% CI), 3.09–8.64] for the quartile with the longest telomeres. Furthermore, RTL carried prognostic information for patients with advanced disease. Node positive (N+) patients with short telomeres ( $\leq$ median) showed an increased survival compared with N+ patients with long telomeres ( $P = 0.001$ ). For patients with ages  $<50$  years with tumors  $>16$  mm (median tumor diameter), short telomeres were associated with a significantly better outcome than longer telomeres ( $P = 0.006$ ). Cox regression analysis showed that long RTL was a significant independent negative prognostic factor (hazards ratio, 2.92; 95% CI, 1.33–6.39;  $P = 0.007$ ). Our results indicate that blood RTL may serve as a prognostic indicator in breast cancer patients with advanced disease. [Cancer Res 2008;68(10):3618–23]**

## Introduction

Telomeres are capping end structures of eukaryotic chromosomes essential for protecting chromosomal integrity. Each telomere is composed of a noncoding sequence consisting of (TTAGGG)<sub>n</sub> repeats, in complex with specific proteins (1, 2). Normally, telomeres shorten with each cell division until a critical length is reached and the cell enters cell cycle arrest (3). Permanent cell growth requires telomere maintenance, and certain human cell populations, along with most malignant cells, possess activity of telomerase for telomere elongation (4). Despite telomerase activity, the majority of tumor cells have shorter telomeres than the corresponding normal tissue and there is a relationship between short telomeres and genetic instability (5). Wu et al. previously

reported an association between short telomeres in peripheral blood and increased risks for head and neck, bladder, lung, and renal cell cancers (6). In another study, telomere length was shorter in buccal cells from patients with bladder cancer compared with controls (7). These data implicate that individuals with shorter telomeres may be at higher risk of developing different forms of cancer.

Breast cancer is the most common cancer in women of the Western world, affecting ~1 in 10 women (8). In a previous study on blood cell telomere length in spontaneous breast cancers, no difference was seen compared with controls (9). Another report in sister sets has indicated an association between breast cancer risk and shorter telomeres in high-risk breast cancer families (10). In the present study, we evaluated blood telomere length as a possible biological marker for breast cancer risk and prognosis. Using real-time PCR, we measured relative telomere length (RTL) in peripheral blood cells of 265 newly diagnosed unselected breast cancer patients and 446 female controls. Interestingly, we observed longer blood telomeres in the patient group relative to controls. Furthermore, we found that blood RTL may serve as a predictor for survival in patients with advanced disease.

## Patients and Methods

**Study material.** Samples were consecutively collected from breast cancer cases in Västerbotten county, Northern Sweden, referred to the Oncology Clinic, Umeå University Hospital. The majority of patients with newly diagnosed breast cancer within this area are referred to the clinic, with the exception of very old cases or subjects otherwise in bad general condition. Thus, the degree of selection in the enrollment of cases could be considered to be very small in this study. The study material originally consisted of 272 unselected breast cancer patients diagnosed between 1990 and 2006. Seven patients were excluded from the study because they, before blood sampling, had received chemotherapy or hormone therapy or, earlier, had been diagnosed with another cancer form. Hence, 265 patients, untreated except for surgical removal of their tumor, were included in the analysis. Buffy coats from all patients were collected at the Oncology Clinic within 3 mo after morphologic diagnosis.

Data regarding tumor size, nodal status, and estrogen receptor (ER) expression were collected from the clinical charts at the Oncology Clinic, Umeå University Hospital. Two techniques for ER determination were used during the sample collection period. Before the year of 2000, ER expression was analyzed using an enzyme immunoassay, and samples with values of  $\geq 0.1$  fmol/ $\mu$ g DNA were classified as positive. After 2000, ER status has been determined by immunohistochemistry. Four patients were classified as "uncertain," and these patients were not included in the statistical analysis regarding ER. There was no difference in the intention to treat strategy, comparing patients with long versus short telomeres. The cause of death was obtained from clinical charts and death certificates with the last follow up in May 2007.

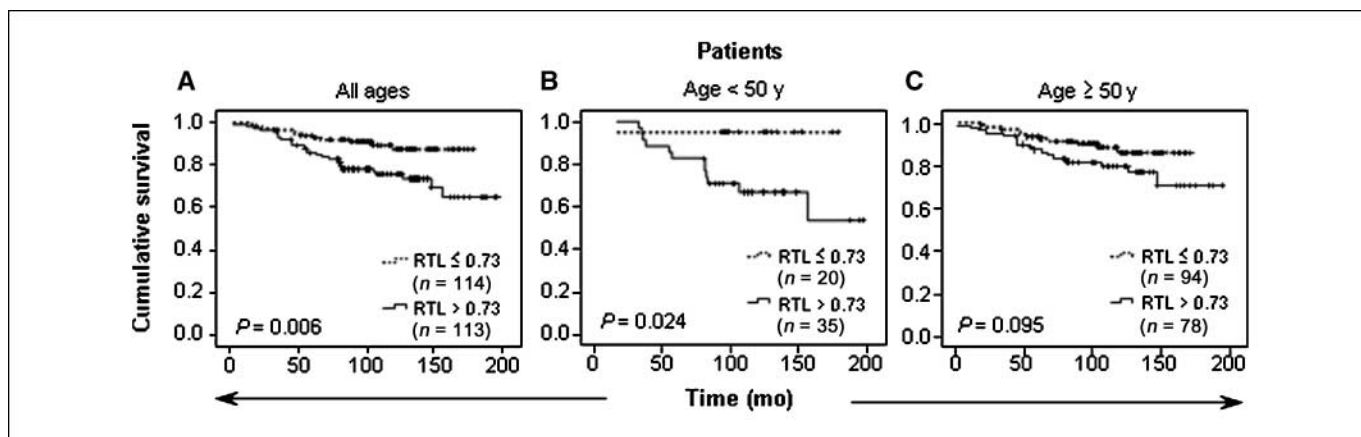
Two population-based control collections were used, one comprising buffy coat samples ( $n = 300$ ) from the MONICA (Multinational Monitoring

**Note:** U. Svenson and K. Nordfjäll contributed equally to this work.

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**Figure 2.** Survival analysis for patients with long (RTL, >0.73) versus short (RTL, ≤0.73) blood telomeres using Kaplan-Meier with the log-rank test. The analysis included patients diagnosed from 1990 through 2000 ( $n = 227$ ; seven cases missing due to no survival data). Patients diagnosed after 2000 were not included in the survival analysis ( $n = 31$ ). A, all ages. B, patients of ages <50 y. C, patients of ages ≥50 y.

age-adjusted ORs were calculated after subdividing the whole material ( $n = 711$ ) into quartiles based on the RTL distribution among controls. Breast cancer risk increased with increasing RTL ( $P_{\text{trend}} < 0.001$ ) with a maximal OR of 5.17 (95% CI, 3.09–8.64) for the quartile with the longest telomeres (Fig. 1C).

**Blood RTL predicts survival in patients with advanced disease.** We also examined blood RTL as a potential predictive factor for cancer-specific death. Before performing survival analysis, the patients were dichotomized into two groups based on the median RTL value (0.73). Interestingly, blood RTL carried significant prognostic information ( $P = 0.006$  for all patients; Fig. 2A–C). Patients of ages <50 years (approximate menopausal age) with short telomeres (≤median value) had a significantly better outcome than patients with long telomeres ( $P = 0.024$ ; Fig. 2B), and for patients of ages ≥50 years, a borderline value was achieved ( $P = 0.095$ ; Fig. 2C).

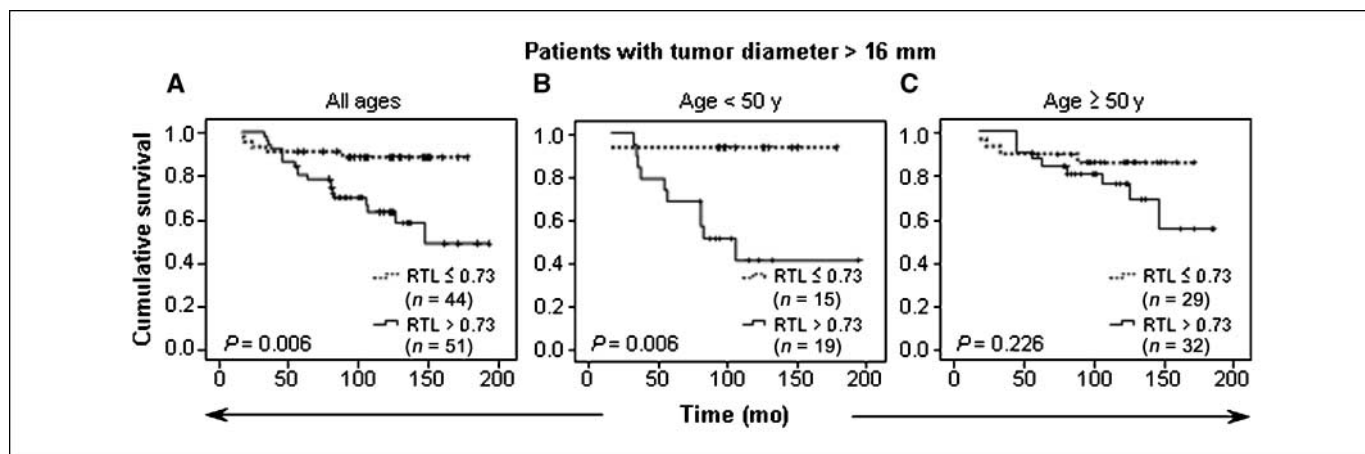
We next addressed whether blood RTL was of prognostic value for any patient subgroup. First, tumor size and nodal status were evaluated, two established prognostic indicators showing expected

outcomes in our patient cohort (Table 1). For patients with tumors larger than 16 mm (median tumor diameter), a significant association was found between blood RTL and survival ( $P = 0.006$ ; Fig. 3A). This connection proved to be significant for patients of ages <50 years, for whom short telomeres were strongly related to an increased survival time ( $P = 0.006$ ; Fig. 3B). The association could not be shown for patients of ages ≥50 years ( $P = 0.226$ ; Fig. 3C) or for patients with a tumor size of ≤16 mm ( $P = 0.529$ ; all ages; not shown in figure). Also for node-positive (N+) patients of all ages, of <50 years, and of ≥50 years, short RTL indicated a better prognosis ( $P = 0.001$ , 0.039, and 0.008, respectively; Fig. 4A–C). Survival was not coupled to blood RTL in node-negative (N0) patients ( $P = 0.444$ ; all ages; not shown in figure). However, the statistics for the ≤16 mm and N0 tumor groups should be taken with caution due to few events in these patient groups.

To verify blood RTL as an independent prognostic indicator, multivariate Cox regression analysis was performed, including age, nodal status, tumor size, and blood RTL. The results revealed that long blood RTL (RTL, >0.73) was a significant independent negative

Table 1. Survival analysis in relation to tumor size and nodal status						
Patients		Tumor diameter, ≤16 mm	Tumor diameter, >16 mm	$P^*$	Valid cases	Missing cases
All ages	Total	110	95	0.001	205	60
	Deaths	9 (8.2%)	24 (25.3%)			
<50 y	Total	18	34	0.027	52	17
	Deaths	1 (5.6%)	11 (32.4%)			
≥50 y	Total	92	61	0.032	153	43
	Deaths	8 (8.7%)	13 (21.3%)			
Patients		N0	N+	$P^*$	Valid cases	Missing cases
All ages	Total	123	66	<0.001	189	76
	Deaths	14 (11.4%)	23 (34.8%)			
<50 y	Total	29	25	0.539	54	15
	Deaths	6 (20.7%)	7 (28.0%)			
≥50 y	Total	94	41	<0.001	135	61
	Deaths	8 (8.5%)	16 (39.0%)			

\* $P$  value using Kaplan-Meier with the log-rank test.



**Figure 3.** Survival analysis (long versus short blood telomeres) restricted to patients with large tumors (tumor  $\varnothing$ , >16 mm;  $n = 95$ ). A, all ages. B, patients of ages <50 y. C, patients of ages  $\geq$ 50 y.

factor for survival (HR, 2.92; 95% CI, 1.33–6.39;  $P = 0.007$ ; Table 2). There were too few events to perform a Cox analysis restricted to patients of ages <50 or  $\geq$ 50 years.

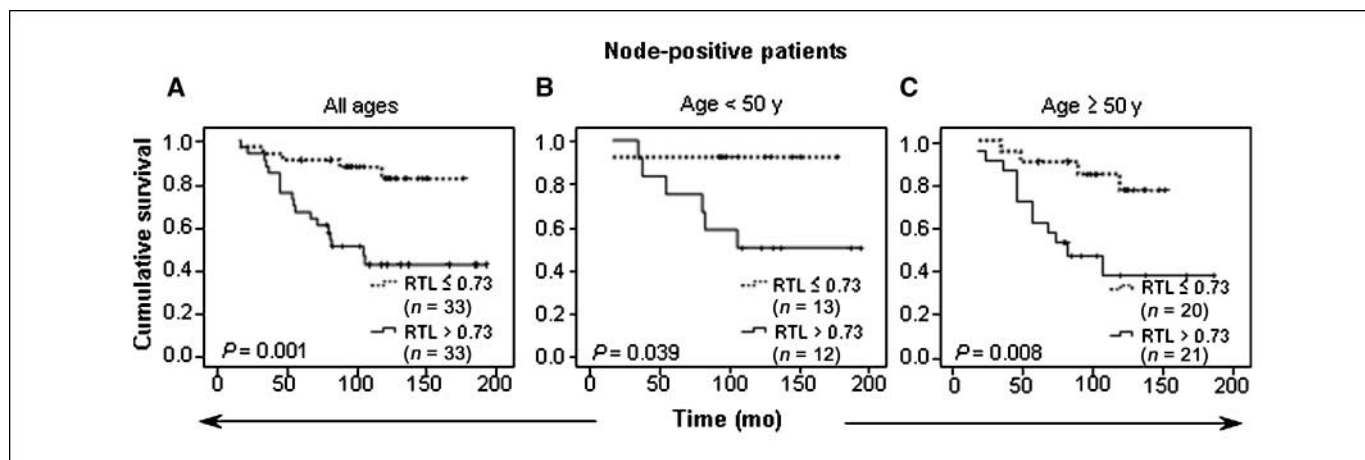
Finally, blood RTL was evaluated in relation to ER status and survival. Data on ER expression were available for 200 of 265 patients, and the majority (78%) were ER+. After adjusting for age, no statistically significant difference in RTL was observed between ER+ and ER– patients ( $P = 0.191$ ; not shown in figure), although mean RTL was slightly higher for ER+ (0.73; 95% CI, 0.70–0.75) compared with ER– patients (0.69; 95% CI, 0.65–0.74). There was no difference in survival for patients classified as ER+ versus ER– ( $P = 0.909$ ; not shown in figure), but again short RTL was associated with a better prognosis in both ER+ and ER– patients ( $P = 0.005$  and  $P = 0.025$ , respectively, all ages; not shown in figures). ER+ cases had significantly longer telomeres than controls ( $P < 0.001$ ), whereas a nearly significant  $P$  value was achieved for the ER– cases ( $P = 0.055$ ).

## Discussion

In the present study, we report novel findings showing that newly diagnosed breast cancer patients had longer blood telomeres compared with controls. Notably, blood RTL was found to

represent a strong independent prognostic indicator in patients with advanced disease (>16 mm tumor diameter and/or N+).

The observation that telomeres shorten with age is consistent with previous studies (15–18). Regarding blood telomere length in cancer patients, the findings in the present study were unexpected and in contrast with previous reports on head and neck, bladder, lung, and renal cell cancers (6). Concerning breast cancer, a report on sisters in high-risk breast cancer families showed a weak, nonsignificant association between breast cancer risk and shorter telomeres (10). Our study involved newly diagnosed, spontaneous tumors encompassing a wide age span and unrelated controls, which makes comparison between the studies difficult. No difference in blood telomere length between breast cancer patients, treated or untreated, and controls was shown in a study using Southern blotting for telomere length estimation (9). We and others have shown a good correlation comparing Southern blot data with PCR-based results (13, 19), but methodologic differences cannot be excluded as one reason for the discrepancies. In contrast to the PCR technique, which can amplify very short TTAGGG tracts, it is difficult to assess the sensitivity of Southern blotting at lower kilobase ranges. Recently, the existence of extremely short telomeres in human cancer cells, “t-stumps,” was shown indicating that telomeres can vary considerably more in size than previously



**Figure 4.** Survival analysis (long versus short blood telomeres) restricted to node-positive patients ( $n = 66$ ). A, all ages. B, patients of ages <50 y. C, patients of ages  $\geq$ 50 y.

**Table 2.** Multivariate Cox regression survival analysis

Patients	HR (95% CI)	P
Blood telomere length		
RTL ≤ 0.73	1.0	
RTL > 0.73	2.92 (1.33–6.39)	0.007*
Tumor size		
Tumor Ø ≤ 16 mm	1.0	
Tumor Ø > 16 mm	2.04 (0.88–4.71)	0.093*
Node status		
N0	1.0	
N+	3.36 (1.56–7.21)	0.002*

NOTE: Valid cases (with complete data on all included variables) = 176; missing cases = 89.  
\*Adjusted for age.

anticipated (20). The presence of such short telomeres can in theory differ between patients and controls, and if so, the methodology for telomere length estimation is likely of significance. Irrespective of the reasons for the conflicting data regarding blood RTL status in breast cancer patients, future confirmatory investigations are warranted.

In general, malignant tumors show shorter telomeres than corresponding normal tissues and telomere dysfunction has been indicated as a negative prognostic marker in solid tumors, including breast cancer (21, 22). Also, *in situ* telomere length assessments showed that telomere attrition occur early during breast carcinogenesis (23). In contrast, we surprisingly found longer blood telomeres in breast cancer patients compared with controls. More importantly, in our breast cancer cohort, longer telomeres were an independent negative prognostic marker. What are the potential explanations for these discrepancies with regard to most previous publications?

Telomere length maintenance is a complex process governed by a multitude of telomere elongating and shortening factors, many of which are differentially expressed in diverse cell types. It is not inconceivable that normal and malignant cells behave differently in this respect, as exemplified in the present study anticipating that the tumors of our patients exhibited shortened telomeres in line with other studies. It should be remembered that breast cancer is thought to develop in cells with shortened telomeres that thereafter are maintained through the action of telomerase. Normal hematopoietic cells differ from, e.g., epithelial cells in that they are telomerase competent upon mitogenic stimulation, a difference likely to be of biological importance in the present study.

It is well established that epigenetic alterations can modulate telomere length (24). CTCF (CCCTC-binding factor), a known chromatin insulator protein, is a negative regulator of *human telomerase reverse transcriptase (hTERT)* gene expression (25), whereas brother of the regulator of imprinted sites (BORIS), a protein activated in various cancers, can inhibit CTCF binding (26). BORIS may thus act as a positive regulator of *hTERT* transcription, and interestingly, high levels of BORIS have been detected in blood leukocytes from breast cancer patients compared with controls (27). Another observation is that the levels of a number of potentially telomerase activating cytokines, like interleukin 2 (IL-2), IL-4, IL-6, IL-7, IL-10, and IL13 (28–32), were found at higher serum levels in breast cancer patients compared with controls (33).

In contrast to malignancies previously associated with shortened blood telomeres (6), breast cancer is generally classified as a hormone-related cancer. Prolonged estrogen exposure is a well-established risk factor for breast cancer (34). Estrogen can also influence telomere dynamics (35). A number of studies have reported that women have longer telomeres than men, and estrogen exposure has been suggested as the most likely reason for this gender difference (15, 16, 36, 37). In support of this hypothesis, postmenopausal women with a history of long-term hormone replacement therapy had longer blood telomeres than postmenopausal women without hormone replacement therapy (38). Influences on telomere length by estrogen can occur by activation of the *hTERT* gene promoter, which contains an estrogen-responsive element (39), and by posttranscriptional regulation of *hTERT* via Akt-dependent phosphorylation (40). Moreover, estrogen has antioxidative capacity (35), and because oxidative stress is known to increase telomere attrition (41), this property might be of relevance in telomere maintenance. Our findings may hence reflect a combined effect of epigenetic regulation, cytokine, and hormonal effects.

In conclusion, we here show that telomere length in peripheral blood cells differ between breast cancer patients and control subjects and may serve as a significant prognostic biological marker.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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