Research Article

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Shorter Leukocyte Telomere Length Is Independently Associated with Poor Survival in Patients with Bladder Cancer

Alessia Russo[1,2], Federica Modica[1,2], Simonetta Guarrera[1,2], Giovanni Fiorito[1,2], Barbara Pardini[1,2], Clara Viberti[1,2], Alessandra Allione[1,2], Rossana Critelli[2,3], Andrea Bosio[4], Giovanni Casetta[4], Giuseppina Cucchiarale[5], Paolo Destefanis[4], Paolo Gontero[4], Luigi Rolle[4], Andrea Zitella[4], Dario Fontana[4], Bruno Frea[4], Paolo Vineis[3,6], Carlotta Sacerdote[7], and Giuseppe Matullo[1,2]

Abstract

Background: Shorter telomere length (TL) has been reported to be associated with increased risk of early death in elder individuals. Telomere shortening has been also related to chromosomal instability, which may possibly contribute to the development of several types of digestive or urogenital system cancers and smoking-related tumors. Therefore, we investigated the impact of TL on bladder cancer survival.

Methods: TL was measured in leukocyte DNA from whole peripheral blood using quantitative real-time PCR in 463 patients with bladder cancer from a total 726 cases who were followed for up to 18 years.

Results: Patients with muscle-invasive tumor/any grade had shorter telomere than patients with non–muscle-invasive tumor/high-grade and with non–muscle-invasive tumor/non–high-grade (TL reference 0.7 ± 0.2; vs. respectively, 0.8 ± 0.2, $P = 3.4 \times 10^{-2}$ and 0.8 ± 0.2, $P = 3.6 \times 10^{-2}$). Moreover, patients in the lowest quartiles of TL were associated with decreased survival after diagnosis (log-rank test, $P = 3.9 \times 10^{-4}$). A Cox regression adjusted by age, cancer aggressiveness, Bacillus Calmette-Guérin, radical cystectomy, radiotherapy, and chemotherapy showed an independent effect of TL on bladder cancer survival (HR, 3.9; 95% confidence interval, 1.7–9.1; $P = 1.2 \times 10^{-3}$).

Conclusions: Our results suggest that leukocyte TL is only partly related to tumor aggressiveness and that shorter telomeres act as independent prognostic predictor of survival in patients with bladder cancer. TL information may allow to better select therapeutic approaches in patients with the same stage and grade.

Impact: Blood leukocyte TL levels could provide an additional noninvasive prognostic marker to better predict survival and personalize therapies in patients with bladder cancer. *Cancer Epidemiol Biomarkers Prev; 23(11); 2439–46. ©2014 AACR.*

Introduction

Telomeres are the specialized DNA protein structures that cap the ends of linear eukaryotic chromosomes. They have an essential role in protecting the chromosome ends from fusions and degradation (1). Telomeres shorten every cell division due to the fact that during replication the DNA polymerase is not able to complete replication until the end of a linear DNA molecule (2). Excessive or accelerated telomere shortening results in chromosomal instability that may contribute to the development of several types of cancer (3, 4). Various case–control studies previously showed that shorter telomere length (TL) in lymphocytes, buffy coats, and buccal cells are associated with an increased risk of bladder cancer (5–7). Telomere shortening depends also on tissue mitotic activity and lifestyle (8).

Shorter TL, used as a marker of cumulative cellular aging, may be associated with increased rate of mortality, as described by Cawthon and colleagues in a study on elders (9). Therefore, shorter TL may also be associated with increased risk of death after cancer. Recently, several studies on different types of tumors reported a poor survival in patients with cancer with shorter TL measured in non–small cell lung cancer and leukocytes (10, 11). Notably, the study of Weischer and colleagues (11) examining 47,102 cancer individuals followed for 20 years observed that shorter leukocyte TL was associated with

[1]Genomic Variation in Human Population and Complex Diseases Unit, Human Genetics Foundation, Turin, Italy. [2]Department of Medical Sciences, University of Turin, Turin, Italy. [3]Molecular and Epigenetic Unit, Human Genetics Foundation, Turin, Italy. [4]Department of Urology, Città della Salute e della Scienza, Turin, Italy. [5]Department of Urology, Clinica Cellini, Turin, Italy. [6]Imperial College London, London, United Kingdom. [7]CPO Piemonte, Turin, Italy.

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Corresponding Author: Giuseppe Matullo, University of Turin and Human Genetics Foundation, Via Nizza 52, 10126 Turin, Italy. Phone: +390116709542; Fax: +390116709546; E-mail: giuseppe.matullo@unito.it

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reduced survival after cancer, but no risk of early death was reported after stratification for cancer types for 131 urinary tract cancers included in the study.

To further investigate the impact of TL on bladder cancer survival, we analyzed leukocyte TL in a large population of 463 patients with bladder cancer sampled at the time of diagnosis and with up to 18-year follow-up to test whether shorter TL may be associated with increased risk of early death.

Materials and Methods

Patients

Patients belong to a hospital-based case–control study led at A. O. La Città della Salute e della Scienza, formerly S. Giovanni Battista hospital, in Turin, and were recruited during the years 1994 to 2008 (12, 13). In total, 726 patients were recruited, followed up, and included in a survival analysis to investigate the effect on clinical outcome of patients' characteristics. For 463 male bladder cancer cases (ages 40–75 years), DNA from blood was available, and this subgroup was included in the study for TL measurement.

All cases were newly diagnosed, histologically confirmed bladder cancers, and were blood-sampled before any treatment at three urology departments of S. Giovanni Battista hospital. Participants were interviewed to fill a questionnaire with information on past and present lifestyle. For each patient, the therapy (Bacillus Calmette-Guérin—BCG, chemotherapy, radiotherapy, and/or radical cystectomy) was recorded through the perusal of clinical records in collaboration with urologists. The chemotherapy group included patients treated with systemic or intravesical chemotherapy except for those who received one instillation of chemotherapy immediately after transurethral resection.

Information on patients vital status was obtained through the local demographic office, and death certificates were retrieved to identify the specific causes of death (last follow-up March 31, 2012).

All subjects were informed and provided written consent to participate in the study, according to the Declaration of Helsinki. The design of the study was approved by the Interhospital Ethical Board of San Giovanni Battista/C.T.O./C.R.F./Maria Adelaide hospitals.

TL analysis

DNA was extracted from peripheral blood leukocytes as reported by Sacerdote and colleagues (13). The total amount of telomeric DNA was measured by quantitative real-time PCR as previously described by Zee and colleagues (14). A commercial DNA (Human DNA Male; Applied Biosystems) was included in each PCR run to guarantee comparability between plates and as calibrator. The average C_t values (measured in triplicate) were used to calculate the ΔC_t for each sample (where $\Delta C_t = C_t$ telomere $- C_t$ single-copy gene). The relative quantification (RQ), for each sample, i.e., the amount of telomeric DNA of the sample relative to that of the calibrator (control DNA), was determined using the equation: $RQ = 2^{-\Delta\Delta Ct}$ (where $\Delta\Delta C_t = \Delta C_t$ of each unknown sample $- \Delta C_t$ of the control sample). A 7900HT Fast Real-Time PCR System (Applied Biosystems) with SDS Software version 2.3 (Applied Biosystems) was used. The interassay coefficient of variation (CV) as percentage based on the control sample included in all plates ($N = 17$) was 0.9% and 1.2% for *36B4* PCR and telomere PCR, respectively. Melting (dissociation) curve analysis was performed on every run to verify specificity of the PCR products.

Statistical analysis

The Kolmogorov–Smirnov test and quantile–quantile plot were performed to verify whether TL was normally distributed. The patients' characteristics were described as mean and SD for the quantitative variable, and absolute and relative frequencies were used for qualitative variables. We used the *t* test and Pearson χ^2 test along with the Yates' continuity correction to assess statistical significance. Correlation between TL and age was obtained by the Pearson correlation coefficient.

Tumor stage (T) and grade (G) were also grouped into one variable (TG) as follows: non–muscle-invasive (NMI)/non–high-grade (NHG), NMI/high-grade (HG), and muscle-invasive (MI)/any-grade (anyG). As only 7 patients had MI/NHG tumor and no significant difference was found for TL and survival between MI/NHG and MI/HG groups, the two groups were merged together in the MI/anyG group. Patients with Tis–Ta–T1 tumors were included in the NMI group, whereas patients with T2–4 tumors were included in the MI group (http://www.cancerresearchuk.org). The NHG group consisted of cases with G1 and G2 grades and low-grade diseases according to the World Health Organization (WHO)/1973 and WHO/2004 classifications, respectively. The HG group consisted of cases with G3 diseases (WHO/1973) and high-grade diseases (WHO/2004). Patients with Tis tumors were included in the NMI/HG group (http://www.cancerresearchuk.org).

The Student *t* test was applied to evaluate the association between TL and TG variable as well as TL and therapies. The dependence between TG variable and therapies was evaluated by the Pearson χ^2 test using Yates' continuity correction. The type-I error threshold of significance was set as $\alpha = 0.05$.

Overall survival (OS) was evaluated for each patient calculating the time (in months) between the date of bladder cancer diagnosis and the death date or the date of follow-up termination as the end point. TL was classified both in quartiles and in two groups (below and above the median value), using the longest telomeres category as reference. For TG variable, as defined above, the NMI/NHG class was used as reference. TG and survival data were available for 708 patients of which 463 had leukocyte DNA and TL measurement. The relative risk of death was estimated as HRs using both unadjusted and adjusted Cox regression.

The prognostic role of blood TL on survival was also evaluated using the Kaplan–Meier (K–M) curves, log-rank test, and Tarone test for trend (15) when appropriate.

In the best-fitted model survival analyses, HRs were calculated by the multivariate Cox regression, satisfying their proportional hazards (PH) assumptions.

Finally, a new variable TL-TG, grouping dichotomous TL and TG variable, was examined in survival analyses with the K–M curves, log-rank test, Cox regression model, and Tarone test for trend. In the TL-TG variable, subjects with longer telomeres and NMI/NHG cancer were used as reference.

Associations with $P \leq 0.05$ (two tailed) were considered significant. Individuals with missing data were excluded from the specific analysis.

All analyses were performed using R (R 2.15.2, Survival package; refs. 16, 17).

Results

The total number of patients with bladder cancer consists in 726 cases for which a complete follow-up was available. The initial survival analyses have been performed on this group. For 463 samples, blood DNA was available and leukocyte TL was measured in this subgroup of incident male cases (mean age, 63.7 years) characterized by a median survival time of 16.3 years. Characteristics of the two groups of patients included and not included are presented in Supplementary Table S1. No significant difference between the two groups is evident except only for smoking habits, due probably mainly to differences in ex-smokers proportions. Blood samples were collected at the moment of diagnosis and before any treatment to eliminate any effect due to chemotherapy or other treatments. Clinical and demographic patient characteristics are reported in Table 1. Smoking status did not significantly affect population survival ($P = 6.3 \times 10^{-2}$; Table 1). The percentage of deaths was significantly higher among cases with MI as well as HG cancer ($P = 1.4 \times 10^{-11}$ and 1.4×10^{-5}, respectively; Table 1). Shorter TL significantly affected patients' survival both as continuous and categorical variables ($P = 7.6 \times 10^{-7}$ and 9.9×10^{-6}, respectively; Table 1). Clinical and demographic patient characteristics did not significantly differ among patients stratified for TL above and below the median value (TL $\leq$ 0.8; TL > 0.8; $P > 2.0 \times 10^{-1}$; Table 1).

In the study population, TL was normally distributed. A significantly inverse correlation between age and TL was found in the overall subjects ($r = -0.1$, $P = 1.1 \times 10^{-2}$).

TL inversely correlated also with bladder cancer aggressiveness: patients with MI/anyG cancer had shorter telomeres than patients with NMI/NHG tumor (mean $= 0.7 \pm 0.2$ and 0.8 ± 0.2, respectively, $P = 3.6 \times 10^{-2}$) and with NMI/HG tumor (mean $= 0.7 \pm 0.2$ and 0.8 ± 0.2, respectively, $P = 3.4 \times 10^{-2}$; Fig. 1).

Among patients having an NMI cancer, 44% were treated with chemotherapy (42% with intravesical chemotherapy, 2% with systemic chemotherapy), 39% with BCG therapy, 19% with radical cystectomy, and 6% with radiotherapy. On the other hand, among patients with MI tumor, 88% underwent chemotherapy (74% intravesical chemotherapy, 14% systemic chemotherapy), 80% radical cystectomy, 18% radiotherapy, and only 4% BCG therapy. Ninety patients received more than one therapy.

As expected, significant differences between TG and therapies were found: TG versus all therapies ($P = 2.5 \times 10^{-3}$), TG versus chemotherapy ($P = 2.1 \times 10^{-11}$), TG versus radiotherapy ($P = 4.8 \times 10^{-3}$), TG versus cystectomy ($P = 2.2 \times 10^{-16}$), and TG versus BCG ($P = 2.2 \times 10^{-16}$).

For the 463 samples with TL measurements, a *t* test was performed to evaluate the potential association between TL and therapies. Interestingly, subjects treated with intravesical chemotherapy had on average shorter TL compared with patients who did not undergo chemotherapy (TL $= 0.7 \pm 0.5$ and 0.8 ± 0.5, respectively, $P = 5.0 \times 10^{-3}$).

The two groups of patients with shorter TL (third and fourth quartiles) were associated with a shorter survival (log-rank, $P = 3.9 \times 10^{-4}$; Supplementary Fig. S1). A similar trend was observed in the univariate Cox model survival [HR for the third quartile = 2.5; 95% confidence interval (CI), 1.4–4.7; $P = 3.0 \times 10^{-3}$; HR for the fourth quartile = 2.7; 95% CI, 1.5–5.0; $P = 1.0 \times 10^{-3}$; Tarone test for trend, $P = 2.0 \times 10^{-5}$; Supplementary Table S2]. Furthermore, patients were divided by TL median because the first and the second quartiles showed a similar survival trend as well as the third and the fourth quartiles (Supplementary Fig. S1). Cases with shorter telomeres had a 2.3-fold increased risk of death compared with those having longer telomeres (HR = 2.3; 95% CI; 1.6–3.5; $P = 4.0 \times 10^{-5}$). K–M curves are reported in Fig. 2A.

K–M curves of patients with bladder cancer stratified into the three TG categories are reported in Fig. 2B (log-rank $P = 1.2 \times 10^{-13}$; Tarone test for trend, $P = 1.2 \times 10^{-14}$). Patients with NMI/HG cancer had a worse survival than patients with NMI/NHG cancer, used as reference (HR = 1.7; 95% CI, 1.2–2.5; $P = 4.1 \times 10^{-3}$). Cases with MI/anyG cancer presented even a worse prognosis (HR = 4.2; 95% CI, 2.8–6.1; $P = 4.9 \times 10^{-13}$).

Moreover, using a Cox model adjusted by age, cancer aggressiveness, BCG, radical cystectomy, radiotherapy, and chemotherapy (intravesical or systemic), we observed an independent effect of TL on survival (HR for shorter telomeres = 1.7; 95% CI, 1.1–2.8; $P = 2.1 \times 10^{-2}$; Supplementary Table S3), supported also by a mediation analysis including TL as mediator and tumor invasiveness as predictor of survival (data not shown). As expected, age and tumor aggressiveness also affected survival (Supplementary Table S3). The model fitted the PH conditions in the global and the specific tests ($\chi^2 = 13.0$; $P = 0.07$). Smoking status was not included in the model because it was not significantly affecting survival at the univariate analysis. At the multivariate analysis, similar results were obtained by considering bladder cancer–specific deaths, with TL being a stronger

Table 1. Distribution of patients ($N = 463$) by clinical and demographic characteristics

Variables	Dead, *N* (%)	Alive, *N* (%)	*P*	Shorter TL	Longer TL	*P*
	113 (24.4)	350 (75.6)		238 (51.4)	225 (48.6)	
Age (years)						
Mean (SD)	67.0 (6.9)	62.6 (7.8)	**3.2×10^{-8}**	64.1 (7.5)	63.3 (8.0)	0.2
Smoking status						
Never	2 (1.8)	28 (8.0)		12 (5.0)	18 (8.0)	
Former	58 (51.3)	164 (46.9)		110 (46.2)	112 (49.8)	
Current	53 (46.9)	158 (45.1)	6.3×10^{-2}	116 (48.7)	95 (42.2)	0.2
Tumor stage (T)						
Tis/Ta/T1	81 (71.7)	324 (95)		204 (87.2)	201 (91.4)	
T2+	32 (28.3)	17 (5.0)	**1.4×10^{-11}**	30 (12.8)	19 (8.6)	0.2
Tumor grade (G)						
G1	25 (22.3)	120 (34.6)		68 (28.8)	77 (34.5)	
G2	29 (25.9)	128 (36.9)		81 (34.3)	76 (34.1)	
G3	58 (51.8)	99 (28.5)	**3.7×10^{-5}**	87 (36.9)	70 (31.4)	0.3
Tumor grade (WHO 2004 + WHO 1973)						
NHG	49 (43.4)	234 (66.9)		140 (58.8)	143 (63.6)	
HG	64 (56.6)	116 (33.1)	**1.4×10^{-5}**	98 (41.2)	82 (36.4)	0.3
TG						
NMI/NHG	45 (39.8)	225 (66.0)		133 (56.8)	137 (62.3)	
NMI/HG	36 (31.9)	99 (29.0)		71 (30.3)	64 (29.1)	
MI/NHG	4 (3.5)	1 (0.3)		4 (1.7)	1 (0.5)	
MI/HG	28 (24.8)	16 (4.7)	**1.5×10^{-11}**	26 (11.1)	18 (8.2)	0.4
Chemotherapy						
No	44 (38.9)	190 (54.3)		111 (46.6)	123 (54.7)	
Intravesical	64 (56.6)	150 (42.9)		118 (49.6)	96 (42.7)	
Systemic	5 (4.4)	10 (2.9)	**1.7×10^{-2}**	9 (3.8)	6 (2.7)	0.2
Radiotherapy						
No	95 (84.1)	335 (95.7)		220 (92.4)	210 (93.3)	
Yes	18 (15.9)	15 (4.3)	**7.1×10^{-5}**	18 (7.6)	15 (6.7)	0.9
Radical cystectomy						
No	47 (54)	136 (80.5)		103 (68.7)	80 (75.5)	
Yes	40 (46)	33 (19.5)	**1.8×10^{-5}**	47 (31.3)	26 (24.5)	0.3
BCG						
No	85 (75.2)	218 (62.3)		160 (67.2)	143 (63.6)	
Yes	28 (24.8)	132 (37.7)	**1.6×10^{-2}**	78 (32.8)	82 (36.4)	0.5
Therapy						
No	40 (35.4)	102 (29.1)		74 (31.1)	68 (30.2)	
Yes	73 (64.6)	248 (70.9)	0.3	164 (68.9)	157 (69.8)	0.9
TL						
Mean (SD)	0.7 (0.2)	0.8 (0.2)	**7.6×10^{-7}**	—	—	—
Shorter	79 (69.9)	159 (45.4)				
Longer	34 (30.1)	191 (54.6)	**9.9×10^{-6}**	—	—	—

NOTE: Significant results are given in bold. Shorter TL, TL $\leq$ 0.8 (median value); Longer TL, TL > 0.8.

independent prognostic factor (HR of TL = 3.9; 95% CI, 1.7–9.1; $P = 1.2 \times 10^{-3}$; Table 2)

K–M survival curves in relation to TL-TG variable are depicted in Fig. 2C (log-rank $P = 1.4 \times 10^{-22}$). Performing a Cox regression analysis without any adjustment, risks of death for shorter TL in patients with NMI/NHG cancer and longer TL in patients with NMI/HG cancer were significantly higher than longer TL in patients with NMI/NHG cancer (HR = 2.5; 95% CI, 1.3–4.8; $P = 4.7 \times 10^{-3}$ and HR = 2.3; 95% CI, 1.0–5.1; $P = 4.6 \times 10^{-2}$, respectively; Table 3). An adjusted multivariate Cox regression model (Table 3) showed that cases with shorter telomeres and NMI/HG cancer had an higher risk of death than cases with longer telomeres and NMI/NHG cancer taken as reference (HR = 3.2; 95% CI, 1.4–7.4; $P = 6.1 \times 10^{-3}$). Risks of death for individuals with longer telomeres

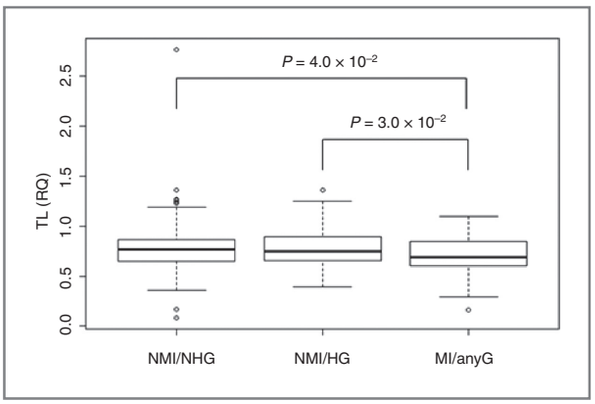

Figure 1. Differences in TL according to TG variable.

and MI/anyG cancer were 4-fold higher compared with the reference group (HR = 4.0; 95% CI, 1.4–11.4; $P = 1.0 \times 10^{-2}$; Table 3). Finally, having shorter telomeres and MI/anyG cancer determined the worst survival (HR = 7.3; 95% CI, 3.1–17.4; $P = 8.0 \times 10^{-6}$; Table 3). Moreover, considering all TL-TG groups, a strongly significant trend was shown (Tarone test, $P = 6.2 \times 10^{-28}$).

Figure 2A–C graphically showed that survival varied accordingly when considering TL alone, tumor aggressiveness alone, and also when stratifying patients on the basis of both TL and tumor aggressiveness.

Discussion

In the present investigation, we examined the relationship between leukocyte TL and survival within a large population of 463 patients with incident bladder cancer. To the best of our knowledge, this is the first study reporting a significant association between telomeres shortening in peripheral blood cells and worse survival after bladder cancer before any treatment. Multivariate analysis revealed that shorter telomeres were an independent prognostic factor, as supported also by a mediation analysis considering also the relationship with tumor invasiveness (data not shown). These results are in agreement with a recent study from Weischer and colleagues (11), in which 47,102 individuals were followed prospectively for up to 20 years after blood sampling and 3,142 of them developed a cancer. Among patients with cancer, those in the shortest telomeres quartile had a 1.3-fold

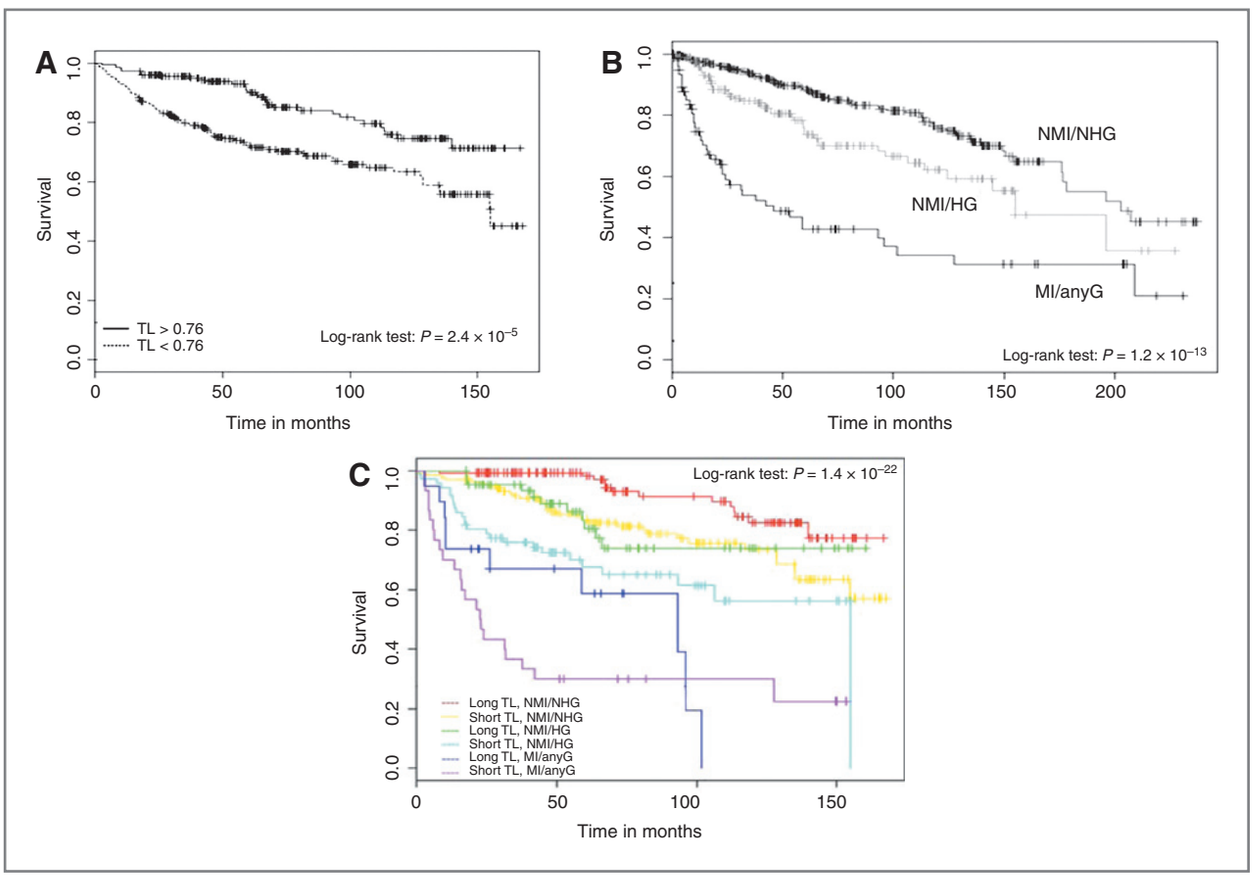

Figure 2. Kaplan–Meier curves for OS of patients with bladder cancer: A, stratified for longest versus shortest telomeres; B, stratified into three TG categories; C, stratified according to the TL-TG model. Long TL, NMI/NHG, $N = 137$, Exp = 38.5, Obs = 13; short TL, NMI/NHG, $N = 133$, Exp = 37.4, Obs = 32; long TL, NMI/HG, $N = 64$, Exp = 14.7, Obs = 11; short TL, NMI/HG, $N = 71$, Exp = 15.0, Obs = 25; long TL, MI/anyG, $N = 19$, Exp = 3.0, Obs = 10; short TL, MI/anyG, $N = 30$, Exp = 4.4, Obs = 22. Exp, expected deaths; Obs, observed deaths.

Table 2. Multivariate Cox regression survival analysis, including TL, age, TG, BCG, radical cystectomy, radiotherapy, and chemotherapy; death for bladder cancer

Variables	HR (95% CI)	*P*
TL (shorter telomeres)	3.9 (1.7–9.1)	**1.2×10^{-3}**
Age	1.1 (1.0–1.1)	**2.0×10^{-2}**
TG (NMI/HG)	1.8 (0.6–5.7)	0.3
TG (MI/anyG)	6.1 (2.6–14.1)	**3.0×10^{-5}**
BCG (Yes)	0.9 (0.3–2.8)	0.8
Radical cystectomy (Yes)	2.5 (1.1–5.3)	**2.0×10^{-2}**
Radiotherapy (Yes)	5.6 (2.3–13.6)	**1.6×10^{-4}**
Intravesical chemotherapy (Yes)	1.6 (0.6–3.8)	0.3
Systemic chemotherapy (Yes)	0.3 (0.1–2.0)	0.2

NOTE: Significant results are given in bold.

higher risk of early death compared with patients in the longest telomeres quartile. Shorter TL was also significantly associated with risk of early death after lung, melanoma, leukemia, and esophagus cancer when the analysis was stratified for cancer type. On the other hand, risk of early death after urinary tract cancer did not reach statistical significance, possibly due to the limited sample size (11). Willeit and colleagues (18) conducted a prospective population-based cohort study where shorter leukocyte TL was found to be strongly associated with an increased mortality risk after developing cancer. Poor prognosis was also described in a subgroup of patients with multiple myeloma characterized by high telomerase activity and shorter TL (19), as well as a subgroup of chronic lymphocytic leukemia cases with shorter TL (20).

In addition, in our sample, when performing survival analysis by using a combined variable including dichotomous TL and TG, survival was gradually decreasing with telomere shortening and increase of tumor grade and invasiveness. Interestingly, differences in survival were observed among patients affected by the same TG and presenting TL below and above median level.

Telomere shortening could also result from a mechanism of action that is common to various chemotherapeutic agents, such as induction of an accelerated replication rate of hematopoietic cells or induction of proliferative stress on marrow progenitors (21). Several published studies showed a reduction in TL after chemotherapeutic treatment in patients affected by solid tumors or hematologic malignancies (22–25). In the current study, the majority of patients presented a superficial bladder cancer which usually does not spread into the muscle; nevertheless it is more likely to relapse. For this reason, patients with an NMI cancer are usually treated by intravesical chemotherapy. Here, we observed telomere shortening in leukocytes of intravesical chemotherapy–treated patients compared with those from individuals who did not receive any chemotherapy treatment. However, this result should be carefully considered because, in our study population, TL was measured in blood samples from patients before they underwent any treatment. Therefore, it is possible that our results on telomere shortening in treated individuals may be more related to the tumor itself rather than to the effects of chemotherapy, being eventually of course both correlated. Indeed, patients with MI/anyG tumor had shorter TL compared with the other groups, although the difference had only a borderline significance. This is consistent with a previous report from Lin and colleagues (26) who observed increased telomeres attrition occurring in patients with chronic lymphocytic leukemia that was proportional to the disease stage and prognosis, indicating that telomeres dysfunction may drive progression in later stages. However, prospective studies are needed to ascertain a causative

Table 3. Multivariate Cox regression survival analysis of patients with bladder cancer stratified according to the TL-TG model and associated with OS; all cause of death

Variable	HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Longer TL, NMI/NHG	Ref.	Ref.	Ref.	Ref.
Shorter TL, NMI/NHG	2.5 (1.3–4.8)	**4.7×10^{-3}**	1.7 (0.8–3.5)	0.2
Longer TL, NMI/HG	2.3 (1.01–5.1)	**4.6×10^{-2}**	1.9 (0.7–5.1)	0.2
Shorter TL, NMI/HG	5.1 (2.6–10.0)	**2.2×10^{-6}**	3.2 (1.4–7.4)	**6.1×10^{-3}**
Longer TL, MI/anyG	10.5 (4.5–24.1)	**3.3×10^{-8}**	4.0 (1.4–11.4)	**1.0×10^{-2}**
Shorter TL, MI/anyG	15.2 (7.6–30.3)	**1.0×10^{-14}**	7.3 (3.1–17.4)	**8.0×10^{-6}**
Age	—	—	1.1 (1.1–1.2)	**2.5×10^{-10}**
BCG	—	—	0.6 (0.3–1.2)	0.1
Radical cystectomy	—	—	1.5 (0.8–2.7)	0.2
Radiotherapy	—	—	2.7 (1.5–4.9)	**1.0×10^{-3}**
Intravesical chemotherapy	—	—	1.2 (0.7–2.0)	0.5
Systemic chemotherapy	—	—	1.5 (0.5–4.4)	0.5

NOTE: Significant results are given in bold. Abbreviation: Ref, reference.

role of telomere attrition in the tumor progression so we cannot exclude, at the present time, that TL could be a host characteristic that is permissive for tumor aggressiveness. On the basis of these observations, we may hypothesize that patients having a more advanced cancer in combination with shorter telomeres in blood before they undergo chemotherapy might experience a worse tolerance and higher chemotoxicity than patients with advanced tumor and longer telomeres, thus affecting their prognosis. Because of molecular size, any of the chemotherapeutic agents administered intravesically is hardly absorbed into the blood. Nevertheless, peripheral blood leukocytes are often used as surrogate cells for the detection of telomeres dysfunction considering that TL was found to be correlated among different tissues (27) and that interindividual TL variation outclasses the variation observed between different tissues of the same individual (28, 29). However, it is not yet known whether TL in leukocytes accurately reflects those of other tissues, thus making useful in the future to analyze TL also in bladder cancer cells from our population study.

Another limitation could be linked to substantial differences to correctly identify the cause of death for specific cancer types so we could observe a bias toward: (i) an underestimation of bladder cancer deaths in case of secondary causes related to bladder cancer and (ii) an overestimation of bladder cancer deaths if secondary causes are not correctly assessed and deaths are attributed to bladder cancer. However, biases clearly go in opposite directions, limiting probably the effects of the misclassification.

Moreover, although smoking is recognized as the most important risk factor for urothelial bladder cancer and smoking cessation has been suggested to benefit urothelial bladder cancer outcomes, its role as an independent prognostic factor is still controversial (30), being smoking also in our study not associated with survival.

In conclusion, in our bladder cancer case series, we reported an association between shorter leukocyte TL and increased overall mortality, with a stronger association for bladder cancer–specific mortality. Moreover, the independent effect of TL on bladder cancer survival before any treatment suggests that TL measurements may help in selecting the best therapeutic approach in patients with the same stage and grade. Further studies on TL as prognostic factor may improve the understanding of the mechanisms underlying bladder cancer progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Guarrera, P. Vineis, C. Sacerdote, G. Matullo
Development of methodology: S. Guarrera
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Guarrera, A. Bosio, G. Casetta, G. Cucchiarale, P. Destefanis, P. Gontero, L. Rolle, A. Zitella, B. Frea, C. Sacerdote
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Russo, F. Modica, S. Guarrera, G. Fiorito, B. Pardini, C. Viberti, G. Matullo
Writing, review, and/or revision of the manuscript: A. Russo, F. Modica, S. Guarrera, B. Pardini, A. Allione, A. Bosio, G. Casetta, P. Destefanis, P. Gontero, A. Zitella, P. Vineis, C. Sacerdote, G. Matullo
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Critelli, L. Rolle
Study supervision: S. Guarrera, D. Fontana, G. Matullo
Other (performed laboratory experiments): A. Russo

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