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Shorter Treatment-Naïve Leukocyte Telomere Length is Associated with Poorer Overall Survival of Patients with Pancreatic Ductal Adenocarcinoma

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ABSTRACT

Background: Critically shortened telomeres contribute to chromosomal instability and neoplastic transformation and are associated with early death of patients with certain cancer types. Shorter leukocyte telomere length (LTL) has been associated with higher risk for pancreatic ductal adenocarcinoma (PDAC) and might be associated also with survival of patients with PDAC. We investigated the association between treatment-naïve LTL and overall survival of patients with incident PDAC.

Methods: The study included 642 consecutively enrolled PDAC patients in the Mayo Clinic Biospecimen Resource for Pancreas Research. Blood samples were obtained at the time of diagnosis, before the start of cancer treatment, from which LTL was assayed by qRT-PCR. LTL was first modeled as a continuous variable (per-interquartile range decrease in LTL) and then as a categorized variable (short, medium, long). Multivariable-adjusted HRs and 95% confidence intervals (CI) were calculated for overall mortality using Cox proportional hazard models.

Results: Shorter treatment-naïve LTL was associated with higher mortality among patients with PDAC ($HR_{continuous}$ = 1.13, 95% CI: 1.01–1.28, P = 0.03; $HR_{shortest\ vs.\ longest\ LTL}$ = 1.29, 95% CI: 1.05–1.59, P_{trend} = 0.01). There was a difference in the association between LTL and overall mortality by tumor stage at diagnosis; resectable tumors ($HR_{continuous}$ = 0.91; 95% CI: 0.73–1.12), locally advanced tumors ($HR_{continuous}$ = 1.29; 95% CI: 1.07–1.56), and metastatic tumors ($HR_{continuous}$ = 1.17; 95% CI: 0.96–1.42), $P_{interaction}$ = 0.04.

Conclusion: Shorter treatment-naïve LTL is associated with poorer overall survival of patients with incident PDAC.

Impact: Peripheral blood LTL might be a prognostic marker for PDAC.

Introduction

Despite substantial progress in understanding the etiology of pancreatic ductal adenocarcinoma (PDAC), it remains a rapidly fatal malignancy with the highest mortality rate of all gastrointestinal malignancies (1, 2). The 5-year mortality rate for PDAC is about 90% (2), due mainly to its frequently late stage of detection, aggressive clinical course, and chemo-resistance in many patients despite the availability of newer therapies (3–6). The poor prognosis of PDAC highlights the need for better understanding of the factors associated with survival, toward the goal of improving outcomes for persons with PDAC. Initial evidence linking telomeres with survival of patients with PDAC has been reported (7).

Telomeres, the hexanucleotide sequence repeats $(TTAGGG)_n$ and an associated protein complex (shelterin/telosome) that cap the ends of eukaryotic chromosomes are essential for maintaining chromosomal and genomic stability (8–10). Telomeres shorten progressively with each successive cell division due to incomplete replication by DNA polymerases (10). Consequently, telomere length is considered a marker of cellular aging, replicative capacity, and senescence across human cell types (8, 9, 11). In addition to aging, other host factors, such as cigarette smoking, obesity, and chronic health conditions (e.g., diabetes) tend to accelerate telomere shortening further to a point where telomeres become critically short and dysfunctional (8, 10). Because fully functional telomeres are essential for maintaining genomic stability, they tend to protect against cancer development (12). Cells with critically short telomere length undergo cell-cycle arrest, apoptosis, or replicative senescence (8, 9, 11). Cells that evade these cell-cycle checkpoint mechanisms and continue to divide despite dysfunctional telomeres lead to the accumulation of aberrant cells, ultimately contributing to genomic instability and age-related pathologies, such as cancer (8, 9, 11–13). In line with this, tissue-based studies have reported progressive telomere shortening during the early stages of PDAC development (14, 15). PDAC tissues have also been found to have shorter telomeres than normal pancreatic duct epithelium (15). These observations suggest that telomere shortening might be a fundamental process in PDAC development, and might play a role in the prognosis of patients with PDAC. Indeed, studies suggest that critically shortened telomeres and associated chromosomal abnormalities facilitate cancer progression (11–13), and shortened telomeres have been associated with early death of patients with certain cancer types (16–18), including PDAC (7).

Epidemiologic studies have found that shorter peripheral blood leukocyte telomere length (LTL), a reflection of overall telomere status, is associated with poorer survival of patients with bladder cancer (19), hematologic malignancies (20), and renal cell carcinoma (18). To our knowledge, two studies have investigated the association between LTL

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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and survival of patients with PDAC. One of these studies reported a null finding (16), and the other found that shorter LTL measured in blood samples collected a median of 6 years before PDAC diagnosis is associated with poorer overall survival after PDAC diagnosis (7). No study has yet examined the association between LTL assayed in blood samples collected at the time of PDAC diagnosis and survival of patients. To clarify the relationship between LTL and survival of patients with PDAC, we performed a large, clinic-based study to test the hypothesis that shorter treatment-naïve LTL in blood samples obtained at diagnosis is associated with poorer overall survival of patients with incident PDAC. We further explored whether treatment-naïve LTL interacts with PDAC patient characteristics (e.g., tumor stage at diagnosis) to influence overall survival.

Materials and Methods

Study sample and data collection

The study was reviewed and approved by the Mayo Clinic Institutional Review Board (IRB) under protocol number 06–004892. Mayo Clinic patients included in this study had previously provided written informed consent under IRB protocol number 354–06. The Mayo Clinic IRB is compliant with the requirements of the U.S. FDA regulations 21 CFR Parts 50 and 56 and the U.S. Department of Health and Human Services (HHS) regulations 45 CFR 46, which are guided by the Belmont Report. In addition, the Mayo Clinic IRB complies with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines on Good Clinical Practices and are consistent with FDA and HHS regulations.

Following IRB approval, we obtained data and biospecimen from the Mayo Clinic Biospecimen Resource for Pancreas Research, a prospective patient registry supported by the Mayo Clinic Specialized Program of Research Excellence (SPORE) in pancreatic cancer (21–23). The registry utilizes an ultrarapid case finding and recruitment process that ensures that about 86% of patients diagnosed with PDAC at Mayo Clinic are recruited within one month of diagnosis, with an overall 2-week average between first contact and recruitment. The patients were recruited between October 2000 and June 2016, and all patients provided blood samples at the time of diagnosis before starting cancer treatment. The participation rate among those approached was approximately 70% (21). In our experience, the main reasons for nonparticipation in the patient registry are the rapid demise of patients following PDAC diagnosis and severe debilitation caused by PDAC (22). Included in this study were data and biospecimens from 642 consecutively enrolled patients with pathologically confirmed (95%) or clinically confirmed PDAC.

At the time of enrollment, the patients were asked to complete a standardized risk factor questionnaire that solicited information on demographics (age, sex, race), smoking history, personal history of diabetes, and usual adult weight and height. We categorized smoking history as never, former, and current, and calculated body mass index (BMI) as weight in kilograms divided by height in meters squared (kg/m^2). Information on cancer stage at diagnosis was obtained from medical records by medical oncologist in the pancreatic cancer SPORE, and were grouped as surgically resected, locally advanced, or metastatic. Vital status was ascertained from multiple sources including medical records, periodic correspondence with patients, direct contact with next of kin, linkage with Mayo Clinic tumor registry data, and use of online sources (e.g., LexisNexis Accurint; ref. 24). We were able to ascertain vital status for all patients included in this study.

LTL measurement

The methods used for measuring LTL have been described previously (23). In brief, the patients donated peripheral blood samples that were fractionated into plasma, serum, and leukocytes (buffy coat). DNA was extracted from buffy coat leukocytes using the Maxwell RSC Instrument with appropriate kit (Promega) and quantitated with a Qubit fluorometer. LTL was measured by qRT-PCR. The qPCR assay measures telomere length as the relative ratio of telomere copy number to single-gene copy number (T/S ratio; ref. 25). Each sample was assayed in triplicate in relation to a reference sample. The final T/S ratio was based on the average of the three measurements. The coefficient of variation (CV) within triplicate samples was less than 3% and the median CV among all samples was 5.5%, both of which are indicative of high assay reproducibility. Laboratory personnel were blinded to all clinical data (age, sex, vital status, etc.).

Statistical analysis

Descriptive statistics were performed using means and medians for continuous variables and proportions for categorical variables. The outcome of interest was overall survival. Overall survival was determined starting with the date of PDAC diagnosis to date of death or date of last follow-up, whichever came first. Cox proportional hazard models were used to calculate HRs and 95% confidence intervals (CI). The proportional hazards assumption was assessed for all variables using residual plots and was determined to have been satisfied. In the Cox model, we modeled LTL as the predictor and time-to-death as outcome, with initial adjustment for age at diagnosis (continuous), sex, and tumor stage at diagnosis (resectable, locally advanced, metastatic). Subsequently, we performed additional adjustments for BMI (continuous), self-reported personal history of diabetes (yes, no), and smoking status (never, former, current). In the primary analysis, we treated LTL as a continuous variable, with HRs reflecting one interquartile range decrease in LTL. We assessed potential nonlinearity of the association between the continuous LTL variable and overall survival with restricted cubic splines in the Cox proportional hazard model (26). On the basis of residual analysis (plots of residuals against continuous LTL variable) and the Bayesian information criterion, we determined that there was no evidence of a nonlinear relationship between LTL and overall survival. Hence, for parsimony and ease of interpretation, we retained LTL as a continuous variable in the primary analyses.

In secondary analyses and for visualization of association trends, we treated LTL as a categorical variable grouped into tertiles (short, medium, long) based on the distribution in the entire cohort. The Kaplan–Meier estimator was used to calculate median survival time with corresponding 95% CIs. For the categorical LTL variable, we used the longest LTL group as the reference to calculate HRs and 95% CIs for the medium and shortest LTL groups. Linear trend across categories was assessed using the ordinal method.

In exploratory analyses, we assessed whether the association between LTL and overall survival differed by tumor stage at diagnosis, sex, usual adult BMI (≤24.9, 25–29.9, ≥30 kg/m^2), personal history of diabetes mellitus (yes, no), and smoking status (never, former, current) by assessing a multiplicative interaction between the stratifying variable and LTL. For these analyses, the continuous LTL variable was used to conserve statistical power. A forest plot was generated to visualize an interaction observed between tumor stage at diagnosis and LTL on overall survival using the continuous LTL variable. We also used the categorical LTL variable to glean additional insights into the interaction between tumor stage at diagnosis and LTL. All statistical analyses were performed using SAS version 9.4 (SAS Institute) and a two-sided $P < 0.05$ was considered statistically significant.

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Data Availability Statement

The data used in this study may be made available to researchers upon request to Dr. Gloria M. Petersen (Mayo Clinic, Rochester, MN, Petersen.gloria@mayo.edu). However, ethical and legal restrictions apply to these data.

Results

Characteristics of the 642 patients with incident PDAC are summarized in **Table 1**. The patients were predominantly White (98%) and majority were male (56%). The average age at PDAC diagnosis was 67 years. Twenty-nine percent of the patients self-reported a personal history of diabetes mellitus, 17% were current smokers, and 32% were obese (BMI ≥ 30 kg/m^2). The proportion of patients with resectable, locally advanced, and metastatic tumors at diagnosis were 28%, 38%, and 33%, respectively. The quantitated LTL (T/S ratio) ranged from 0.341 to 1.280, with a mean of 0.775 and standard deviation of 0.162. As expected, the LTLs correlated inversely with age at diagnosis (Spearman $r = -0.31$, $P < 0.001$). By the end of follow-up, 605 (94%) deaths had occurred. Overall median survival time was 10.8 months. Median survival was longer for patients with resectable PDAC (20.5 months) than patients with locally advanced (9.5 months) or metastatic (7.8 months) PDAC. When patients' characteristics were stratified by categorical LTL, patients in the shortest LTL category were found to be older than those in the longest LTL category (mean age: 70 years. vs. 63 years.). In addition, patients in the shortest LTL category included greater proportions of males, former and current smokers, self-reported diabetics, and obese individuals compared with those in the longest LTL. The patients in the longest LTL category had longer overall survival than those in the shortest LTL category (12.7 months vs. 8.7 months).

The association between treatment-naïve LTL and overall survival is summarized in **Table 2**. For analyses that were based on continuous LTL variable, the HRs reflect an interquartile range (0.216 T/S ratio) decrease in LTL. Shorter treatment-naïve LTL was associated with a higher risk of death after adjusting for age, sex, and tumor stage at diagnosis ($HR_{\text{continuous LTL}} = 1.15$, 95% CI: 1.02–1.29, $P = 0.02$; $HR_{\text{shortest vs. longest LTL}} = 1.23$, 95% CI: 1.01–1.50, $P_{\text{trend}} = 0.04$). After additional adjustment for BMI, diabetes, and smoking history, the association between shorter treatment-naïve LTL and higher risk of death remained significant ($HR_{\text{continuous LTL}} = 1.13$, 95% CI: 1.01–1.28, $P = 0.03$; $HR_{\text{shortest vs. longest LTL}} = 1.29$, 95% CI: 1.05–1.59, $P_{\text{trend}} = 0.01$). Survival probabilities plotted by Kaplan–Meier survival curves also show poorer overall survival for patients in the shortest LTL groups, compared with those in the longest LTL group (**Fig. 1**).

We observed an interaction between treatment-naïve LTL and tumor stage at diagnosis ($P_{\text{interaction}} = 0.04$; **Table 3**). The interaction was such that shorter LTL was significantly associated with higher mortality among patients with locally advanced PDAC ($HR_{\text{continuous}}$ LTL = 1.29; 95% CI: 1.07–1.56), but not those with resectable PDAC ($HR_{\text{continuous}}$ LTL = 0.91; 95% CI: 0.73–1.12) or metastatic PDAC ($HR_{\text{continuous}}$ LTL = 1.17; 95% CI: 0.96–1.42), as illustrated graphically in Supplementary Fig. S1. We further explored the interaction by tumor stage using the categorical LTL variable (**Table 3**). Although no association was found among patients with resectable PDAC ($HR_{\text{shortest vs. longest LTL}} = 0.92$; 95% CI: 0.62–1.35), shorter LTL was significantly associated with higher mortality among patients with locally advanced PDAC ($HR_{\text{shortest vs. longest LTL}} = 1.42$; 95% CI: 1.01–1.98) and those with metastatic PDAC ($HR_{\text{shortest vs. longest LTL}} = 1.46$; 95% CI: 1.06–2.01). However, interaction P value based on the categorical LTL was not significant ($P_{\text{interaction}} = 0.16$).

There also were instances where no statistically significant interaction was observed, but associations were observed in stratified analyses among certain subgroup (Supplementary Table S1), such as patients with BMI ≥ 30 kg/m^2 ($HR_{\text{continuous LTL}} = 1.31$; 95% CI: 1.07–1.61) and those with personal history of diabetes mellitus ($HR_{\text{continuous LTL}} = 1.25$; 95% CI: 1.01–1.54). A marginal association was also observed among never smokers ($HR_{\text{continuous LTL}} = 1.20$; 95% CI: 0.99–1.45). These associations were not observed among patients with BMI < 30 kg/m^2, nondiabetics, and former or current smokers (Supplementary Table S1).

Discussion

PDAC is a rapidly fatal malignancy; thus, improved understanding of the molecular processes associated with survival of patients with PDAC could offer insights into the pathobiology of the tumor for prognostication, treatment selection, and response monitoring (27, 28). In this study, we investigated the association between treatment-naïve LTL and overall survival of 642 patients with PDAC consecutively enrolled in the Mayo Clinic Biospecimen Resource for Pancreas Research. Our results show that shorter treatment-naïve LTL is associated with poorer overall survival of patients with PDAC. In interaction analyses, we found that shorter treatment-naïve LTL was associated with poorer survival among patients with locally advanced PDAC, and possibly patients with metastatic PDAC, but not those with resectable PDAC. Our findings suggest that LTL plays a plausible role in the survival of patients with PDAC.

In previous studies, we and others found that shorter LTL is associated with higher risk for PDAC (23, 29). Here, we provide additional evidence that shorter LTL is associated also with poorer survival of patients with PDAC. Consistent with our findings are the results of a prospective study of pooled data from four independent cohorts, in which investigators assessed the association between prediagnostic LTL and overall mortality among 423 patients ($HR_{\text{shortest vs. longest LTL}} = 1.39$; 95% CI: 1.01–1.93; $P_{\text{trend}} = 0.04$; ref. 7). In addition to having a smaller sample size than our study, the blood samples used in this prior study were collected a median of 6 years before cancer diagnosis (7). In our study, blood samples were collected at the time of cancer diagnosis, before cancer treatment. Hence, our results are more germane to clinical settings during initial evaluation of patients with PDAC, seeking to aid in the assessment of patient prognosis and decisions regarding patient management. In another prospective study in Denmark, the association between LTL and overall survival was assessed among 124 patients with PDAC and a null finding was reported (per kilobase decrease in LTL, HR = 1.02; 95% CI: 0.83–1.25; ref. 20). However, this study was limited substantially by small sample size and lack of control of confounding by lifestyle factors, such as obesity and cigarette smoking history (16).

Our study further suggests that among patients with locally advanced PDAC, shorter LTL is associated with poorer survival based on a continuous LTL variable, whereas no association was found among patients with resectable PDAC or metastatic PDAC. When a categorical LTL variable was used, we found a significant association between shorter LTL and poorer survival among patients with locally advanced PDAC and patients with metastatic PDAC, but not those with resectable PDAC. However, statistical interaction was not significant when using categorical LTL, which may be due to reduced statistical power associated with categorization of continuous variables (30, 31). There also was a suggestion that

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Table 1. Characteristics of patients with pancreatic ductal adenocarcinoma.

Characteristics	Total $n = 642$	Telomere length categories[a] Long $n = 215$	Medium $n = 212$	Short $n = 215$
Age at diagnosis				
Mean (SD)	66.7 (10.3)	63.4 (10.4)	66 (10.0)	70.4 (9.2)
Range	38–89	38–87	43–87	41–89
Male	360 (56.1%)	114 (53.0%)	107 (50.5%)	139 (64.7%)
Whites	632 (98.4%)	213 (99.1%)	208 (98.1%)	211 (98.1%)
Smoking status				
Never	248 (38.6%)	91 (42.3%)	84 (39.6%)	73 (34.0%)
Former	284 (44.2%)	92 (42.8%)	89 (42.0%)	103 (47.9%)
Current	110 (17.1%)	32 (14.9%)	39 (18.4%)	39 (18.1%)
Self-reported history of DM	187 (29.1%)	55 (25.6%)	64 (30.2%)	68 (31.6%)
BMI, kg/m^2				
≤ 24.9	167 (26.0%)	60 (27.9%)	50 (23.6%)	57 (26.5%)
25–29.9	272 (42.4%)	95 (44.2%)	92 (43.4%)	85 (39.5%)
≥30	203 (31.6%)	60 (27.9%)	70 (33.0%)	73 (34.0%)
Mean (SD)	28.5 (5.3)	27.875 (6.0)	28.7 (5.2)	28.8 (5.4)
Range	17.6–53.0	(17.6–53.0)	(18.2–48.8)	(18.0–49.9)
Tumor stage at diagnosis				
Resectable	181 (28.2%)	61 (28.4%)	61 (28.8%)	59 (27.4%)
Locally advanced	247 (38.5%)	73 (34.0%)	94 (44.3%)	80 (37.2%)
Metastatic	214 (33.3%)	81 (37.7%)	57 (26.9%)	76 (35.3%)
Telomere length				
Mean (SD)	0.755 (0.162)	0.937 (0.100)	0.742 (0.040)	0.587 (0.070)
Range	0.341–1.280	(0.817–1.280)	(0.678–0.815)	(0.341–0.677)
Median survival, months (95% CI)				
Overall (all stages combined)	10.8 (10.1–12.5)	12.7 (11.4–13.9)	11.3 (10.1–13.3)	8.7 (8.2–10.0)
Resectable	20.5 (16.7–23.5)	20.4 (16.8–24.1)	22.8 (16.7–24.0)	20.5 (16.4–23.7)
Locally advanced	9.5 (8.2–10.6)	10.7 (9.6–13.2)	10.6 (9.2–13.0)	7.9 (6.9–8.8)
Metastatic	7.8 (6.8–8.6)	10.3 (8.1–11.6)	8.0 (7.3–9.3)	6.3 (5.2–7.7)

Abbreviation: DM, diabetes mellitus.
[a]LTL was measured as the ratio of telomere to single-copy gene (T/S ratio) and categorized as short (≤0.677), medium (0.678–0.815), and long (>0.815).

shorter LTL is associated with poorer overall survival among patients BMI ≥ 30 kg/m^2, diabetic patients, and never smokers, but no significant interaction was found by these factors, and therefore require verification in larger studies.

Importantly, having a shorter LTL has been associated with higher mortality in the general population, with much higher mortality among individuals with certain age-related health conditions (32–34), lending support to the hypothesis that telomere shortening contributes to premature death from certain age-related diseases (16, 32). It is thought that the presence of fully functional telomeres may delay the progression of age-related pathologies and thereby prevent early death (12), which may explain our finding that shorter LTL is associated with poorer survival of patients with PDAC. Alternatively, telomere shortening may not contribute directly to mortality but may be a marker for a higher burden of terminally arrested senescent cells. Excessive accumulation of dysfunctional senescent cells tends to alter gene expression patterns, disrupt tissue renewal processes, and obstruct the functions of normal cells (12, 35–37). Telomere

Table 2. Association between treatment-naïve LTL and overall survival of patients with pancreatic ductal adenocarcinoma.

Telomere length (T/S ratio)[a]	Deaths/No. at risk	Median survival, months (95% CI)	Adjusted[d] HR (95% CI)	*P*	Adjusted[e] HR (95% CI)	*P*
Continuous[b]	605/642	10.8 (10.1–12.5)	1.15 (1.02–1.29)	0.02	1.13 (1.01–1.28)	0.03
Categorical[c]						
Long	197/215	12.7 (11.4–13.9)	1.00 (ref)	0.04[f]	1.00 (ref)	0.01[f]
Medium	198/212	11.3 (10.1–13.3)	0.98 (0.80–1.19)		0.97 (0.79–1.18)	
Short	210/215	8.7 (8.2–10.0)	1.23 (1.01–1.50)		1.29 (1.05–1.59)	

[a]Telomere length was measured as the ratio of telomere to single-copy gene (T/S ratio).
[b]HRs for continuous telomere length reflects per interquartile range (0.216 T/S ratio) decrease in LTL.
[c]Categorical telomere length was based on distribution in the entire cohort, categorized as short (≤0.677), medium (0.678–0.815), and long (>0.815).
[d]Adjusted for age at diagnosis (continuous), sex, and tumor stage at diagnosis (resectable, locally advanced, metastatic).
[e]Additional adjustment for BMI (continuous), diabetes (yes, no), and smoking status (never, former, current).
[f]P_{trend} across tertiles

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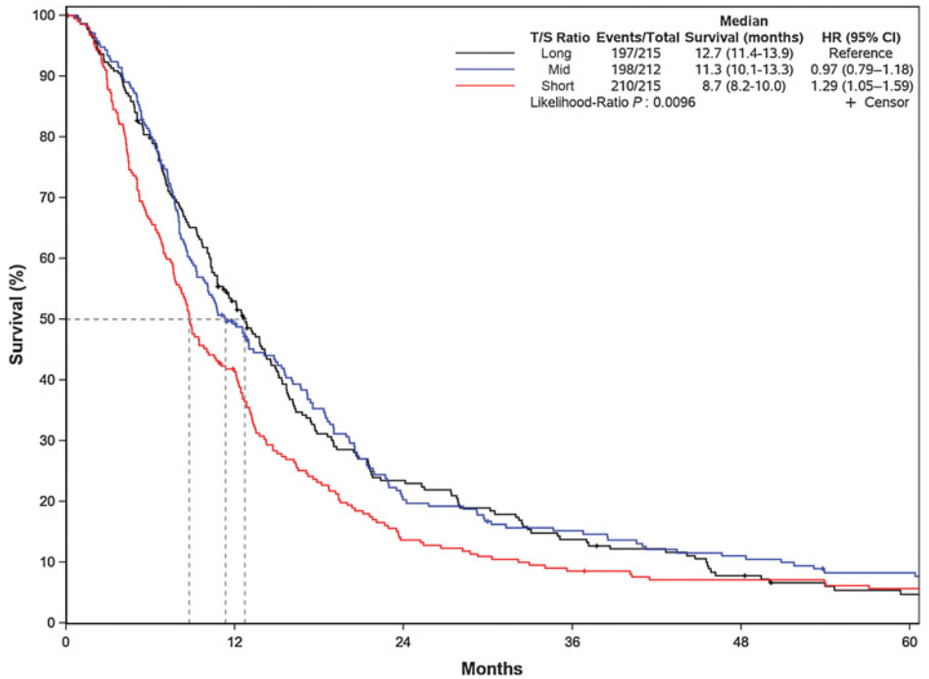

Figure 1.
Kaplan–Meier survival curves, multivariable-adjusted HRs and 95% CIs for the association between treatment-naive LTL and pancreatic ductal adenocarcinoma. Telomere length was measured as the ratio of telomere to single-copy gene (T/S ratio) and categorized as short (≤0.677), medium (0.678–0.815), and long (>0.815). HRs and 95% CIs were calculated from Cox proportional hazard models, adjusting for age at diagnosis (continuous), sex, tumor stage at diagnosis (resectable, locally advanced, metastatic), BMI (continuous), personal history of diabetes mellitus (yes, no), and smoking history (never, former, current).

shortening may also compromise the capacity to repair double-strand DNA breaks (38), which has multiple phenotypic consequences, including susceptibility to cancer and poor prognosis after cancer diagnosis. In addition, senescent cells often secrete deleterious substances, such as proinflammatory cytokines, epithelial growth factors, and extracellular matrix remodeling enzymes that can alter tissue microenvironment, thereby compromising tissue structure and function, culminating in deleterious effects on health and limiting longevity (10, 12).

Our study has several important strengths and some limitations. Strengths of our study include the relatively larger sample size compared with prior studies, and the prospective assessment of LTL in blood samples collected before the start of treatment and therefore avoids confounding by cancer therapy, particularly chemotherapeutic

Table 3. Association between treatment-naïve LTL and overall survival, stratified by tumor stage at diagnosis.

Telomere length by tumor stage	Deaths/No. at risk	Median survival, months (95% CI)	HR (95% CI)[c]	$P_{interaction}$[d]
Continuous LTL[a]				0.04
Resectable	159/181	20.5 (16.7–23.5)	0.91 (0.73–1.12)	
Locally advanced	233/247	9.5 (8.2–10.6)	1.29 (1.07–1.56)	
Metastatic	213/214	7.8 (6.8–8.6)	1.17 (0.96–1.42)	
Categorical LTL[b]				0.16
Resectable				
Long	50/61	20.4 (16.8–24.1)	1.00 (ref)	
Medium	54/61	22.8 (16.7–24.0)	0.96 (0.65–1.42	
Short	55/59	20.5 (16.4–23.7)	0.92 (0.62–1.35)	
Locally advanced				
Long	67/73	10.7 (9.6–13.2)	1.00 (ref)	
Medium	87/94	10.6 (9.2–13.0)	0.86 (0.62–1.18)	
Short	79/80	7.9 (6.9–8.8)	1.42 (1.01–1.98)	
Metastatic				
Long	80/81	10.3 (8.1–11.6)	1.00 (ref)	
Medium	57/57	8.0 (7.3–9.3)	1.06 (0.75–1.50)	
Short	76/76	6.3 (5.2–7.7)	1.46 (1.06–2.01)	

[a]LTL was measured as the ratio of telomere to single-copy gene (T/S ratio), with HR for continuous LTL reflecting per interquartile range (0.216 T/S ratio) decrease in LTL.
[b]Categorical LTL was based on the distribution in the entire cohort, categorized as short (≤0.677), medium (0.678–0.815), or long (>0.815).
[c]Adjustment for age (continuous), sex, BMI (continuous), diabetes (yes, no), and smoking status (never, former, current).
[d]For continuous LTL, $P_{interaction}$ was calculated using a continuous T/S ratio and a categorical stage variable (resectable, locally advanced, metastatic). For categorical LTL, $P_{interaction}$ was calculated using a categorical T/S ratio variable (short, medium, long) and a categorical stage variable (resectable, locally advanced, metastatic).

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agents, which are known to influence telomere length (39–41). Biospecimens were processed and stored by standard protocol in a core lab, ensuring quality, and each sample was assayed in triplicate with an overall high level of reproducibility. The assessment of LTL at the time of diagnosis is reflective of telomere dynamics in the setting of PDAC and has potential clinical application for prognostication, disease monitoring, and future development of targeted therapies, as discussed elsewhere (42, 43). In additon, we used data from a prospective patient registry that utilizes an ultrarapid case ascertainment process with the majority of patients recruited within one month of diagnosis with a high participation rate (∼70%). We also controlled for potential confounding by multiple clinical and patient factors that have been associated with both LTL and PDAC survival.

Limitations of our study include the predominantly White population, limiting racial generalizability of the findings. We also did not have data on PDAC-specific mortality. However, because PDAC is a rapidly fatal cancer with most patients (∼90%) dying within 5 years of diagnosis, overall survival is a reasonable surrogate for PDAC-specific survival and is widely used in PDAC survival studies (7, 44). Vital status was ascertained from multiple sources. This could have introduced some level of bias into the study, influencing effect estimates to some extent, either toward or away from null. Moreover, we measured average telomere length among all leukocyte subtypes. Because telomere length varies by leukocyte subtype (20), the average telomere length among all subtypes may be influenced by differential proportions of certain subtypes in the sample. Future studies that are based on cell type–specific telomere length will avoid this limitation. Additionally, we had limited data on treatments administered to the patients, which precluded its use in our study. However, blood samples were collected before cancer treatment, and treatment-naïve LTL is unlikely to have been affected by treatment choice. It is also worth noting that tumor stage at diagnosis is a major determinant of treatment choice. We had reasonable numbers of patients with resectable, locally advanced, and metastatic tumor, reflective of what is commonly seen in most clinics (1), and we adjusted for stage at diagnosis as a surrogate for treatment. We also acknowledge that peripheral blood LTL may not faithfully represent telomere length of pancreatic tumor epithelium. However, studies have shown that telomere length of peripheral blood leukocytes correlates strongly with telomere length of matching somatic tissues, including the skin, subcutaneous fat, and skeletal muscle (Pearson's $r = 0.83–0.84$; $P < 0.0001$; ref. 45).

In conclusion, our study shows that shorter treatment-naïve LTL is associated with poorer overall survival of patients with PDAC. The findings suggest that treatment-naïve LTL may have prognostic relevance in PDAC and could be useful for patient stratification for treatment selection and prioritization toward the goal of improving outcomes for patients with PDAC. Additional investigations into leukocyte cell type–specific telomere length and PDAC survival would advance our understanding of telomere kinetics in PDAC.

Authors' Disclosures

R.M. Cawthon reports a patent for telomere measurement by quantitative PCR issued and with royalties paid from Telomere Diagnostics, Inc. A. Mahipal reports other from Exelixis and grants from Taiho Oncology outside the submitted work. A.L. Oberg reports grants from NCI during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

S.O. Antwi: Conceptualization, funding acquisition, writing-original draft, writing-review and editing. **W.R. Bamlet:** Conceptualization, resources, data curation, formal analysis, methodology, writing-review and editing. **R.M. Cawthon:** Data curation, methodology, writing-review and editing. **K.G. Rabe:** Resources, data curation, writing-review and editing. **B.R. Druliner:** Data curation, methodology, writing-review and editing. **H. Sicotte:** Writing-review and editing. **A. Jatoi:** Funding acquisition, writing-review and editing. **A. Mahipal:** Resources, writing-review and editing. **L.A. Boardman:** Resources, data curation, funding acquisition, methodology, writing-review and editing. **A.L. Oberg:** Resources, data curation, formal analysis, supervision, methodology, project administration, writing-review and editing. **G.M. Petersen:** Conceptualization, resources, data curation, supervision, funding acquisition, methodology, project administration, writing-review and editing.

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