



Age-adjusted leukocyte telomere length predicts long-term mortality in older patients discharged from acute care hospitals

Rosanna Maniscalco · Deborah Ramini · Mirko Di Rosa · Maurizio Cardelli · Rina Recchioni · Francesca Marchegiani · Francesco Piacenza · Robertina Giacconi · Chiara Giordani · Giulia Matacchione · Matilde Sbriscia · Sonia Fantone · Stefania Fumarola · Salvatore Vaiasicca · Monia Cecati · Adrianapia Maria Lamedica · Leonardo Biscetti · Antonio Cherubini · Francesco Spannella · Luca Soraci · Anna Rita Bonfigli · Fabrizia Lattanzio · Jacopo Sabbatinelli · Fabiola Olivieri · Angelica Giuliani

Received: 11 April 2026 / Accepted: 17 June 2026
© The Author(s) 2026

Abstract Leukocyte telomere length (LTL) is a widely studied biomarker of biological aging, reflecting cumulative cellular damage beyond chronological age. However, its prognostic relevance in clinically complex populations such as hospitalized older adults remains uncertain. In this prospective observational study, 872 patients aged ≥ 65 years admitted to acute care wards of IRCCS INRCA within the Report-AGE cohort were included. LTL was measured by

quantitative real-time PCR and expressed as age-adjusted residuals derived from an external reference population. The primary outcome was 10-year all-cause mortality. Associations between LTL residuals and mortality were assessed using Cox proportional hazards models, both as continuous variables and using a data-driven cut-point. Multivariable models adjusted for demographic characteristics, comorbidity burden, laboratory parameters, and polypharmacy

R. Maniscalco · D. Ramini · M. Cardelli · F. Piacenza · R. Giacconi · M. Sbriscia · S. Fantone · S. Fumarola · F. Olivieri (✉)
Center of Biogerontology, IRCCS INRCA, Ancona, Italy
e-mail: f.olivieri@staff.univpm.it

M. Di Rosa
Centre for Biostatistics and Applied Geriatric Clinical Epidemiology, IRCCS INRCA, Cosenza, Italy

R. Recchioni · F. Marchegiani · C. Giordani · G. Matacchione · J. Sabbatinelli (✉) · A. Giuliani
Clinic of Laboratory and Precision Medicine, IRCCS INRCA, Ancona, Italy
e-mail: j.sabbatinelli@staff.univpm.it

S. Vaiasicca · A. M. Lamedica · A. R. Bonfigli · F. Lattanzio
Scientific Direction, IRCCS INRCA, Ancona, Italy

M. Cecati
Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma University, Rome, Italy

L. Biscetti
Neurology Unit, IRCCS INRCA, Ancona, Italy

A. Cherubini
Geriatrics, Accettazione Geriatrica e Centro Di Ricerca Per L'Invecchiamento, IRCCS INRCA, Ancona, Italy

A. Cherubini · F. Spannella · J. Sabbatinelli · F. Olivieri · A. Giuliani
Department of Clinical and Molecular Sciences (DISCLIMO), Università Politecnica Delle Marche, Via Tronto 10/A, 60126 Ancona, Italy

F. Spannella
Internal Medicine and Geriatrics, IRCCS INRCA, Ancona, Italy

L. Soraci
Unit of Geriatric Medicine, IRCCS INRCA, Cosenza, Italy

were used. Effect modification by frailty was evaluated through interaction and stratified analyses. Over a median follow-up of 126.8 months, 713 patients died. Shorter age-adjusted LTL was associated with increased mortality risk in univariable analysis (HR per 1 SD decrease = 1.10, 95% CI 1.02–1.19; $p=0.012$). When dichotomized using an optimal cut-point, patients with shorter-than-expected telomeres exhibited significantly higher mortality (adjusted HR = 1.30, 95% CI 1.05–1.60; $p=0.016$), regardless of clinical and laboratory covariates. A significant interaction with frailty was observed (p for interaction = 0.031), with the association between LTL and mortality being evident among patients without frailty. Age-adjusted LTL provides independent prognostic information in hospitalized older adults and may reflect biologically meaningful heterogeneity associated with aging, particularly among individuals without frailty.

Keywords Telomere length · Biological aging · Frailty · Mortality · Older patients

Introduction

Aging is a complex biological process driven by multiple factors, characterized by progressive molecular and cellular changes that contribute to a decline in physiological function and a higher vulnerability to disease and death. While chronological age is widely used as a measure of aging, it is an imperfect indicator of biological aging, as it does not account for the considerable variability in health, functional decline, and survival observed among individuals, especially at older ages [1, 2]. Individuals of the same chronological age may exhibit markedly different trajectories of aging, reflecting differences in cumulative biological damage, resilience, and adaptive capacity [3].

This heterogeneity has driven increasing interest in biomarkers of biological aging, which aim to capture the cumulative burden of molecular and cellular damage beyond the mere passage of time. Among the proposed biomarkers, leukocyte telomere length (LTL) has received considerable attention [4]. Telomeres are repetitive DNA-protein structures that protect chromosome ends from degradation and inappropriate DNA damage responses. With successive cell divisions, telomeres progressively shorten, eventually

contributing to cellular senescence, genomic instability, and altered tissue homeostasis [5]. As a result, LTL is widely considered a proxy of biological aging, reflecting cumulative lifelong exposure to genetic, environmental, and lifestyle factors rather than chronological age per se [6–8]. Importantly, telomere length varies substantially even among individuals of the same chronological age, further underscoring heterogeneity in biological aging trajectories [9].

To disentangle biological from chronological aging, telomere length is increasingly operationalized as an age-adjusted measure. In this framework, LTL is modeled as a function of chronological age, and individual deviations from the expected value, expressed as residuals, serve as indicators of biological age. Positive residuals (longer telomeres than expected for a given age) may reflect slower biological aging or greater biological resilience, whereas negative residuals suggest accelerated biological aging relative to chronological age. This approach has proven informative in large longitudinal cohorts, where shorter residual LTL has been associated with increased mortality risk, particularly from non-cancer causes [10].

In longitudinal studies, residual LTL has been shown to predict survival independently of chronological age, with stronger associations observed for cardiovascular and other non-cancer causes of death [11]. Similar associations have been reported in cohorts of older adults, including individuals aged > 65 years, where shorter telomeres were associated with substantially higher mortality risk even after adjustment for major cardiovascular risk factors and comorbidities [12]. Recently, it was reported that frailty mediates the effect of LTL on mortality, suggesting that measuring LTL and frailty together may help to identify patients at higher risk of death. This in turn supports the implementation of preventive strategies in patients with accelerated biological aging and frailty, according to the modern principles of predictive, preventive, and personalized medicine (PPPM/3PM) [13].

However, the majority of studies about the predictive accuracy of LTL in geriatric subjects involved outpatient population. On the other hand, relatively little is known about the prognostic value of telomere length in hospitalized geriatric populations, who represent a particularly vulnerable subgroup of older adults. Hospitalized older patients are characterized

by high prevalence of multimorbidity, frailty, and functional impairment, conditions that may amplify the biological consequences of accelerated aging. In this context, biological age markers may offer additional prognostic information beyond traditional clinical variables and chronological age alone. In one of the few studies conducted in acutely ill geriatric patients, no significant association between LTL and post-discharge mortality was observed, with comorbidity, functional status, and nutritional impairment emerging as the main determinants of survival [14]. However, that study relied on raw telomere length measurements and did not account for age-related telomere attrition, which may have obscured biologically meaningful inter-individual variability. Moreover, potential interactions with key geriatric constructs such as frailty were not explored.

The present study investigates the association between LTL, expressed as residuals relative to chronological age, and 10-year all-cause mortality in a cohort of hospitalized geriatric patients. By focusing on long-term survival and on a clinically complex population, this study aims to clarify whether telomere length captures biologically meaningful heterogeneity in aging that remains relevant even in late life and under conditions of frailty and high disease burden. In addition, we specifically investigated whether frailty modifies the association between LTL and mortality, testing the hypothesis that the prognostic relevance of telomere-based biological aging is amplified in individuals with the greatest reduction in physiological reserve.

Materials and methods

Study design and participants

This study is a prospective observational analysis conducted within the framework of the Report-AGE project (ClinicalTrials.gov identifier: NCT01397682), an ongoing multicenter epidemiological cohort aimed at investigating clinical and biological determinants of outcomes in hospitalized older adults. The Report-AGE project has been previously described in detail [15]. Participants were consecutively recruited among patients aged 65 years or older admitted to acute care wards, including geriatric medicine, cardiology, urology, surgery, and neurology, at three Italian National

Institute on Health and Aging (Istituto Nazionale di Ricovero e Cura dell'Anziano, Istituto a carattere scientifico, IRCCS INRCA) hospitals between September 2011 and October 2021. During the overall enrollment period, more than 5000 patients were included in the parent cohort, with written informed consent obtained from over 90% of eligible individuals.

Eligibility criteria for inclusion in the Report-AGE cohort comprised age ≥ 65 years, hospitalization lasting at least 24 h, and the ability to provide informed consent. Patients with hematological malignancies or active SARS-CoV-2 infection were excluded. For the purposes of the present analysis, patients who died during the index hospitalization were excluded to minimize potential bias related to acute terminal conditions and to focus on post-discharge mortality.

The present study was conducted as a predefined substudy of the Report-AGE cohort and included patients discharged alive for whom biological samples were available and suitable for LTL assessment. Blood samples were collected at hospital admission and processed and stored according to standardized protocols. The resulting analytical sample consisted of 872 patients and was broadly representative of the overall cohort with respect to age distribution, sex, and long-term vital status. Vital status and mortality outcomes were ascertained through hospital discharge records and linkage with national population registries, ensuring complete follow-up without the need for active participant contact. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committees of the IRCCS INRCA network (CE INRCA 20031, 04/02/2021). All participants provided written informed consent prior to enrollment; consequently, individuals with severe cognitive impairment or impaired consciousness may be under-represented in the study population.

Telomere length assessment

High molecular weight genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (Qiagen, Milan, Italy), following the manufacturer's instructions. DNA purity and concentration were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) by measuring absorbance at 260 and 280 nm. LTL was quantified as the telomere-to-single copy gene (T/S)

ratio by quantitative real-time polymerase chain reaction (PCR), according to the method originally described by Cawthon [16], with minor modifications.

Briefly, each reaction was performed using 20 ng of DNA in a 5 μ L volume combined with 10 μ L of master mix. A reference DNA sample was serially diluted (1.68-fold) to generate a standard curve consisting of five concentrations ranging from 30 to 2 ng per reaction. To minimize inter-assay variability, telomere and single-copy gene (36B4) amplifications were performed on the same plate. The 36B4 gene, encoding the acidic ribosomal phosphoprotein P0, served as the reference gene in the conventional qPCR assay for telomere length measurement. Primer sequences and concentrations were as previously reported [16].

The thermal cycling conditions consisted of an initial denaturation step at 95 °C for 10 s, followed by 30 cycles of 95 °C for 5 s, 57 °C for 15 s, and 72 °C for 20 s. All samples were analyzed in duplicate, and results were expressed as T/S ratios normalized to a calibrator sample (Roche, Milan, Italy), allowing comparison across different runs. Quantitative PCR reactions were performed using a Rotor-Gene real-time PCR system (Qiagen).

All samples were analyzed in duplicate, and measurements were accepted only when the difference between duplicate Ct values was below 0.5 cycles. Samples exceeding this threshold were reanalyzed. To further reduce technical variability, telomere and single-copy gene amplifications were performed on the same plate using the same calibrator sample across runs whenever possible.

Derivation of age-adjusted telomere length residuals

Age-adjusted telomere length residuals were derived using an external reference population of apparently healthy individuals aged 40–90 years. This population comprised 593 subjects (median age of 58 years (IQR 52–67), 38.4% males) drawn from a previously published cohort of community-dwelling individuals recruited in central Italy within prevention-oriented studies [17]. Participants underwent standardized clinical evaluation, including detailed medical history, physical examination, and laboratory testing. Individuals with major chronic conditions were excluded, including diabetes (based on clinical history or fasting glucose criteria), cardiovascular

disease (myocardial infarction, stroke, or peripheral vascular disease), and other relevant comorbidities, and were not receiving medications known to interfere with metabolic or inflammatory pathways. All participants were Caucasian, sampled under fasting conditions, and derived from a geographically and lifestyle-homogeneous setting, broadly adhering to a Mediterranean dietary pattern. This reference cohort represents a geographically comparable reference to the Report-AGE study population [18].

Linear regression models were fitted with LTL as the dependent variable and chronological age as the independent variable. For each participant in the hospitalized geriatric cohort, telomere length residuals were calculated as the difference between observed telomere length and the value predicted by the age-specific regression line derived from the healthy reference population. Positive residuals indicate longer-than-expected telomeres for age, whereas negative residuals indicate shorter-than-expected telomeres. The same age-adjusted telomere residuals derived from the healthy reference population were used across all survival analyses, including exploratory analyses focused on 1-year, 5-year, and 10-year mortality.

Covariates

Comorbidities were recorded at admission and coded according to the International Classification of Diseases, Ninth Revision (ICD-9). Multimorbidity was assessed using the Charlson comorbidity index (CCI). Frailty was quantified as previously described [19] using a frailty index (FI) based on the deficit accumulation model [20, 21]. Frailty was defined as a $FI \geq 0.20$ according to established thresholds [22]. Laboratory variables were assessed using standard commercial methods. The glomerular filtration rate (GFR) was estimated by the creatinine-based Berlin Initiative Study-1 (BIS1) equation specifically used to estimate GFR in older adults [23]. All covariates were assessed at hospital admission.

Statistical analysis

Continuous variables are described as medians with interquartile ranges (IQRs), while categorical variables are reported as absolute numbers and percentages. The distribution of continuous variables was

assessed using the Shapiro–Wilk test. Comparisons between survivors and non-survivors were performed using the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables, as appropriate.

LTL was analyzed as an age-adjusted measure. Age-adjusted telomere length residuals were obtained as described above and analyzed both as a continuous variable (per standard deviation decrease) and as a categorical variable based on a data-driven cut-point derived within the study population using maximally selected rank statistics, as implemented in the `surv_cutpoint` function of the `survminer` R package. This approach identifies the threshold that maximizes separation in survival between groups while enforcing a minimum proportion of observations in each group ($\text{minprop}=0.10$). This cut-point was used to dichotomize participants into groups with shorter versus longer telomere length relative to chronological age.

The association between age-adjusted telomere length residuals and all-cause mortality was investigated using Cox proportional hazards regression models. Univariable Cox models were first fitted to evaluate the crude association between telomere length residuals and mortality. Multivariable Cox models were then constructed to adjust for potential confounders, including demographic characteristics, comorbidity burden, polypharmacy, and laboratory parameters. Covariates were selected based on their clinical relevance and on significant differences observed between survivors and non-survivors at baseline. Continuous covariates were modeled per clinically meaningful increments where appropriate. Results of Cox regression analyses are presented as hazard ratios (HRs) with corresponding 95% confidence intervals (CIs). The proportional hazards assumption was assessed using Schoenfeld residuals and was not violated.

To investigate whether the association between telomere length residuals and mortality differed according to frailty status, interaction terms between telomere length residuals and frailty strata were included in multivariable Cox models. Stratified analyses were subsequently performed to explore the association between telomere length and mortality within different frailty strata.

All statistical analyses were performed using R software (version 4.5.2). A two-sided p value <0.05 was considered statistically significant.

Results

Characteristics of the study population

The study population consisted of 872 hospitalized geriatric patients, of whom 159 were alive and 713 died during follow-up. Median follow-up time, estimated using the reverse Kaplan–Meier method, was 126.8 months (IQR 111.5–134.6). Using the conventional approach, median follow-up was 50.6 months (IQR 14.9–95.1). The median age of the cohort was 84 years (IQR 80–88), with deceased patients being significantly older than survivors (85 vs. 79 years, $p<0.001$). Women accounted for 53.6% of the population, with no significant sex differences between survivors and non-survivors. Compared with survivors, patients who died during follow-up exhibited a less favorable clinical and laboratory profile at admission. Specifically, deceased patients had lower red blood cell count and hemoglobin levels, lower serum albumin, reduced renal function as assessed by eGFR, and higher blood urea nitrogen levels (all $p<0.001$). They also had a higher comorbidity burden, as reflected by higher Charlson comorbidity index scores, and a greater number of prescribed medications ($p<0.001$). Frailty was highly prevalent in the cohort and strongly associated with mortality. Patients with frailty ($\text{FI}\geq 0.20$) accounted for more than half of deaths (50.8%), whereas they represented only 13.8% of survivors ($p<0.001$). Detailed baseline characteristics stratified by vital status are reported in Table 1.

Distribution of telomere length residuals

LTL was expressed as age-adjusted residuals, calculated as the difference between observed values and those expected for a given age based on a healthy reference population previously characterized by our group [17]. In this framework, residuals reflect deviations from physiological age-related telomere shortening, with negative values indicating shorter-than-expected telomeres and positive values indicating longer-than-expected telomeres.

Telomere length values in the hospitalized geriatric cohort showed substantial dispersion around the age-specific regression line derived from the healthy reference population. This variability was observed across the entire age range, indicating

Table 1 Baseline characteristics of the study population according to 10-year survival status

Variables	Total (<i>n</i> = 872)	Survivors (<i>n</i> = 159)	Deceased (<i>n</i> = 713)	<i>P</i> -value
Age (years)	84.0 (80.0–88.0)	79.0 (73.5–83.0)	85.0 (81.0–89.0)	<0.001
Sex, <i>n</i> (%)				0.229
Female	467 (53.6%)	92 (57.9%)	375 (52.6%)	
Male	405 (46.4%)	67 (42.1%)	338 (47.4%)	
WBC ($\times 10^3/\mu\text{L}$)	7.33 (5.77–9.61)	6.96 (5.76–8.93)	7.40 (5.78–9.79)	0.121
RBC ($\times 10^6/\mu\text{L}$)	4.06 (3.63–4.50)	4.35 (3.92–4.65)	4.01 (3.56–4.45)	<0.001
Hemoglobin (g/dL)	12.0 (10.6–13.4)	13.1 (11.7–14.0)	11.8 (10.3–13.1)	<0.001
Platelets ($\times 10^3/\mu\text{L}$)	205 (164–263)	203 (168–249)	207 (163–267)	0.361
AST (U/L)	17.0 (14.0–24.0)	18.0 (15.0–27.0)	17.0 (14.0–24.0)	0.041
ALT (U/L)	13.0 (10.0–21.0)	14.0 (11.0–22.5)	12.0 (10.0–20.0)	0.001
Gamma-GT (U/L)	24.0 (15.0–46.0)	21.0 (14.0–45.5)	25.0 (15.0–47.0)	0.493
Albumin (g/dL)	3.60 (3.20–4.00)	4.00 (3.50–4.30)	3.55 (3.20–3.90)	<0.001
CRP (mg/dL)	1.12 (0.22–5.46)	0.40 (0.15–1.71)	1.50 (0.27–5.90)	<0.001
CRP-to-albumin ratio	0.31 (0.06–1.66)	0.10 (0.03–0.47)	0.41 (0.07–1.82)	<0.001
LDH (IU/L)	193 (153–261)	170 (139–239)	197 (156–262)	0.223
Glucose (mg/dL)	102.0 (89.0–125.0)	103.0 (92.0–123.5)	101.0 (88.0–125.0)	0.315
Sodium (mEq/L)	139.0 (136.0–141.0)	139.0 (137.8–141.0)	139.0 (136.0–141.0)	0.074
Potassium (mEq/L)	4.10 (3.75–4.50)	4.10 (3.77–4.40)	4.10 (3.75–4.50)	0.459
Chloride (mEq/L)	101.0 (98.0–103.9)	101.0 (99.0–103.1)	101.0 (98.0–104.0)	0.417
eGFR (mL/min/1.73 m ²)	60.0 (43.0–78.0)	70.0 (54.8–83.3)	58.0 (39.0–76.0)	<0.001
Azotemia (mg/dL)	46 (34–69)	38 (31–49)	48 (35–75)	<0.001
Polypharmacy, <i>n</i> (%)	548 (62.8%)	68 (42.8%)	480 (67.3%)	<0.001
Charlson index, <i>n</i> (%)				<0.001
0	199 (22.8%)	57 (35.8%)	142 (19.9%)	
1	225 (25.8%)	43 (27.0%)	182 (25.5%)	
≥ 2	448 (51.4%)	59 (37.1%)	389 (54.6%)	
Frailty, <i>n</i> (%)	384 (44.0%)	22 (13.8%)	362 (50.8%)	<0.001

Continuous variables are presented as median (interquartile range, IQR), and categorical variables as number (percentage). Comparisons between survivors and deceased patients were performed using the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables. *WBC*, white blood cells; *RBC*, red blood cells; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *CRP*, C-reactive protein; *LDH*, lactate dehydrogenase; *eGFR*, estimated glomerular filtration rate

marked heterogeneity in biological aging among patients of similar chronological age.

When stratified by vital status, individuals who died during follow-up tended to exhibit more negative telomere length residuals compared with survivors ($p < 0.001$). This pattern was evident for overall mortality and became progressively more pronounced when mortality within 1 year (Fig. 1A) and within 5 years (Fig. 1B) was considered. Patients who died within 1 year after hospitalization showed the greatest downward deviation from the healthy reference trajectory.

Association between telomere length residuals and all-cause mortality

In univariable Cox proportional hazards models, shorter age-adjusted telomere length residuals were associated with increased all-cause mortality (HR per 1 SD decrease = 1.10, 95% CI 1.02–1.19; $p = 0.012$). After multivariable adjustment, this association was attenuated and did not reach statistical significance (HR per 1 SD decrease = 1.08, 95% CI 1.00–1.16; $p = 0.062$).

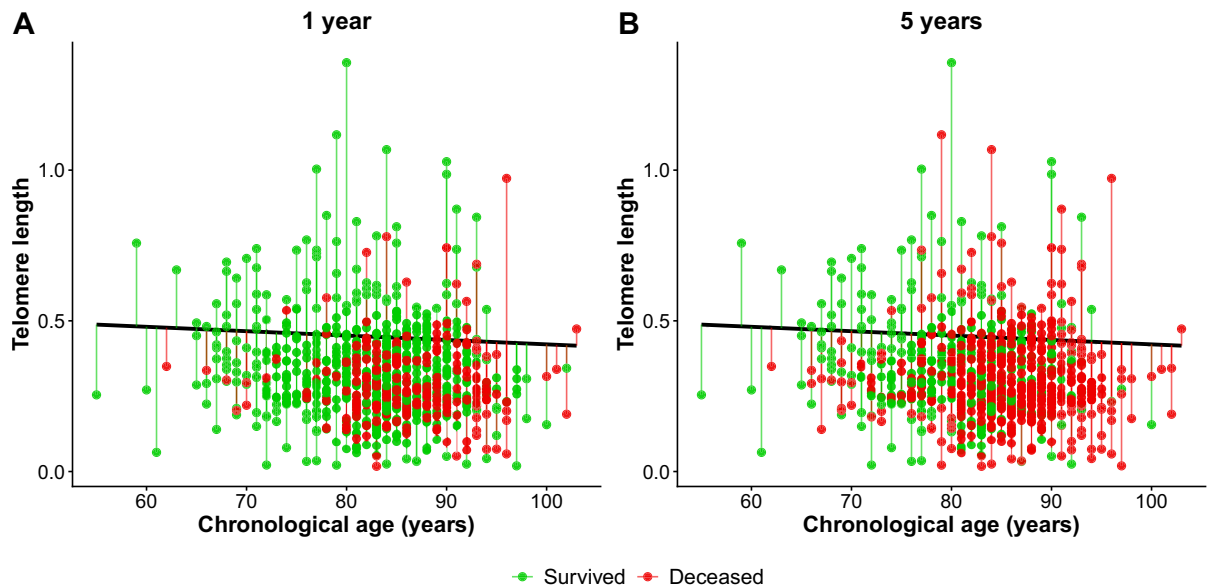


Fig. 1 Telomere length in relation to age and survival at 1 and 5 years. Scatter plots showing telomere length according to chronological age in hospitalized older adults. The black line represents the age-specific regression derived from a healthy reference population. Each point represents an individual

patient, and vertical segments indicate the deviation from the expected telomere length based on age (residuals from the reference line). Patients are stratified by survival status (green = survived, red = deceased)

When analyzed using a data-driven cut-point, shorter telomere length was independently associated with higher all-cause mortality (HR=1.30,

95% CI 1.05–1.60; $p=0.016$; Table 2). This association remained significant after adjustment for demographic variables, comorbidity burden, laboratory

Table 2 Association between age-adjusted telomere length residuals and 10-year all-cause mortality assessed using univariable and multivariable Cox proportional hazards models

Variable	Univariable HR (95% CI)	<i>p</i> -value	Multivariable HR (95% CI)	<i>p</i> -value
Short telomeres (vs long)	1.36 (1.10–1.68)	0.004	1.30 (1.05–1.60)	0.016
Male sex	1.16 (1.02–1.32)	0.026	1.36 (1.16–1.59)	<0.001
Hemoglobin (g/dL)	0.82 (0.79–0.85)	<0.001	0.85 (0.81–0.89)	<0.001
Charlson index = 1	1.24 (1.02–1.52)	0.033	1.09 (0.86–1.36)	0.481
Charlson index ≥ 2	1.79 (1.50–2.14)	<0.001	1.31 (1.07–1.61)	0.010
Platelets (per + $10 \times 10^3/\mu\text{L}$)	1.01 (1.00–1.02)	0.046	1.01 (1.00–1.02)	0.203
Neutrophil-to-lymphocyte ratio	1.02 (1.02–1.03)	<0.001	1.01 (0.99–1.02)	0.359
Mononuclear cells (monocytes + lymphocytes, per + 10%)	0.81 (0.77–0.86)	<0.001	0.99 (0.90–1.07)	0.733
White blood cells	1.05 (1.03–1.06)	<0.001	1.02 (1.00–1.05)	0.116
eGFR (per + 10 mL/min/1.73 m ²)	0.85 (0.82–0.87)	<0.001	0.93 (0.90–0.97)	<0.001
Polypharmacy	1.01 (1.00–1.02)	0.239	1.36 (1.15–1.60)	<0.001
ALT (per + 10 U/L)	1.00 (0.99–1.01)	0.095	1.00 (0.99–1.01)	0.535
CRP-to-albumin ratio	1.08 (1.05–1.10)	<0.001	0.99 (0.95–1.02)	0.453

Telomere length was analyzed as a categorical variable using a data-driven cut-point (residual=0.00518), with longer telomeres as the reference category. The multivariable model was adjusted for sex, hemoglobin levels, Charlson comorbidity index, platelet count, neutrophil-to-lymphocyte ratio, white blood cell count, estimated glomerular filtration rate (eGFR), and polypharmacy.

parameters, renal function, and polypharmacy. Laboratory covariates were chosen among those significantly associated with mortality in univariable analyses while avoiding collinearity by including only one variable among highly correlated measures. In multivariable models, male sex, higher comorbidity burden, and polypharmacy were associated with increased mortality, whereas higher hemoglobin levels and eGFR were protective. Additional adjustment for neutrophil-to-lymphocyte ratio and mononuclear cell percentage, included to account for differences in leukocyte subset composition, did not materially alter the association between telomere length and mortality.

In sensitivity analyses using sex-specific data-driven cut-points, the association between shorter telomere length and mortality was consistent in both

men (HR 1.24, 95% CI 1.00–1.55; $p=0.054$) and women (HR 1.34, 95% CI 0.97–1.83; $p=0.073$), although it did not reach statistical significance, likely due to reduced sample size.

To facilitate clinical interpretation and visualization of survival differences, age-adjusted telomere length residuals were dichotomized using a data-driven optimal cut-point. The optimal threshold was identified at a residual value of 0.00518, which maximized discrimination between survival groups. Patients were therefore stratified into two groups: those with shorter-than-expected telomeres (residuals ≤ 0.00518 , $n=737$) and those with longer-than-expected telomeres (residuals > 0.00518 , $n=135$). Kaplan–Meier survival curves were constructed to compare long-term mortality between these groups (Fig. 2), and differences between curves were assessed using the log-rank test ($p=0.004$).

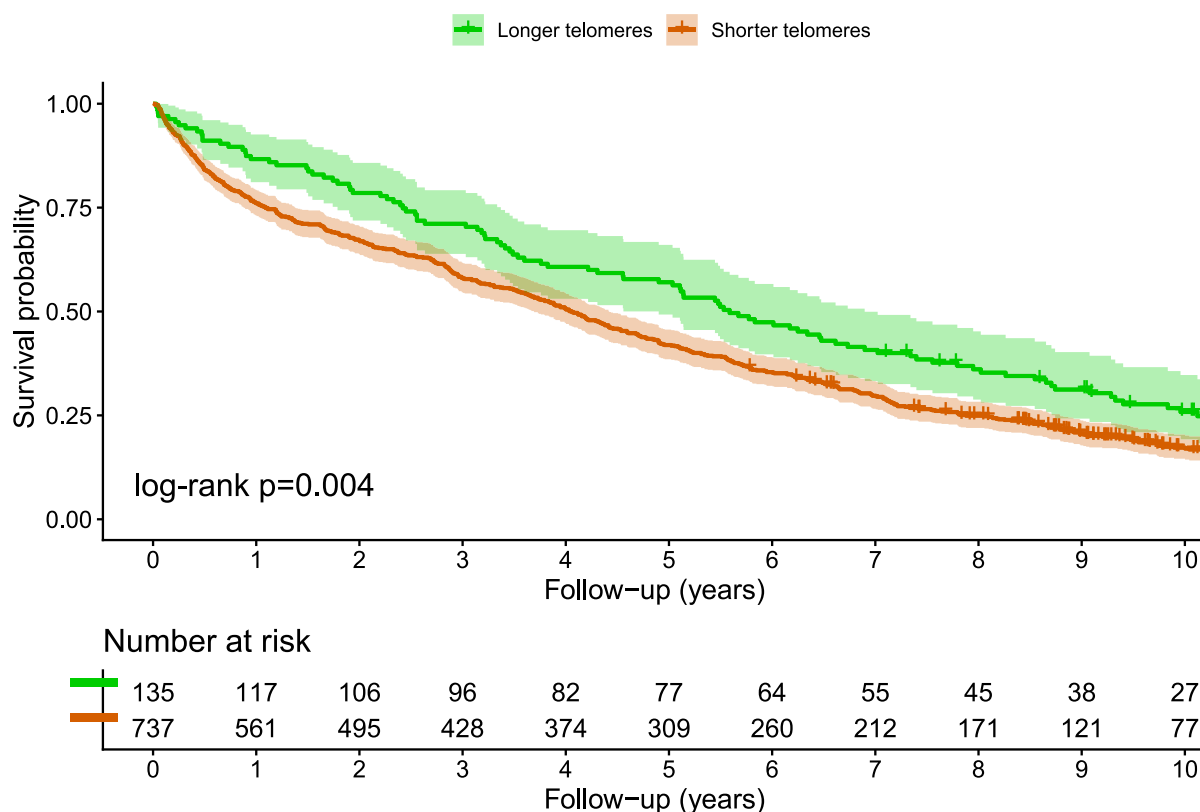


Fig. 2 Kaplan–Meier survival curves for 10-year all-cause mortality according to age-adjusted telomere length residuals. Patients were stratified using a data-driven optimal cut-

point (residual = 0.00518) into shorter ($\leq$ cut-point) and longer ($>$ cut-point) telomere groups. Shaded areas represent 95% confidence intervals. Numbers at risk are shown below the plot

Interaction between telomere length and frailty

In models including standardized telomere length residuals and their interaction with frailty, a significant interaction was observed (p for interaction = 0.031). A 1 SD decrease in telomere length was associated with increased mortality among patients without frailty (HR 1.13, 95% CI 1.01–1.25), whereas no significant association was observed in those with frailty (HR 0.95, 95% CI 0.85–1.06; Table 3).

Discussion

In this study, leukocyte telomere length (LTL), expressed as age-adjusted residuals derived from a healthy reference population, was independently associated with long-term all-cause mortality in a cohort of hospitalized geriatric patients. Individuals with shorter-than-expected telomeres for their chronological age exhibited a significantly higher risk of death in an analysis based on a data-driven survival threshold, even after adjustment for demographic characteristics, comorbidity burden, laboratory parameters, and pharmacological treatment, although the association was attenuated in continuous multivariable models. These findings suggest that LTL is associated with inter-individual differences in aging-related and clinical factors that are not fully explained by chronological age or traditional clinical predictors [4, 24].

Our results are consistent with previous longitudinal studies showing an association between shorter telomeres and increased mortality, particularly from non-cancer causes. Large population-based cohorts have demonstrated that residual LTL predicts survival independently of chronological age, with shorter telomeres associated with increased mortality risk, supporting the idea that telomere dynamics may reflect

fundamental biological constraints on human lifespan [11, 25, 26]. Importantly, our study extends these observations to a clinically complex population of hospitalized older adults, in whom chronological age alone is often insufficient to discriminate prognosis due to high variability in health status, multimorbidity, and functional reserve.

A key methodological strength of the present work lies in the derivation of telomere length residuals using an external population of healthy individuals. LTL residuals represent the variation in telomere length remaining after statistical adjustment for age, allowing the separation of age-related effects from those attributable to genetic, environmental, or disease-related factors [27]. This approach enabled us to contextualize telomere length relative to expected age-related attrition and to interpret residual telomere length as a marker potentially reflecting biological heterogeneity beyond chronological age alone. However, LTL is influenced by multiple factors, including inflammation, immune activation, comorbidity burden, and leukocyte composition, which may be particularly relevant in hospitalized older adults [5, 28, 29]. By anchoring telomere length to a healthy reference trajectory, we aimed to improve the interpretation of telomere length variability relative to chronological age in this geriatric population. Nevertheless, age-adjusted telomere residuals should not be interpreted as a direct measure of biological aging and likely reflect the influence of multiple aging-related and disease-related processes, including inflammation, immune system dynamics, and comorbidity burden [4].

Notably, the prognostic value of telomere length was modified by frailty status. The interaction analysis showed that shorter LTL was associated with increased mortality among patients without frailty, whereas no significant association was observed in those with frailty. These findings suggest that telomere length may retain greater prognostic value among individuals without established frailty, whereas its prognostic relevance appears attenuated in frail patients, in whom multisystem impairment may already dominate risk [30].

Telomere shortening has been linked to cellular senescence, impaired tissue repair, immune dysregulation, and chronic low-grade inflammation, processes that contribute to the development of frailty and related conditions such as sarcopenia [13, 31]. However, once frailty is established, these downstream

Table 3 Association between standardized leukocyte telomere length (per 1 SD decrease) and all-cause mortality by frailty status. HRs (95% CIs) were estimated from multivariable Cox models including an interaction term. P for interaction was derived from the likelihood ratio test

Frailty status	HR (95% CI)	p
Fit (FI < 0.20)	1.13 (1.01–1.25)	0.031
Frail (FI ≥ 0.20)	0.95 (0.85–1.06)	0.361
P for interaction		0.031

alterations may already be sufficiently advanced to reduce the ability of telomere length to further discriminate risk. Conversely, in non-frail individuals, telomere length may retain prognostic discrimination that is no longer evident once frailty is established [32]. More broadly, LTL should be interpreted within the evolving landscape of biological aging biomarkers. Although telomere length has been extensively studied as a marker of biological aging, emerging approaches including epigenetic clocks [33], proteomic signatures [34], inflammatory indices [19], and multimodal biological age models [35] may capture complementary dimensions of aging biology and may provide greater prognostic resolution in some settings. In this context, TL may represent one component of a multidimensional assessment of biological aging rather than a standalone biomarker. The present findings should primarily be interpreted within the context of an observational prognostic biomarker study and not as direct evidence of specific biological mechanisms.

Several limitations should be acknowledged. First, telomere length was measured at a single time point, precluding assessment of telomere attrition rates over time. Second, although residualization against a healthy reference population strengthens biological interpretation, unmeasured confounding and cohort differences cannot be entirely excluded. Indeed, LTL remains influenced by multiple biological and clinical factors beyond aging processes per se, including inflammation, immune activation, comorbidity burden, renal dysfunction, and leukocyte subset composition. Third, the study population consisted of hospitalized geriatric patients, which may limit generalizability to community-dwelling older adults. However, this clinical specificity may also be viewed as a strength, as it targets a population in which improved biological risk stratification is particularly needed. Fourth, LTL was assessed using a quantitative PCR-based method, which, despite being widely adopted in research settings, may be subject to inter-assay variability and requires careful standardization. Differences in DNA extraction procedures, assay conditions, calibrator selection, and data normalization strategies may further affect measurement consistency [36]. Further studies are warranted to confirm the reproducibility and analytical robustness of this approach across laboratories. An additional

limitation relates to the use of a data-driven cut-point for telomere length stratification. Although this approach improved visualization and facilitated interpretation of survival differences, thresholds derived within the same cohort may be sensitive to sample-specific characteristics and may not generalize to external populations. Therefore, the identified cut-point should be considered exploratory and requires validation in independent cohorts before potential clinical application. Accordingly, the survival differences observed using this threshold should be interpreted cautiously until external validation is available.

In conclusion, shorter age-adjusted LTL was associated with long-term mortality in hospitalized older adults and interacted with frailty, with its prognostic value being evident in individuals without frailty but attenuated in those with established frailty. These findings support the potential relevance of telomere length as a biomarker of biological heterogeneity and vulnerability in late life and highlight the importance of integrating molecular aging markers with the assessment of clinical geriatric constructs. Future studies should investigate whether combining telomere-based measures with multidimensional frailty assessments can improve prognostic accuracy and inform personalized approaches to care in older populations.

Author contribution D.R. and F.O. conceived and designed the study. R.M., D.R., M.D.R., M.C., R.R., F.M., F.P., R.G., C.G., G.M., M.S., S.F., and S.Fu. contributed to data acquisition and curation. A.G., J.S., and M.D.R. performed the statistical analyses. D.R., A.G., and J.S. drafted the manuscript. J.S. and F.O. contributed to data interpretation and critically revised the manuscript. A.M.L., L.B., A.C., F.S., L.S., F.L., and A.R.B. contributed to clinical interpretation and provided important intellectual content. F.O. and F.L. supervised the study. All authors contributed to the interpretation of the data, revised the manuscript critically, and approved the final version.

Funding Open access funding provided by Università Politecnica delle Marche within the CRUI-CARE Agreement. This study was funded by the Italian Ministry of Health under the grants Ricerca Corrente to IRCCS INRCA and Ricerca Finalizzata 2021, project code SG-2021-12372949, to D.R.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Iuorio MS, De Vincentis A, Lelli D, Bandinelli S, Ferrucci L, Incalzi RA, et al. PhenoAge and PhenoAgeAccel do not outperform chronological age in predicting physical performance decline or mortality in community-dwelling older adults. *J Gerontol A Biol Sci Med Sci*. 2026. <https://doi.org/10.1093/gerona/glaf285>.
- Sun Y, Wu W, Sun X, Ren L, Ren Z, Gong G. Aging acceleration, not chronological age, is associated with cognitive performance in older adults: a cross-sectional study on the protective role of physical activity. *Medicine (Baltimore)*. 2025;104(29):e43431.
- Salimi S, Raftery D, Ferrucci L. Genomic perspective on heterogeneity of organs and body aging. *Aging Cell*. 2026;25(1):e70353.
- Vaiserman A, Krasnienkov D. Telomere length as a marker of biological age: state-of-the-art, open issues, and future perspectives. *Front Genet*. 2020;11:630186.
- Rossiello F, Jurk D, Passos JF, d'Adda di Fagagna F. Telomere dysfunction in ageing and age-related diseases. *Nat Cell Biol*. 2022;24(2):135–47.
- Tilekli MM, Yilmaz AK, Yasul Y, Con N, Mercan S, Tek N. Influence of diet and exercise on leukocyte telomere length, markers of oxidative stress and inflammation in rats. *Exp Mol Pathol*. 2024;140:104947.
- Valeanu A, Margina D, Moreno-Villanueva M, Blasco M, Sikora E, Mosieniak G, Capri M, Breusing N, Bernhardt J, Schön C, Toussaint O, Debacq-Chainiaux F, Grubeck-Loebenstien B, Weinberger B, Fiegl S, Gonos ES, Hervonen A, Slagboom EP, de Craen A, Dollé MET, Jansen EHJM, Mocchegiani E, Giacconi R, Piacenza F, Malavolta M, Weber D, Stuetz W, Grune T, Franceschi C, Bürkle A, Gradinaru D. Causal Inference Approaches Reveal Associations Between LDL Oxidation, NO Metabolism, Telomere Length and DNA Integrity Within the MARK-AGE Study. *Antioxidants (Basel)*. 2025 Jul 30;14(8):933. <https://doi.org/10.3390/antiox14080933>.
- Zhang W, Peng SF, Chen L, Chen HM, Cheng XE, Tang YH. Association between the oxidative balance score and telomere length from the national health and nutrition examination survey 1999–2002. *Oxid Med Cell Longev*. 2022;2022:1345071.
- Xiong L, Yang G, Guo T, Zeng Z, Liao T, Li Y, et al. 17-year follow-up of association between telomere length and all-cause mortality, cardiovascular mortality in individuals with metabolic syndrome: results from the NHANES database prospective cohort study. *Diabetol Metab Syndr*. 2023;15(1):247.
- Schneider CV, Schneider KM, Teumer A, Rudolph KL, Hartmann D, Rader DJ, et al. Association of telomere length with risk of disease and mortality. *JAMA Intern Med*. 2022;182(3):291–300.
- Arbeev KG, Verhulst S, Steenstrup T, Kark JD, Bagley O, Kooperberg C, et al. Association of leukocyte telomere length with mortality among adult participants in 3 longitudinal studies. *JAMA Netw Open*. 2020;3(2):e200023.
- Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, Jenny NS, et al. Leukocyte telomere length and mortality in the cardiovascular health study. *J Gerontol A Biol Sci Med Sci*. 2011;66(4):421–9.
- Jian X, Sun W, Zhang J, Zhang Q, Meng X, Lu H, et al. Frailty mediating the causality between leukocyte telomere length and mortality: a cohort study of 440,551 UK Biobank participants. *EPMA J*. 2024;15(1):99–110.
- Zekry D, Krause KH, Irminger-Finger I, Graf CE, Genet C, Vitale AM, et al. Telomere length, comorbidity, functional, nutritional and cognitive status as predictors of 5 years post hospital discharge survival in the oldest old. *J Nutr Health Aging*. 2012;16(3):225–30.
- Bustacchini S, Abbatecola AM, Bonfigli AR, Chiatti C, Corsonello A, Di Stefano G, et al. The Report-AGE project: a permanent epidemiological observatory to identify clinical and biological markers of health outcomes in elderly hospitalized patients in Italy. *Aging Clin Exp Res*. 2015;27(6):893–901.
- Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):47e–47.
- Testa R, Bonfigli AR, Salvioli S, Invidia L, Pierini M, Sirolla C, et al. The Pro/Pro genotype of the p53 codon 72 polymorphism modulates PAI-1 plasma levels in ageing. *Mech Ageing Dev*. 2009;130(8):497–500.
- Bonfigli AR, Spazzafumo L, Prattichizzo F, Bonafe M, Mensa E, Micolucci L, et al. Leukocyte telomere length and mortality risk in patients with type 2 diabetes. *Oncotarget*. 2016;7(32):50835–44.
- Sbriscia M, Fantone S, Di Rosa M, Marchegiani F, Recchioni R, Maccacchione G, et al. Association of an inflammation score based on IL-6, IL-10 and CXCL9, and frailty with long-term mortality in hospitalized older adults. *Immun Ageing*. 2025;22(1):56.
- Theou O, Haviva C, Wallace L, Searle SD, Rockwood K. How to construct a frailty index from an existing dataset in 10 steps. *Age Ageing*. 2023. <https://doi.org/10.1093/ageing/afad221>.
- Johnson L, Guthrie B, Anand A, Marshall A, Seth S. Measuring frailty: a comparison of the cumulative deficit model of frailty in survey and routine data. *Eur Geriatr Med*. 2025;16(5):1871–80.

22. Kim DH, Rockwood K. Frailty in older adults. *N Engl J Med*. 2024;391(6):538–48.
23. Schaeffner ES, Ebert N, Delanaye P, Frei U, Gaedeke J, Jakob O, et al. Two novel equations to estimate kidney function in persons aged 70 years or older. *Ann Intern Med*. 2012;157(7):471–81.
24. Emami M, Agbaedeng TA, Thomas G, Middeldorp ME, Thiagarajah A, Wong CX, et al. Accelerated biological aging secondary to cardiometabolic risk factors is a predictor of cardiovascular mortality: a systematic review and meta-analysis. *Can J Cardiol*. 2022;38(3):365–75.
25. Thai NQN, LaCroix AZ, Haring B, Wactawski-Wende J, Manson JE, Posis AIB, et al. The association of leukocyte telomere length with exceptional longevity among older women. *Geroscience*. 2024;46(2):2083–92.
26. Steenstrup T, Kark JD, Verhulst S, Thinggaard M, Hjelmborg JVB, Dalgard C, et al. Telomeres and the natural lifespan limit in humans. *Aging (Albany NY)*. 2017;9(4):1130–42.
27. Wang Q, Codd V, Raisi-Estabragh Z, Musicha C, Bountziouka V, Kaptoge S, et al. Shorter leukocyte telomere length is associated with adverse COVID-19 outcomes: a cohort study in UK Biobank. *EBioMedicine*. 2021;70:103485.
28. Hoffmann J, Tabata N, Mas-Peiro S, Spyridopoulos I, Sinning JM, Berkowitsch A, et al. Longer leukocyte telomere length is associated with myeloid inflammation and increased mortality after transcatheter aortic valve replacement. *Eur Heart J Open*. 2022;2(4):oeac045.
29. Heba AC, Toupance S, Arnone D, Peyrin-Biroulet L, Benetos A, Ndiaye NC. Telomeres: new players in immune-mediated inflammatory diseases? *J Autoimmun*. 2021;123:102699.
30. Araujo Carvalho AC, Tavares Mendes ML, da Silva Reis MC, Santos VS, Tanajura DM, Martins-Filho PRS. Telomere length and frailty in older adults-a systematic review and meta-analysis. *Ageing Res Rev*. 2019;54:100914.
31. Bountziouka V, Nelson CP, Codd V, Wang Q, Musicha C, Allara E, et al. Association of shorter leukocyte telomere length with risk of frailty. *J Cachexia Sarcopenia Muscle*. 2022;13(3):1741–51.
32. Boccardi V, Polom J. Searching for beauty and health: aging in women, nutrition, and the secret in telomeres. *Nutrients*. 2024. <https://doi.org/10.3390/nu16183111>.
33. Zalesky A, Wen J, Tian YE. From whole-body to organ-specific biological age clocks. *Nat Aging*. 2026;6(5):961–9.
34. Argentieri MA, Xiao S, Bennett D, Winchester L, Nevado-Holgado AJ, Ghose U, et al. Proteomic aging clock predicts mortality and risk of common age-related diseases in diverse populations. *Nat Med*. 2024;30(9):2450–60.
35. Wang J, Gao Y, Wang F, Zeng S, Li J, Miao H, et al. Accurate estimation of biological age and its application in disease prediction using a multimodal image transformer system. *Proc Natl Acad Sci U S A*. 2024;121(3):e2308812120.
36. Raschenberger J, Lamina C, Haun M, Kollerits B, Coassin S, Boes E, et al. Influence of DNA extraction methods on relative telomere length measurements and its impact on epidemiological studies. *Sci Rep*. 2016;6:25398.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.