SHORT COMMUNICATION

Relative telomere length impact on mortality of COVID-19: Sex differences

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Abstract

Increasing age is associated with severity and higher mortality of COVID-19. Telomere shortening is associated with higher risk of infections and may be used to identify those patients who are more likely to die. We evaluated the association between relative telomere length (RTL) and COVID-19 mortality. RTL was measured in patients hospitalized because of COVID-19. We used Kaplan–Meier method to analyze survival probabilities, and Cox regression to investigate the association between RTL and mortality (30 and 90 days). Six hundred and eight patients were included in the analysis (mean age =72.5 years, 41.1% women, and 53.8% Caucasic). During the study period, 75 people died from COVID-19 and 533 survived. Lower RTL was associated with a higher risk of death in women either at 30 (adjusted hazard ratio [HR] (aHR) = 3.33; 95% confidence interval [CI] = 1.05–10.00; p = 0.040) and at 90 days (aHR = 3.57; 95%CI = 1.23–11.11; p = 0.019). Lower RTL was associated with a higher risk of dying of COVID-19 in women. This finding suggests that RTL has an essential role in the prognosis of this subset of the population.

KEYWORDS

COVID-19, mortality, relative telomere length, SARS-CoV2

Ana Virseda-Berdices and Leyre Concostrina-Martinez contributed equally to this study.

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1 | INTRODUCTION

Population of all ages is susceptible to SARS-CoV-2 infection, but elderly people tend to develop severe respiratory disease that usually requires hospitalization and, in some cases, leads to the death of the patient.[1] Telomere shortening is associated with an impairment of the immune system, and, therefore a higher risk of infection,[2] which is explained by the progressive decline in physiological homeostasis associated with aging, which results in an impaired function of immune system and a greater predisposition to diseases.[3] Some studies have addressed the impact of telomere attrition on COVID-19 pathogenesis. In this line, COVID-19 patients seem to display shorter telomeres than the control population,[4] which is also associated with a higher risk of critical disease.[5] However, no available report has addressed the role in the survival of the relative telomere length (RTL) measured in whole blood in a large cohort of COVID-19 patients.

Sex also affects both SARS-CoV-2 infection and COVID-19 mortality. However, there is a lack of sex-disaggregated results in the majority of COVID-19 studies and clinical trials.[6] Sex differences have been found for many traits, and potential masking in sex-agnostic analyses has been identified.[7] Therefore, the consideration of sex as a variable appears essential to uncover possible sex-related molecular mechanisms of diseases such as COVID-19.[6]

Thus, we aimed to study the impact of telomere length in whole blood from COVID-19 patients at disease onset with mortality, considering sex bias.

2 | METHODS

2.1 | Design and study population

A cross-sectional prospective study enrolling patients with symptomatic COVID-19 recruited at Infanta Leonor University Hospital and Tajo University Hospital was carried out from March to September 2020. Patients diagnosed by laboratory confirmation (RNA detection by polymerase chain reaction [PCR] or serology-based methodology), or by consideration of clinical manifestations compatible with COVID-19 were included. The STROBE-ID checklist was followed.

2.2 | Outcome variables

Two censoring points for COVID-19-related mortality were studied: (i) 30-day mortality and (ii) 90-day mortality after COVID-19 diagnosis. When the date of clinical diagnosis was not available, the hospitalization date was used.

2.3 | Clinical data

Epidemiological and clinical variables were collected from medical records with REDCap.[8]

2.4 | Sample collection

Blood samples were obtained during hospitalization. Peripheral blood was extracted in EDTA tubes and DNA was isolated with the chemagic Prepito® (2022-0030) instrument (PerkinElmer).

2.5 | Telomere relative quantification

RTL quantification was carried out by monochromatic multiplex real-time quantitative PCR (MMqPCR) assay as previously described[9] (see Appendix Data 1 for extended information).

2.6 | Statistical analysis

For the descriptive analysis, the Chi-squared test for categorical variables and the Mann–Whitney U test for continuous variables were used. Regarding RTL measurement, outlier identification was performed using the interquartile range (IQR) method. A survival analysis was used to evaluate mortality in the first 30 (early mortality) and 90 days (late mortality) since COVID-19 diagnosis. Survival probabilities were estimated by the Kaplan–Meier product-limit method using RTL percentile 10 as the cutoff, which would reflect a critical telomere length. Groups were compared using the log-rank test. Cox regression models were also performed adjusting for the most relevant patients' characteristics in each comparison using a stepwise procedure.

Statistical software R (v.4.0.5) was used to perform the statistical analysis. All p-values were two-tailed, and the statistical significance was defined as $p \leq 0.05$.

3 | RESULTS

3.1 | Patient characteristics

Figure 1 shows the study design and patient selection. As a small negative significant correlation ($p = 0.019$; R = −0.086) was found between diagnosis time until blood extraction for RTL determination (Appendix Data 2A). Thus, only patients whose sample was collected in an interval less than 20 days between COVID-19 diagnosis and sample extraction were included. These patients showed no differences in RTL with respect to the day of extraction (Appendix Data S2B and S2C). Next, a total of 20 outliers were filtered off, leaving 608 patients as the final sample size for subsequent analysis (Figure 1).

Clinical and epidemiological characteristics of the 608 enrolled patients by mortality status are described in Appendix Data S3 and by sex in Table 1. RTL was significantly lower as age increased (rho = −0.328; $p < 0.001$), while no significant correlation was observed for either days of hospitalization or intensive care stay.
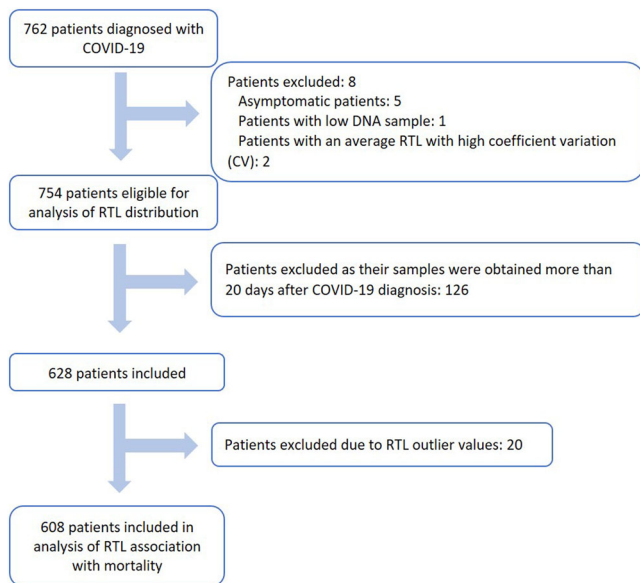
FIGURE 1 Flow chart of patient selection.

3.2 | Association between RTL and mortality

Firstly, we evaluated RTL differences between the COVID-19 mortality groups stratifying by sex (Figure 2, Appendix Data S4). Women who died showed significantly lower RTL (median 1.34; IQR = 0.92–1.46) than women who survived (1.49; (IQR = 1.24–1.80); $p = 0.027$) (Figure 2). In contrast, we did not observe differences regarding age.

Next, we evaluated the effect of RTL on survival time at 30 and 90 days since diagnosis (Table 2). There were differences in survival probabilities among all patients and the women subgroup ($p < 0.05$).

Additionally, we performed a Cox proportional hazards regression model for all patients adjusted by stepwise algorithm with age, sex, chronic renal disease, chronic neurological disease, neoplasia, and smoking status. For sex-stratified analyses, we first carried out univariate Cox regression models to select the sex-specific confounding factors, which were subsequently used to adjust the multivariate Cox regression models (Appendix Data S5 and S6). An extremely low RTL value was a negative factor for survival time in women, being associated with a higher risk of death either at 30 days (adjusted HR (aHR) = 3.33; 95% CI = 1.05–10.00; $p = 0.040$) and at 90 days (aHR = 3.57; 95%CI = 1.23–11.11; $p = 0.019$) (Appendix Data S7).

4 | DISCUSSION

Using a large cohort of patients ($n = 608$), we found that lower whole blood RTL was associated with increased risk of death from COVID-19 in women, being this risk more prominent in older women.

To date, RTL has been evaluated mainly in peripheral blood cells (PBMCs) of many diseases,[2] including COVID-19.[10] However, the need for a density gradient separation becomes a disadvantage, increasing sample processing time and technical requirements. This makes the use of whole blood constitutes an improvement over the use of PBMCs, since RTL measurement is technically easier and additional immune cells (neutrophils and eosinophils) are considered.

Lower RTL has been previously associated with a higher hazard of all-cause mortality in general population,[11] but no evidence of sex bias has yet been shown. In this setting, although sex appears to be an important factor for mortality risk and immunologic responses to COVID-19,[6] few studies have accounted for sex in this condition. Brady et al. observed that only a minority of clinical trials designed to develop new therapies and vaccines for COVID-19 include sex as an analytical variable. Recently, a large genome-wide study identified novel genes and sex differences involved in COVID-19 severity,[12] therefore, analyzing sex differences is essential to expose new mechanisms associated with COVID-19 mortality.

In this study, we observed that lower RTL significantly increased the odds of dying of COVID-19 up to three to four times in women, while no association was found in men. In general, female patients present less severe disease and are more likely to survive COVID-19. The sex disparity of COVID-19–related morbidity and mortality is likely explained by a combination of biological sex differences (differences in chromosomes and related sex steroids) and gender-specific factors (differential behaviors and activities). Based on the available literature, biological sex differences may affect the pathogenic mechanisms of COVID-19, the risk for infection, and the severity of the disease, its outcomes, and its biomarkers.[13] Furthermore, at similar ages women generally possess longer telomeres than men,[14] which may be a result of a different rate of telomere attrition among sexes.[15] Estrogen, which has antioxidant properties, could contribute to a longer RTL since estrogen increases telomerase activity.[16] In addition to scavenge free radicals, estrogen inhibit its production and stimulate some enzymes involved in detoxification.[17] Furthermore, women present lower expression of angiotensin-converting enzyme 2 (ACE2), the functional receptor for SARS-CoV-2, which leads to a milder clinical outcome.[18]

Several limitations should be considered. First, this is a cross-sectional study, which does not allow an inference of causality as a longitudinal design would, but enables for hypothesis generation and limits the possible reverse-causation bias.[19] Secondly, the criteria followed for hospital or ICU stay during the first wave of the COVID-19 pandemic in different countries such as Spain could have limited the possibility of finding statistical differences in some of the analyses carried out, given that the high pressure on the healthcare system resulted in older patients being less likely to be admitted in ICU and a prioritization of ICU beds for younger patients.[20] Third, additional confounders could be affecting our results, however, we accounted for the most important such as gender and sex.[19] Despite this, our study also has several strengths such as a large sample size and the fact that we measured RTL in whole blood, which is a much simpler method of measuring RTL and could be an advantage for transferring this method to routine clinical practice. In addition, we

TABLE 1 Clinical and epidemiological characteristics of patients with COVID-19, stratified by sex

	Male	Female	p-value
No.	358	250	
Age (years)	66.00 (56.00–78.00)	73.00 (59.00–83.75)	**0.003**
Ethnicity (n = 443)			0.781
Caucasian	202/275 (73.5)	125/168 (74.4)	
Hispanic	64/275 (23.3)	41/168 (24.4)	
BMI (kg/m^2) (n = 175)	29.35 (25.63–32.65)	28.98 (26.29–34.98)	0.379
BMI ≥ 25 (kg/m^2) (n = 175)	92/116 (79.3)	49/59 (83.1)	0.697
Smoker status (n = 608)			**<0.001**
Ex-smoker	93/358 (26.0)	24/250 (9.6)	
Smoker	29/358 (8.1)	10/250 (4.0)	
Comorbidities			
Hypertension (n = 608)	187/358 (52.2)	152/250 (60.8)	0.064
Cardiopathy (n = 608)	84/358 (23.5)	56/250 (22.4)	0.950
Chronic pulmonary disease (n = 608)	62/358 (17.3)	17/250 (6.8)	**0.001**
Chronic renal disease (n = 608)	27/358 (7.5)	24/250 (9.6)	0.436
Chronic liver disease (n = 608)	11/358 (3.1)	6/250 (2.4)	0.730
Chronic neurological disease (n = 608)	35/358 (9.8)	28/250 (11.2)	0.801
Neoplasia (n = 608)	34/358 (9.5)	22/250 (8.8)	0.935
Obesity (IMC > 30) (n = 608)	71/358 (19.8)	48/250 (19.2)	0.880
Diabetes (n = 608)	88/358 (24.6)	63/250 (25.2)	0.984
Chronic inflammatory disease (n = 608)	20/358 (5.6)	18/250 (7.2)	0.607
Autoimmune diseases (n = 608)	14/358 (3.9)	16/250 (6.4)	**<0.001**
Therapy			
Basal			
NSAIDs (n = 605)	7/356 (2.0)	12/249 (4.8)	0.070
ACE inhibitors (n = 603)	102/355 (28.7)	51/248 (20.6)	0.071
ARA II (n = 604)	49/355 (13.8)	46/249 (18.5)	0.264
Corticoids (n = 608)	42/358 (11.7)	35/250 (14.0)	0.634
Treatment received during hospitalization			
Chloroquine and hidroxychloroquine (n = 608)	343/358 (95.8)	239/250 (95.6)	0.675
Tocilizumab (n = 600)	114/351 (32.5)	40/249 (16.1)	**<0.001**
Corticoids (n = 603)	220/355 (62.0)	118/248 (47.6)	**0.001**
COVID-19-related symptoms			
Dyspnea (n = 608)	280/358 (78.2)	185/250 (74.0)	0.268
Cough (n = 607)	277/358 (77.4)	181/249 (72.7)	0.221
Headache (n = 607)	86/357 (24.1)	92/250 (36.8)	**0.001**
Diarrhea or abdominal pain (n = 608)	154/358 (43.0)	133/250 (53.2)	**0.036**
Hospitalization			
Hospital stay (days) (n = 607)	13.00 (8.00–20.00)	11.00 (7.00–18.00)	**0.009**
Maximum temperature (n = 588)	38.10 (37.30–38.70)	37.70 (37.10–38.32)	**<0.001**
Oxygen therapy (n = 608)	315/358 (88.0)	205/250 (82.0)	0.051

TABLE 1 (Continued)

	Male	Female	p-value
Invasive mechanical ventilation (n = 608)	32/358 (8.9)	13/250 (5.2)	0.115
Noninvasive mechanical ventilation (n = 608)	79/358 (22.1)	23/250 (9.2)	**<0.001**
Infiltrates (n = 608)	328/358 (91.6)	231/250 (92.4)	0.844
ICU (n = 608)	36/358 (10.1)	17/250 (6.8)	0.210
ICU days (n = 53)	13.00 (5.75–29.25)	9.00 (4.00–19.00)	0.256

Note: Values for continuous variables are expressed as median (interquartile range) and values for categorical variables are expressed as absolute number (percentage). *p*-values for continuous variables were calculated by Mann–Whitney *U* test and *p*-values for categorical variables were calculated by Chi-square tests. Statistically significant differences are shown in bold. Abbreviations: ACE, angiotensin-converting enzyme; ARA II, angiotensin II receptor antagonists; BMI, body mass index; HIV, human immunodeficiency virus; ICU, intensive care unit; NSAIDs, nonsteroidal anti-inflammatory drugs.
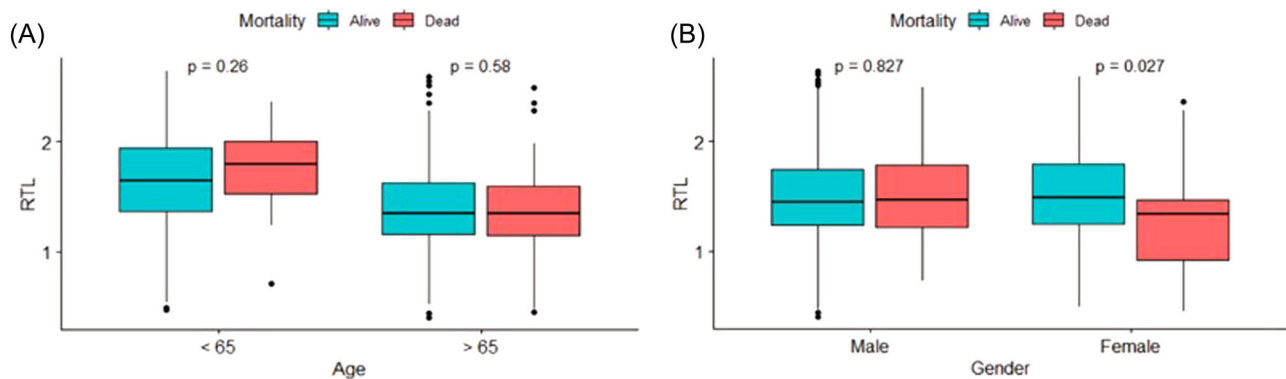
FIGURE 2 Relative telomere length (RTL) association with mortality in all patients (n = 608), according to age (A) and sex (B). *p*-values between mortality groups for each sex were calculated by a Mann–Whitney *U* test. Statistical significance was defined as *p* ≤ 0.05.

TABLE 2 Survival probabilities at 30 and 90 days (Kaplan–Meier product-limit method) for relative telomere length regarding percentile 10.

Patients	Percentile	N	30 days Deaths	p	90 days Deaths	p
All	<10	61	11 (18.03%)	**0.012**	13 (21.31%)	**0.015**
	>10	547	47 (8.59%)		61 (11.51%)	
Men	<10	36	5 (13.89%)	0.384	6 (16.67%)	0.537
	>10	322	31 (9.63%)		43 (13.35%)	
Women	<10	25	6 (24.00%)	**0.003**	7 (28.00%)	**0.001**
	>10	225	16 (7.11%)		18 (8.00%)	
<65	<10	26	1 (3.85%)	0.918	1 (3.85%)	0.661
	>10	231	10 (4.33%)		14 (6.06%)	
>65	<10	35	10 (28.57%)	**0.003**	12 (34.29%)	**0.002**
	>10	316	37 (11.71%)		47 (14.87%)	
Men < 65	<10	17	0 (0.00%)	0.365	0 (0.00%)	0.276
	>10	148	7 (4.73%)		10 (6.76%)	

(Continues)

TABLE 2 (Continued)

Patients	Percentile	N	30 days		90 days	
			Deaths	p	Deaths	p
Men > 65	<10	20	5 (25.00%)	0.152	6 (30.00%)	0.200
	>10	173	24 (13.87%)		33 (19.08%)	
Women < 65	<10	10	1 (10.00%)	0.344	1 (10.00%)	0.484
	>10	82	3 (3.66%)		4 (4.88%)	
Women > 65	<10	16	5 (31.25%)	**0.005**	6 (37.50%)	**0.001**
	>10	142	13 (9.15%)		14 (9.86%)	

Note: Values are expressed as absolute count and percentage. *p*-values were calculated by log-rank tests. Significant differences are shown in bold.

analyzed RTL in the first days of the infection, which allows identifying the RTL as a predictor of mortality.

5 | CONCLUSIONS

An extremely low RTL value was associated with a higher risk of dying of COVID-19 in women. This finding suggests that RTL has an essential role in the prognosis of this subset of the population.

CONFLICT OF INTEREST
The authors declare no conflict of interest

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. All data are available upon request to corresponding authors.

ETHICS STATEMENT
The study protocol was approved by the Ethics Committee of the Institute of Health Carlos III (CEI PI 33_2020-v3-Enmienda_2020-v2) and the Ethics Committee of each hospital. Written, oral, or delegated informed consent was obtained from patients or legal representatives whenever possible. The ethics committee, in some cases, authorized an informed consent waiver.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.