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## Telomere Shortening, Regenerative Capacity, and Cardiovascular Outcomes

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### Abstract

**Rationale**—Leucocyte telomere length (LTL) is a biological marker of aging, and shorter LTL is associated with adverse cardiovascular outcomes. Reduced regenerative capacity has been proposed as a mechanism. Bone marrow-derived circulating progenitor cells (PCs) are involved in tissue repair and regeneration.

**Objective**—To examine the relationship between LTL and PCs, and their impact on adverse cardiovascular outcomes.

**Methods and Results**—We measured LTL by quantitative PCR in 566 outpatients (age 63±9 years, 76% male) with coronary artery disease (CAD). Circulating PCs were enumerated by flow

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### DISCLOSURES

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cytometry. After adjustment for age, gender, race, BMI, smoking and previous myocardial infarction, a shorter LTL was associated with a lower CD34<sup>+</sup> cell count: for each 10% shorter LTL, CD34<sup>+</sup> levels were 5.2% lower (p<0.001). After adjustment for the aforementioned factors, both short LTL (<Q1) and low CD34<sup>+</sup> levels (<Q1) predicted adverse cardiovascular outcomes (death, myocardial infarction, coronary revascularization or cerebrovascular events) independently of each other, with a hazards ratio (HR) of 1.8, 95% confidence interval (CI), 1.1–2.0, and a HR of 2.1, 95% CI, 1.3–3.0, respectively, comparing Q1 to Q2–4. Patients who had both short LTL (<Q1) and low CD34<sup>+</sup> cell count (<Q1), had the greatest risk of adverse outcomes (HR=3.5, 95% CI, 1.7–7.1).

**Conclusion**—Although shorter LTL is associated with decreased regenerative capacity, both LTL and circulating PC levels are independent and additive predictors of adverse cardiovascular outcomes in CAD patients. Our results suggest that both biological aging and reduced regenerative capacity contribute to cardiovascular events, independent of conventional risk factors.

### Keywords

Telomere length; progenitor cells; CD34; CD133; CXCR4; regenerative capacity; cardiac outcomes; aging; cardiac outcomes; coronary artery disease

## INTRODUCTION

Biological aging has been linked to adverse cardiovascular outcomes, yet the underlying mechanisms are unknown.<sup>1–3</sup> Telomeres are regions of repetitive nucleotide sequences at each end of a chromosome that protect the chromosome from deterioration or from fusion with neighboring chromosomes during cell replication.<sup>4, 5</sup> After each cell division, the length of the telomere shortens, and when a critical shortening is reached, the cell enters senescence or apoptosis.<sup>6, 7</sup> Thus, telomere length is considered a marker of cell senescence and replicative capacity.<sup>6, 8</sup> Leukocyte telomere length (LTL) represents the average telomere length across a heterogeneous population of leukocytes including monocytes, granulocytes and lymphocytes, and can serve as a biological marker of aging.

Decreased regenerative capacity, estimated by circulating levels of progenitor cells (PCs), has been also linked to adverse cardiovascular outcomes.<sup>9–13</sup> Circulating PCs are mononuclear cells that originate primarily from the bone marrow and contribute to vascular repair and regeneration.<sup>14–18</sup> CD34<sup>+</sup> mononuclear cells from the human bone marrow include distinct lineages of both hematopoietic (CD34<sup>+</sup> /CD45<sup>med</sup>) and non-hematopoietic (mesenchymal) progenitors.<sup>19</sup> CD34<sup>+</sup> cells have greater myocardial reparative potential than unselected populations.<sup>20</sup> CD133 is a 5-transmembrane antigen marker of primitive stem cells that is lost during maturation, and cells expressing both markers (CD34<sup>+</sup> /CD133<sup>+</sup>) are thought to be enriched with a vascular PC phenotype.<sup>21, 22</sup> Co-expression of chemokine (C-X-C motif) receptor 4 (CXCR4<sup>+</sup>), which promotes homing of PCs to stromal derived factor-enriched hypoxic environments, may also further characterize PCs with capacity for vascular repair.<sup>23</sup> Although chronological aging is a primary determinant of reduced regenerative capacity, other factors including inflammation, oxidative stress, and genetic predisposition may also influence repair from cellular injury.<sup>24, 25</sup>

Previous studies that have linked LTL to adverse outcomes have postulated that decreased regenerative capacity is a major driver of this effect. However, prior investigations have not specifically addressed this question. Thus, the aim of our study was to examine the association between LTL and PC counts and their mutual and joint relationship with recurrent cardiovascular events and mortality in CAD patients. Our hypothesis was that shorter LTL are associated with lower PC counts and that PCs are a key determinant of the relationship between LTL and adverse cardiovascular outcomes.

## METHODS

### Study population

Patients were enrolled into the Mental Stress Ischemia Prognosis Study (MIPS), a prospective study of patients with stable CAD recruited between June 2011 and August 2014 at Emory University affiliated hospitals. Presence of CAD was defined by an abnormal coronary angiogram demonstrating evidence of atherosclerosis with at least luminal irregularities, documented previous percutaneous or surgical coronary revascularization, documented myocardial infarction (MI), or a positive nuclear stress test. Patients with acute coronary syndromes or decompensated heart failure, and unstable psychiatric conditions other than depression were excluded. Clinical information including previous CAD events, CAD risk factors, results of coronary angiography and current medications were documented using standardized questionnaires and chart reviews. The Emory University Institutional Review Board approved the research protocol, and all participants provided written informed consent. Blood samples were collected after a 12-hour fast.<sup>26</sup> Angiographic CAD severity was calculated using the Gensini score for 490 patients with a median time between the angiogram and enrolment of 2.1 (1.0 – 4.7) years.<sup>27</sup> Adjudicated events (death, MI, coronary revascularization and cerebrovascular accident (CVA)) were ascertained for all subjects after enrollment. Mortality data were collected through follow up clinic visits at 1 and 2 years, phone calls at 3 years, medical records review, and querying the Social Security Death Index. The main outcome of the study was a combined endpoint including death, MI, coronary revascularization and CVA. We also considered a separate endpoint that excluded revascularization to rule out potential bias due to patient referral for coronary procedures.

### Leukocyte telomere length measurement

Genomic DNA samples were extracted from peripheral blood leukocytes, standardized and used for LTL assay following the original method by Cawthon.<sup>8, 28</sup> LTL, measured as the ratio of telomeric product/single copy gene (T/S), was quantified by a high-throughput LTL assay involving quantitative PCR using a serially diluted standard DNA and the standard curve method,<sup>8</sup> as described previously (online Supplement). The T/S ratio reflects the average length of the telomeres across all leukocytes.

### Flow cytometry

Flow cytometry was used for circulating PCs counting as described before.<sup>9</sup> Venous blood was collected via a peripheral vein in EDTA tubes after an overnight fast. Blood samples were processed within 4 hours and incubated with fluorochrome labeled monoclonal antihuman mouse antibodies to identify surface markers expressed on mononuclear cells

before quantification using flow cytometry using a “lyse no-wash” methodology.<sup>9</sup> Mononuclear cells PC that were CD45med and expressed CD34 were enumerated, with subsets of the CD45med, CD34<sup>+</sup> cells, that co-expressed CD133 (stem cell marker) and CXCR4 (homing marker that may direct PC to ischemic tissues) measured. Further details are given in the online Supplement.

### Statistical analyses

Logarithmic base 2 transformations were used for PC counts and LTL. Simple and multiple linear regression models were used to examine the association between PCs and LTL, and to identify factors that were associated with PCs or LTL among several candidate variables, such as age, gender, race, smoking, body mass index (BMI) and history of MI. The Spearman rank correlation and scatter plots were used to test the association between LTL, and PC and Leukocyte levels, respectively. To study the association between LTL, PCs and cardiovascular events, LTL and PCs were examined both as continuous variables and as quartiles using Kaplan-Meier curves and the log-rank test, as well as Cox proportional hazards regression models. LTL and PC were examined both as independent predictors as well as an interaction term in Cox Proportional hazards regression model. The incidence rate of cardiovascular events was calculated and the association with LTL and PC count was examined using chi-squared tests. Statistical analysis was performed using SPSS statistical software (version 23.0; SPSS Inc.).

## RESULTS

Of 695 CAD patients enrolled in MIPS, we had missing PCs for the first 100 patients enrolled, because it was not part of the initial study protocol. This was an ancillary study which begun after MIPS enrollment had already started. Additional patients had missing PCs or LTL because of technical difficulties in sample drawing or processing, or the patient refused. A total of 566 patients had complete data for both LTL and PCs, and were included in this analysis. Patients with missing PC or LTL were less likely to be males or white; there were no other substantial differences in other major demographic or clinical characteristics. The average age was  $63 \pm 9$  years and 76% were male. LTL was normally distributed with a mean  $\pm$  SD of  $0.82 \pm 0.14$  T/S units (Table 1).

### Correlates of LTL

In bivariate analysis, increasing age, male gender, white race, history of smoking and BMI were inversely correlated with LTL (Online Table I). All these factors, except BMI, remained inversely correlated with LTL in a multivariable linear regression model that included CAD risk factors, history of MI, medications (beta blockers, aspirin, and statins), and CAD severity (Gensini angiographic score). There was no statistically significant association between CAD severity and LTL; however, history of MI was associated with shorter LTL in multivariable analysis.

### Correlates of circulating PCs

In bivariate analysis, younger age, male gender, white race and higher BMI were all associated with higher levels of PCs. These factors remained significant in multivariable

regression analysis that included demographic variables, CAD risk factors, history of MI, medications and CAD severity (Online Table II).

### Association between LTL and circulating PCs

Shorter LTL was independently associated with lower PC counts in all subsets, but not other leukocyte counts, including granulocytes, monocytes or lymphocytes (Figure 1, Table 2). For each 10% shorter LTL, PC counts were lower by 5.2%, 8.6%, 5.3% and 9.3 % for CD34<sup>+</sup>, CD34<sup>+</sup>/CD133<sup>+</sup>, CD34<sup>+</sup>/CXCR4<sup>+</sup> and CD34<sup>+</sup>/CD133<sup>+</sup>/CXCR4<sup>+</sup>, respectively. This association remained statistically significant after adjustment for factors associated with LTL and PC counts (age, gender, race, BMI, smoking and previous MI), total white blood cell counts monocyte, neutrophil and lymphocyte counts (Table 2).

### LTL, circulating PCs and outcomes

Patients were followed for a median (IQR) period of 3.0 (2.4 – 3.7) years. A total of 71 patients had adverse events, including 13 deaths, 15 MIs, 48 coronary revascularizations, and 8 cerebrovascular events. In Kaplan Meier analysis, both lower levels of LTL and lower PC counts (CD34<sup>+</sup>, CD34<sup>+</sup>/CD133<sup>+</sup> and CD34<sup>+</sup>/CXCR4<sup>+</sup> cells) were associated with higher rate of adverse cardiovascular events (Figure 2, Online Figure I).

To investigate the interaction between LTL and CD34<sup>+</sup> cell counts, both were dichotomized by the lowest quartile. In bivariate analysis, both LTL<Q1 and CD34<sup>+</sup><Q1 were associated with increased risk of adverse outcomes (Tables 3 and 4). Including both LTL and CD34<sup>+</sup> PCs in one model only slightly weakened their associations and both remained independent predictors of outcome. These associations remained significant after adjustment for age, gender, race, BMI, smoking and previous MI (Table 4). Furthermore, there was an additive effect such that patients with short LTL and low CD34<sup>+</sup> cell counts had the highest risk of adverse outcomes, approximately the sum of the risk for those two factors alone (Figure 3, Table 3 and 4). In adjusted Cox-proportional hazards modeling, the HR for those with both factors, compared to those with neither, was 4.3 (95% CI, 1.7, 10.8), for death, MI, or CVA, and 3.5 (95% CI, 1.7, 7.1) for death, MI, CVA, or revascularizations. However, the interaction between LTL and CD34<sup>+</sup> was not statistically significant (Table 4).

Similar relationships of LTL and other PC counts (CD34<sup>+</sup>/CD133<sup>+</sup>, CD34<sup>+</sup>/CXCR4<sup>+</sup> and CD34<sup>+</sup>/CD133<sup>+</sup>/CXCR4<sup>+</sup>) with cardiovascular events and mortality were observed (Online Figure II).

## DISCUSSION

We found that shorter LTL is associated with lower circulating levels of PCs, independent of age and CAD risk factors. All CD34 expressing PCs that are known to be enriched for hematopoietic and endothelial progenitors were lower in patients with shorter LTL, but similar associations were not present with respect to other leukocyte cell counts. Although there was no statistically significant association between severity of coronary atherosclerosis and either LTL or PC counts, the latter were both significantly associated with future cardiovascular events. Both LTL and PC counts were independent predictors of cardiovascular outcomes. No statistically significant interaction was found between LTL and

PC as binary predictors in the Cox regression model. Our results suggest that accelerated biological aging, reflected by a shorter LTL, is associated with decreased numbers of circulating PC, a measure of reduced regenerative capacity. However, both accelerated aging and reduced regenerative capacity are independent and additive predictors of future cardiovascular endpoints.

The length of telomeres, although in part genetically determined,<sup>29</sup> is thought to be a reflection of the accumulated cellular damage over time, resulting from various mechanical, hemodynamic, metabolic, oxidative and immunological insults.<sup>1, 4, 25, 29, 30</sup> These factors may also affect the replicative capacity of stem cells leading to increase cellular activation and possibly exhaustion.<sup>31</sup> Consistent with this view, several studies have used LTL as a marker of biological aging and have linked it to age-related diseases including atherosclerosis and its consequences,<sup>32</sup> such as peripheral vascular disease,<sup>7, 33</sup> CAD,<sup>34</sup> and importantly, cardiovascular mortality.<sup>2, 32</sup> Since aging is associated with decline in circulating PCs, and atherosclerosis is thought to develop as a result of an imbalance between endogenous repair mechanisms and factors causing cell injury,<sup>35, 36</sup> most of the studies have speculated that these observed clinical associations were related to decreased regenerative capacity. Increased inflammation and oxidative stress, two known key factors in the pathophysiology of atherosclerosis,<sup>37</sup> may result in increased utilization of circulating PCs, which home to the ischemic tissue and denuded endothelium to contribute to vascular and endothelial repair. In turn, this may trigger bone marrow stimulation and hematopoietic PC division and subsequent shorter LTL. Consistent with this hypothesis, Di Cas et al. reported an association between shorter LTL and lower circulating CD34<sup>+</sup> PC counts in a small group of healthy young subjects.<sup>38</sup> In our larger study, we have confirmed and expanded this observation in patients with CAD. Although these findings suggest that LTL might be a determinant of the circulating stem cells pool, it predicts adverse outcomes independent of circulating PCs.<sup>25</sup> Thus, our data indicate that the relationship between LTL and cardiovascular events is only minimally driven by vascular repair processes. Other mechanisms related to biological aging must be at play.

In this study, LTL was significantly correlated with PCs independent of chronological age, which suggests that it may serve as a biological marker of replicative capacity. Others have shown that LTL largely mirrors the telomere length in human bone marrow hematopoietic stem cells that are also the source for circulating PCs.<sup>25, 39</sup> Although LTL represents the average telomere length in a heterogeneous population of leukocytes, there is a robust similarity in the telomere length among different cells. Thus, individuals with relatively short (or long) telomeres in one leukocyte subset have short (or long) telomeres in other leukocyte subsets.<sup>40</sup> Previous reports showed a high correlation between LTL and telomere length of CD34<sup>+</sup> hematopoietic PCs, in both bone marrow<sup>39</sup> and umbilical cord.<sup>40, 41</sup> Thus, shorter LTL is a reflection of similar reductions in telomere length of bone marrow progenitors, implying decreased replicative capacity of hematopoietic stem cells. We did not observe any statistically significant association between LTL and other leukocyte counts, suggesting that shorter LTL is a reflection of bone marrow regenerative capacity rather than global circulating cellular reserve, although this issue remains controversial.<sup>42-48</sup>

Interestingly, we did not find a significant correlation between CAD burden and LTL. This is consistent with findings from the PESA (Progression of Early Subclinical Atherosclerosis) and the Asklepios studies where LTL was not associated with subclinical atherosclerosis.<sup>49, 50</sup> In the Brubeck cohort, LTL was also not associated with subclinical atherosclerosis (non-stenotic carotid atherosclerosis), yet it was associated with development of advanced atherosclerosis (stenotic carotid plaques) and adverse cardiovascular outcomes (vascular death, MI, revascularization and stroke).<sup>51</sup> Our study suggests that, in terms of cardiovascular risk, LTL is a marker of more downstream processes involved in biological aging, rather than the atherosclerotic process in general. Telomere shortening can cause cell senescence, and once a certain threshold is reached, particularly in the setting of inflammation and oxidative stress, such as in presence of CAD plaque or CAD risk factors, LTL shortening may accelerate the decline in circulating PCs. This may lead to less effective endothelial repair, with subsequent increase in inflammation and oxidative stress at the plaque level, which in turn may lead to plaque destabilization and the triggering of cardiovascular events.<sup>52, 53</sup>

The lowest quartile of PCs and LTL had highest risk of cardiovascular events, suggesting threshold effects in injury and repair processes, such that repair mechanisms may fail only when critical levels of oxidative damage face a low pool of PCs. The observed findings could also be related to other factors implicated in the pathophysiology of atherosclerosis, including increased inflammation and oxidative stress.<sup>37</sup> Whether inflammatory and oxidative pathways trigger both biological aging and decline of regenerative capacity, and whether they explain fully the LTL association with adverse cardiovascular outcomes, need to be further investigated.

### Strengths and limitations

Our study is the largest known published study to date investigating the association between LTL and PCs and the only study investigating PCs and LTL in patients with CAD. We used highly accurate assays for both LTL and CD34<sup>+</sup> cell count (coefficient of variation of 2.1% and 2.9%, respectively). We did not assess telomere length in different circulating PCs, which would require cellular isolation with apheresis, or large amount of cells obtained usually by either cell culture or bone marrow stimulation.<sup>39, 54, 55</sup> However, Sakoff et al has previously shown that LTL correlates with the telomere length of CD34<sup>+</sup> cells.<sup>39</sup> We assessed regenerative capacity using circulating CD34<sup>+</sup> cell subsets; however, there may be other PC pools that would also be informative. Furthermore, we did not assess circulating PCs functionality, which would require cell culture and/or other complex methodology which was not feasible in this study. Whether patients with shorter LTL also have decreased circulating PCs function need to be further investigated. We assessed CAD severity using the closest available coronary angiogram by chart review, with a median time between angiogram and enrollment of 2.1 (1.0 – 4.7) years. Thus, angiogram data may not accurately reflect patients' current burden of CAD in our study. As in any study, our measurements carry some degree of error, which, however, is expected to be mostly non-systematic error which would bias the estimates towards the null value. However, the analytical methods we used for our main variables of interest, LTL and PCs, have low coefficient of variation (<10%), and our outcomes were independently adjudicated. Finally, our study included

patients with stable CAD; whether our findings can be generalized to healthy populations need to be further investigated.

### Conclusion and implications

Among patients with stable CAD, LTL is associated with decreased regenerative capacity, independent of age and CAD risk factors. Both LTL and circulating PC levels are independent and incremental predictors of adverse cardiovascular outcomes. Our study suggests that biological aging and regenerative capacity processes, albeit related, index largely independent pathways involved in cardiovascular disease risk.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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### Nonstandard Abbreviations and Acronyms

<b>LTL</b>	Leukocyte telomere length
<b>PC</b>	circulating progenitor cells
<b>MI</b>	Myocardial infarction
<b>CAD</b>	Coronary Artery disease
<b>BMI</b>	Body mass index
<b>CVA</b>	Cerebrovascular accident

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## Novelty and Significance

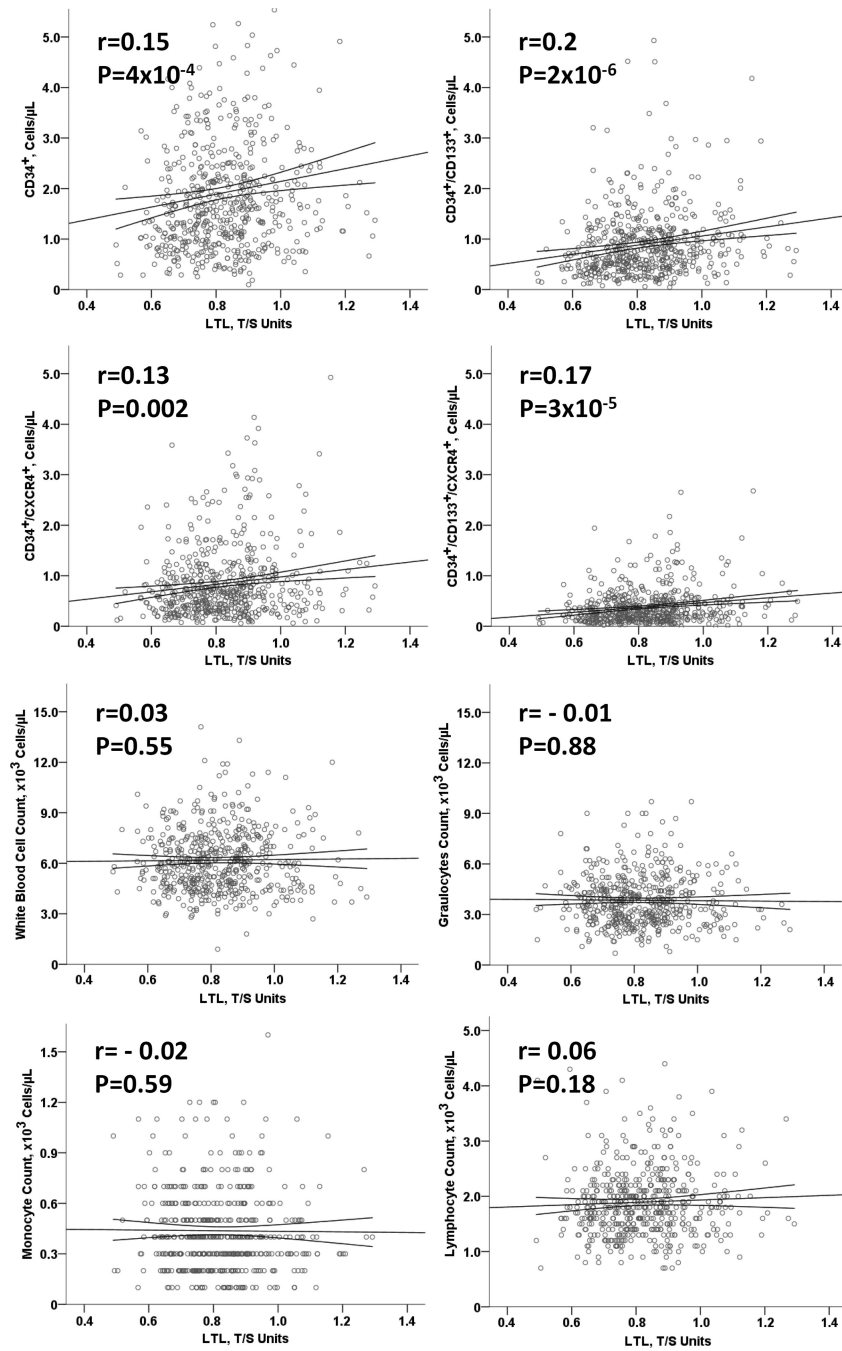
### What Is Known?

- Circulating progenitor cells (PC), promote vascular repair and regeneration and in low levels are linked to adverse cardiovascular outcomes.
- Leukocyte telomere length (LTL) is a biomarker of biological aging that reflects cumulative inflammatory and oxidative cellular injury. Short LTL has been linked to adverse cardiovascular outcomes.
- Previous studies have speculated that the link between biological aging (lower LTL) and adverse cardiovascular outcomes is due to decreased regenerative capacity. However, this hypothesis has not been tested before.

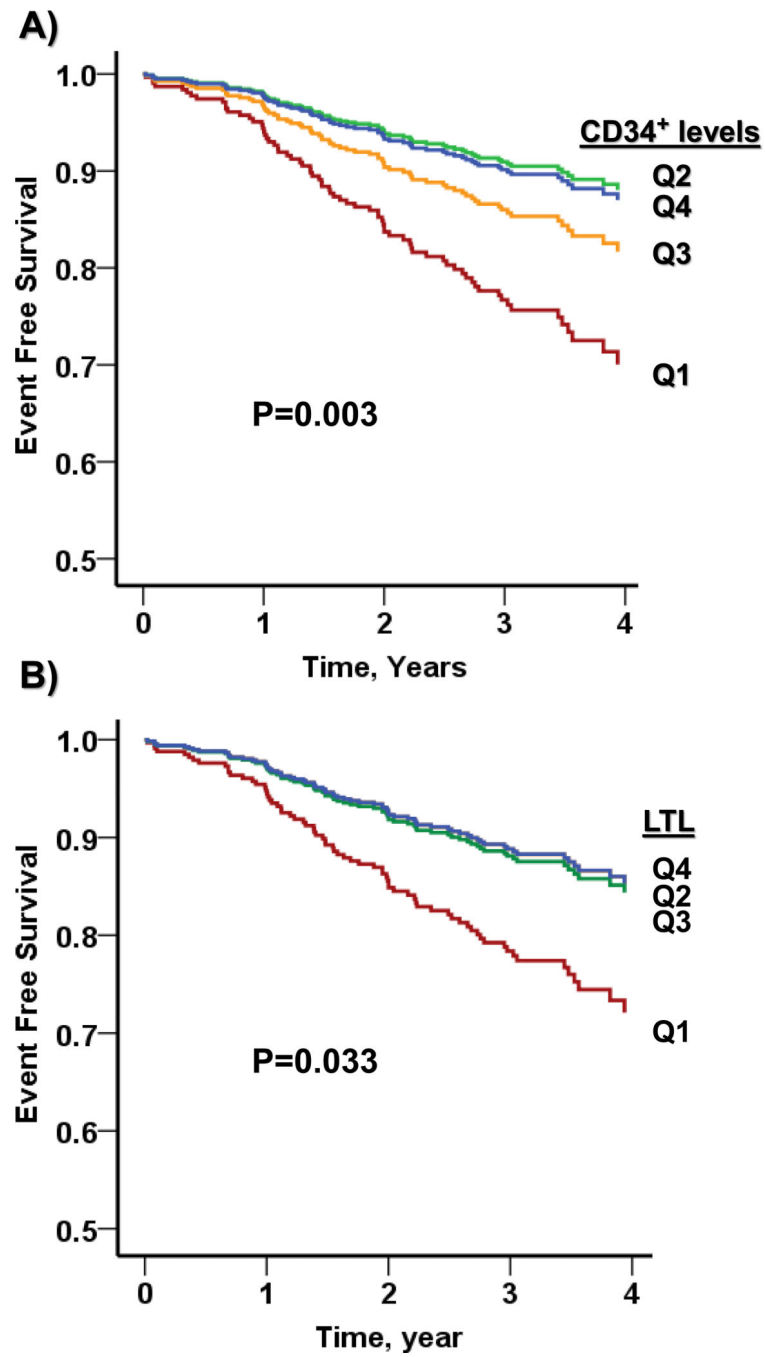
### What New Information Does This Article Contribute?

- Biological aging, measured as LTL, is associated with decreased regenerative capacity, assessed using circulating PCs.
- In relation to adverse cardiovascular events, biological aging and decreased regenerative capacity to be two independent processes, that are additive to each other.

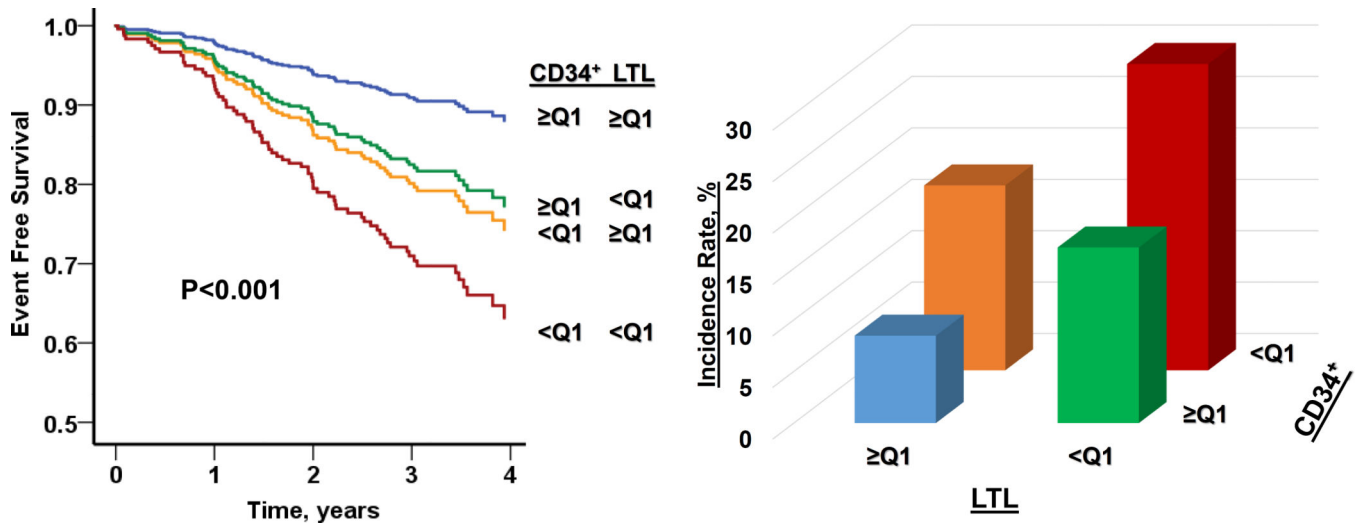
In comparison with chronological aging, biological aging reflects accumulated burden of individual exposure to injurious factors including oxidative stress, inflammation, toxins and environmental factors. Using peripheral blood, we can detect average leukocyte telomere length, which can serve as a biological marker of aging. Shorter leukocyte telomere length predicts adverse cardiovascular outcomes independent of chronological aging. It has been postulated that this effect is due to decreased regenerative capacity, leading to impaired biological repair processes; however, this was not tested directly before. In a cohort of patients with stable coronary artery disease, found a significant correlation between biological aging, measured as leukocyte telomere length, and decreased regenerative capacity, measured as circulating PCs. However, we found biological aging to be independent and additive to decreased regenerative capacity in predicting adverse cardiovascular outcomes. Thus, mechanisms related to biological aging other than vascular regeneration and repair must be at play.



**Figure 1.** Scatter plots showing the association of LTL with PC and Leukocyte levels, respectively, as continuous variables. The graphs show raw data to ease interpretation, with Spearman correlation coefficients and p values.



**Figure 2.** Association of LTL quartiles and CD34<sup>+</sup> cell count quartiles with adverse cardiovascular outcomes. P values were derived from log-rank tests comparing Kaplan Meier survival curves.



**Figure 3.** Additive effect between LTL and CD34+ cell count in predicting cardiovascular outcomes (Death, MI, CVA and revascularization). LTL and CD34+ were dichotomized using the first quartile of the distribution. P values were derived from log-rank tests comparing Kaplan Meier survival curves, and from chi square tests comparing incidence rates.

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**Table 1**

Distribution of LTL, PCs and other characteristics of patients included in the study sample.

	<b>Total</b>
<b>Number of patients</b>	566
<b>Age, year, mean <math>\pm</math> SD</b>	63 $\pm$ 9
<b>Male, %</b>	76
<b>White, %</b>	69
<b>BMI, kg/m<sup>2</sup>, mean <math>\pm</math> SD</b>	30 $\pm$ 5
<b>Hypertension, %</b>	76
<b>Diabetes, %</b>	32
<b>Dyslipidemia, %</b>	82
<b>Current/former smoking, %</b>	60
<b>Previous MI, %</b>	36
<b>Medication use</b>	
<b>ACEI, %</b>	46
<b>ARBs, %</b>	17
<b>Aspirin, %</b>	86
<b>Statins, %</b>	85
<b>Beta blocker, %</b>	76
<b>CAD severity, median (IQR)</b>	24 (9 – 56)
<b>LTL, mean <math>\pm</math> SD</b>	0.82 $\pm$ 0.14
<b><u>CIRCULATING PCS (CELLS/<math>\mu</math>L), MEDIAN (IQR)</u></b>	
<b>CD34<sup>+</sup></b>	1.6 (1 – 2.4)
<b>CD34<sup>+</sup>/CXCR4<sup>+</sup></b>	0.7 (0.5 – 1.1)
<b>CD34<sup>+</sup>/CD133<sup>+</sup></b>	0.7 (0.4 – 1)
<b>CD34<sup>+</sup>/CD133<sup>+</sup>/CXCR4</b>	0.3 (0.2 – 0.5)
<b><u>LEUKOCYTE COUNTS (<math>\times 10^3</math> CELLS/<math>\mu</math>L), MEDIAN (IQR)</u></b>	
<b>White blood cells</b>	6 (4.8 – 7.3)
<b>Monocytes</b>	1.8 (1.5 – 2.2)
<b>Neutrophils</b>	0.4 (0.3 – 0.6)
<b>Lymphocytes</b>	3.7 (2.8 – 4.7)

BMI: Body mass index. ARBs: Angiotensin receptor blockers. ACEI: angiotensin converting enzyme inhibitors; MI: myocardial infarction



**Table 2**

Association between LTL and PCs using linear regression models.

	CD34+		CD34 <sup>+</sup> /CD133 <sup>+</sup>		CD34 <sup>+</sup> /CXCR4 <sup>+</sup>		CD34 <sup>+</sup> /CD133 <sup>+</sup> /CXCR4 <sup>+</sup>	
	*	P value <sup>‡</sup>	*	P value <sup>‡</sup>	*	P value <sup>‡</sup>	*	P value <sup>‡</sup>
<b>Unadjusted</b>	-5.2%	$3.9 \times 10^{-4}$	-8.6%	$7.2 \times 10^{-7}$	-5.3%	0.002	-9.3%	$1.1 \times 10^{-5}$
<b>Adjusted model 1<sup>‡</sup></b>	-5.5%	$4.4 \times 10^{-4}$	-8.3%	$4.7 \times 10^{-6}$	-5.8%	0.002	-9.0%	$6.8 \times 10^{-5}$
<b>Adjusted model 2<sup>§</sup></b>	-5.5%	0.001	-7.7%	$2.5 \times 10^{-5}$	-6.0%	0.002	-8.6%	$1.8 \times 10^{-4}$

\* represents the % change in PCs per 10% decrease in LTL. Linear regression analyses were performed with log base of 2 transformations of LTL and PCs, with PCs as outcomes. was calculated as  $(2^{\beta} - 1) \times 0.10$ .

<sup>‡</sup> P values were derived from linear regression models.

<sup>‡</sup>Model 1: Adjusted for age, gender, race (white vs non-white), BMI, smoking and previous MI.

<sup>§</sup>Model 2: Adjusted for model 1 + absolute monocyte count. P values remained significant when monocyte count was replaced with total leukocyte, lymphocyte, or granulocyte count.

Abbreviations: LTL: Leukocyte telomere length. PCs: Progenitor Cells.

**Table 3**Event rate of adverse cardiovascular outcomes according to LTL and CD34<sup>+</sup> levels.

	Death, MI or CVA (number of events = 36)		Death, MI, CVA or revascularization (number of events = 84)	
	Event rate	P* value	Event rate	P* value
<b>Bivariate analysis</b>				
<i>LTL &lt; Q1 vs Q1</i>	11.1 % vs 4.5 %	0.006	21.5 % vs 10.6 %	0.001
<i>CD34<sup>+</sup> &lt; Q1 vs Q1</i>	11.0 % vs 4.6 %	<0.007	22.1 % vs 10.4 %	0.001
<b>Additive effect</b>				
<i>LTL Q1 &amp; CD34<sup>+</sup> Q1</i>	3.9 %	0.001	8.5 %	<0.001
<i>LTL Q1 &amp; CD34<sup>+</sup> &lt; Q1</i>	6.7 %		18 %	
<i>LTL &lt; Q1 &amp; CD34<sup>+</sup> Q1</i>	6.8 %		17 %	
<i>LTL &lt; Q1 &amp; CD34<sup>+</sup> &lt; Q1</i>	19.1 %		29.8 %	

\* p values were calculated using the Chi Square test.

LTL: leucocyte telomere length. MI: Myocardial infarction, CVA: Cerebrovascular accident. Q1: first quartile

**Table 4**

Multivariable Cox regression analysis of the association of LTL and CD34<sup>+</sup> with adverse cardiovascular outcomes.

Multivariable Cox regression analyses	Death, MI or CVA (number of events = 36)		Death, MI, CVA or revascularization (number of events = 84)	
	HR (95%CI)	P value	HR (95%CI)	P value
<b>Continuous variables*</b>				
<i>LTL (per 10% increase)</i>	0.82 (0.71–0.95)	0.008	0.89 (0.80 – 0.98)	0.018
<i>CD34<sup>+</sup> (per 10% increase)</i>	0.96 (0.93 – 0.99)	0.027	0.97 (0.95–0.99)	0.011
<b>Dichotomous variables</b>				
<b>Bivariate analysis*</b>				
<i>LTL &lt; Q1 vs Q1</i>	2.4 (1.2 – 4.7)	0.013	2.0 (1.3 – 3.2)	0.004
<i>CD34<sup>+</sup> &lt;Q1 vs Q1</i>	2.5 (1.3 – 5.0)	0.008	2.3 (1.4 – 3.6)	0.001
<b>Multivariable Model 1<sup>†</sup></b>				
<i>LTL &lt; Q1 vs Q1</i>	2.1 (1.1 – 4.3)	0.032	1.8 (1.1 – 2.9)	0.017
<i>CD34<sup>+</sup> &lt;Q1 vs Q1</i>	2.3 (1.1 – 4.6)	0.019	2.1 (1.3 – 3.4)	0.003
<b>Multivariable Model 2<sup>‡</sup></b>				
<i>LTL &lt; Q1 vs Q1</i>	2.0 (0.9 – 4.4)	0.067	1.8 (1.04 – 2.9)	0.037
<i>CD34<sup>+</sup> &lt;Q1 vs Q1</i>	2.2 (1.1 – 4.5)	0.030	2.1 (1.3 – 3.5)	0.003
<b>Multivariable Model 3<sup>§</sup></b>				
<i>LTL &lt; Q1 vs Q1</i>	1.7 (0.6 – 4.6)	0.306	2.5 (1.3 – 4.7)	0.005
<i>CD34<sup>+</sup> &lt;Q1 vs Q1</i>	1.6 (0.6 – 4.4)	0.376	2.1 (1.1 – 4)	0.033
<i>Interaction between LTL and CD34<sup>+</sup></i>	1.6 (0.4 – 6.9)	0.519	0.7 (0.3 – 1.8)	0.462

\* LTL and CD34<sup>+</sup> in separate models.

<sup>†</sup> Model 1: CD34<sup>+</sup> cell counts and LTL in same model

<sup>‡</sup> Model 2: Model 1 + age, gender, race (white vs non-white), BMI, smoking and previous MI.

<sup>§</sup> Model 2 + interaction term.

LTL: leucocyte telomere length. MI: Myocardial infarction, CVA: Cerebrovascular accident. Q1: First quartile.