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CLINICAL RESEARCH

Cardiac-Referenced Leukocyte Telomere Length and Outcomes After Cardiovascular Surgery

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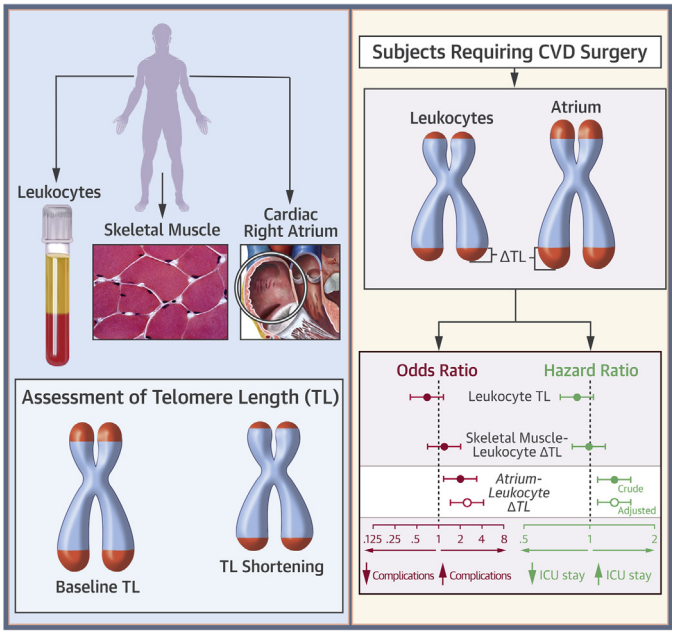
VISUAL ABSTRACT



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HIGHLIGHTS

- **Short leukocyte telomeres have been associated with adverse cardiovascular outcomes in population studies, but this relationship has not translated to patient care. The authors report a telomere length autologous referencing strategy that has the potential to mark biological aging and to identify high-risk individuals.**
- **Among 163 patients who underwent cardiovascular surgery, telomeres in leukocytes and skeletal muscle displayed age-related shortening, whereas the telomere length in the cardiac right atrium was stable during 6 decades of life.**
- **The magnitude of the telomere length gap between cardiac atrial tissue and leukocytes was associated with post-operative complications and length of stay in the intensive care unit.**
- **This study provided proof of concept that a single-time, internally referenced assessment of leukocyte telomere shortening behavior could inform acute risks in patients with cardiovascular disease.**

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ABBREVIATIONS AND ACRONYMS

bp = base pair

CI = confidence interval

HR = hazard ratio

ICU = intensive care unit

OR = odds ratio

PCR = polymerase chain reaction

TL = telomere length

ΔTL^{RA-L} = right atrium-leukocyte TL difference

SUMMARY

Leukocyte telomere shortening reflects stress burdens and has been associated with cardiac events. However, the patient-specific clinical value of telomere assessment remains unknown. Moreover, telomere shortening cannot be inferred from a single telomere length assessment. The authors investigated and developed a novel strategy for gauging leukocyte telomere shortening using autologous cardiac atrial referencing. Using multitissue assessments from 163 patients who underwent cardiovascular surgery, we determined that the cardiac atrium-leukocyte telomere length difference predicted post-operative complexity. This constituted the first evidence that a single-time assessment of telomere dynamics might be salient to acute cardiac care. (J Am Coll Cardiol Basic Trans Science 2018;3:591–600)

Leukocyte telomere length (TL) and leukocyte telomere shortening are 2 genomic DNA attributes that have generated considerable interest because of their relationships with health risks. Leukocyte TL is measured from a single blood sample and has received particular attention in human studies. Several large cross-sectional studies have revealed that adults with short leukocyte telomeres are at increased risk of aging-associated diseases, including coronary artery disease (1–4), heart failure (5), and stroke (2–4). However, despite these associations, the value of knowing an individual's leukocyte TL is unclear, and whether telomere assessment is useful for clinical decision-making is uncertain (6,7).

Leukocyte telomere shortening is an alternative telomere assessment that, in theory, might provide meaningful information for patient care. Leukocyte telomeres shorten in response to replicative and oxidative stresses, and the extent of shortening has been considered to serve as an aggregate indicator of these biological stresses (8–10). Although less studied in humans than leukocyte TL, leukocyte telomere shortening has also been associated with cardiovascular outcomes (11,12). However, leukocyte telomere shortening cannot be inferred from a single leukocyte TL measurement. This is because the major determinant of leukocyte TL is inheritance, which accounts for up to 80% of the TL variation in the population (13–15). In addition, serially measuring TL to ascertain the shortening rate has limited value in the clinical setting. The relatively low leukocyte telomere attrition rate (~20 to 40 base pairs [bps] per year) (2,13,16,17) means that typically 5 to 10 years of follow-up are required to discriminate a change in TL (12,18).

Recently, an alternative approach to assessing leukocyte telomere attrition was proposed based on referencing leukocyte TL to that of a nonreplicating tissue in the same individual (19). A quasi-longitudinal assessment of telomere shortening might thus be made. In the dog, skeletal muscle TL was found to be stable over time, and the difference in TL between skeletal muscle and leukocytes correlated with age better than leukocyte TL alone did (19). Comparing leukocyte and skeletal muscle TL has been termed the "blood-and-muscle model" and has yielded insights into human telomere length dynamics (15,20,21).

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Although conceptually interesting, whether an internal TL referencing approach has the power to inform clinical decision-making is unknown. At a more fundamental level, it is also unclear what the most suitable reference tissue for this purpose might be. Ideally, this would be tissue in which the TL is stable over the course of adulthood. However, there are limited human data on telomere dynamics in tissues other than leukocytes. In addition, although some reports have suggested that human skeletal muscle TL is invariant over time (22,23), recent larger studies have indicated that skeletal muscle telomeres shorten in adults (20,21).

All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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The purpose of this study was to determine if there was a muscle-based strategy for gauging leukocyte TL shortening that was biologically rational and clinically meaningful for individuals with advanced cardiovascular disease. We studied 163 patients who underwent cardiac surgery and measured TL in their leukocytes, skeletal muscle, and cardiac right atrium. We found that, although there was evidence for telomere shortening in adult skeletal muscle, shortening was not evident for telomeres in the right atrium during 6 decades of life. Furthermore, a wide intraindividual difference between right atrium TL and leukocyte TL was associated with a more complicated post-operative course following surgery. The findings raised prospects for a single-time assessment of leukocyte telomere dynamics that could have patient-specific clinical usefulness.

METHODS

STUDY POPULATION AND SAMPLE PREPARATION. Patients who underwent coronary artery bypass grafting, cardiac valve replacement or repair, ascending aortic replacement, or a combination of these procedures were enrolled in the study in accordance with protocols approved by the Institutional Review Board/Research Ethics Committee at The University of Western Ontario. Participants gave written informed consent. Samples from participants enrolled between March 2011 and August 2014 were studied. The samples studied were consecutive, excluding those in which the DNA integrity was insufficient for analysis (identified in 2.5% of subjects). Whole blood samples were obtained preoperatively, and leukocytes were harvested using Histopaque-1077 (Sigma, Oakville, Ontario, Canada) for immediate isolation of genomic DNA. Tissue samples were harvested intraoperatively from trimmed and otherwise discarded fragments of skeletal muscle from the anterior chest wall (suprasternal, supraclavicular, and pectoralis major territories), as well as the right atrial appendage. Tissues were cleaned of adherent adipose tissue and stored at −80°C, within 45 min of surgical resection. A single thaw was undertaken for DNA extraction.

GENOMIC DNA PREPARATION. Genomic DNA was isolated from peripheral blood leukocytes and muscle tissues using the QIAamp DNA mini kit (QIAGEN, Toronto, Ontario, Canada). The integrity of genomic DNA was ascertained by agarose gel electrophoresis. Samples were excluded if there was evidence for loss of integrity (smear >10% of total DNA signal), which was a rare finding that, when present, was exclusively in the muscle tissue. Concentration was determined in a 2-stage procedure, starting with ultraviolet spectrophotometry (NanoDrop Products, Wilmington, Delaware) and then with ultrasensitive fluorescent DNA staining (Quanti-iT PicoGreen dsDNA Assay Kit, Life Technologies, Burlington, Ontario, Canada).

TL MEASUREMENT USING QUANTITATIVE POLYMERASE CHAIN REACTION. TL was measured using quantitative polymerase chain reaction (PCR), based on described methods (24–26). A telomere repeat signal (T) was determined relative to that of the single copy gene, 36B4 (acidic ribosomal phosphoprotein P0, S). PCR was performed in a 10-μl reaction volume containing 5-ng genomic DNA and RT^2 SYBR Green ROX Mastermix (QIAGEN), using an ABI 7500 Real-time PCR system or ViiA 7 Real-time PCR system (ThermoFisher Scientific, Burlington, Ontario, Canada). We used the following primers: GGTTTTT(GAGGGT)$_5$ (T-forward), TCCCGACTA(TCCCTA)$_5$ (T-reverse), CAGCAAGTGGGAAGGTGTAATCC (S-forward), and CCCATTCTATCATCAACGGGTACAA (S-reverse). A standard curve was generated for each run using DNA harvested from a single preparation of HepG2 cells, serially diluted by a factor of 1.68 (0.52 to 19.7 ng/μl).

To control for run-to-run variability, control genomic DNA samples from 7 different cell lines were included in each PCR run (25). Specifically, a single preparation from each of HEK293, HeLa, HT1080, THP1, U87, HITC6 vascular smooth muscle cells that stably expressed hTERT (27,28) and human umbilical vein endothelial cells were used. The respective T/S ratios were divided by the average T/S ratio of that same DNA from 20 PCR runs to yield a normalizing factor. The average normalizing factor from all 7 control DNA preparations was used to correct the patient sample T/S ratios. Patient sample T/S ratios were measured in triplicate in 2 runs, or 3 runs if the first 2 values differed by >15%. The interassay coefficient of variation was 6.3%. Conversion of T/S ratios to absolute length (kilobase pair) was undertaken using DNA oligomer standards for both telomere repeats and 36B4, as described previously (26).

ADVERSE POST-OPERATIVE OUTCOMES. Post-operative complications were ascribed if the patient developed ≥1 of the following: in-hospital mortality, cardiac arrest, life-threatening arrhythmia, need for an intra-aortic balloon pump, myocardial infarction, stroke, delirium, bleeding that necessitated reoperation, new renal failure that required dialysis, respiratory failure, septicemia, or mediastinitis; operational definitions were used for each endpoint as previously reported (29,30). All of these are documented hazards in the post-operative period and

were included as an aggregate endpoint (29). The length of stay in the intensive care unit (ICU) was recorded in days.

STATISTICAL ANALYSIS. The target sample size for this study was based on a post-operative event rate of 15% (29,31) and an assumption that the effect size for telomere shortening on post-operative events would be similar to that of leukocyte TL on mortality (32,33). This yielded a sample size of 119 to detect an odds ratio (OR) of 1.4 per 1 SD change in TL, with a power of 85% and α of 0.05. Descriptive data were expressed as mean $\pm$ SD. Mean tissue TLs were compared using 1-way analysis of variance with the Bonferroni post hoc test. The lengths of telomeres in different tissues within an individual were compared using Pearson's correlation analysis. Relationships between TL and age were evaluated by linear regression analysis. Relationships between TL or ΔTL and sustaining a post-operative complication were evaluated using binary logistic regression analysis. A multivariate logistic regression model was used for non-telomere covariate adjustment (Table 1). Because of the sample size and predicted event rate, 1 covariate was used for multivariate modeling, selected based on the results of univariate analysis. Relationships with lengths of stay in the ICU, excluding 3 patients who died in hospital, were assessed using Cox proportional hazards regression analysis. Risks were adjusted using multivariate proportional hazards regression, and the covariate was selected as per the previously described strategy. The proportional hazard assumption was tested using Schoenfeld residuals, and no violations were observed. Multiple comparisons of different telomere indexes with outcomes were adjusted using the Bonferroni procedure to control the family-wise error rate at 0.05. ICU duration was further assessed in age-stratified tertiles of TL and ΔTL by chi-square analysis. Five age strata were used (<50, 50 to 59, 60 to 69, 70 to 79, and ≥80 years). Statistical analyses were performed using SPSS 19 (IBM Corp., Armonk, New York). All tests were 2-sided.

RESULTS

TELOMERES IN SKELETAL MUSCLE AND RIGHT ATRIUM ARE LONGER THAN THOSE IN CIRCULATING LEUKOCYTES. A total of 163 patients who underwent coronary artery bypass graft, cardiac valve surgery, ascending aortic replacement, or a combination of any of these procedures were enrolled in the study. Demographic, clinical, and operative data are listed in Table 1. No sex differences were identified. The average TLs of peripheral blood leukocytes, chest wall skeletal muscle, and the right atrium are shown in Table 2. Telomeres in skeletal muscle were an average of 77% longer than leukocyte telomeres ($p < 0.001$). Telomeres in the right atrium were 85% longer than leukocyte telomeres ($p < 0.001$).

BIASED SYNCHRONY IN TLs BETWEEN LEUKOCYTE AND MUSCLE TISSUES. There was wide interindividual variation in leukocyte TL, with a coefficient of variation of 24%. The coefficients of variation for skeletal muscle TL and atrial TL were also wide, at 28% and 27%, respectively (Table 2). However, correlation analysis revealed synchrony in TL among tissues from an individual, that is, an individual with relatively long leukocyte telomeres also had relatively long skeletal muscle telomeres and relatively long atrial telomeres, and vice versa (Figures 1A to 1C) ($p < 0.001$). However, there was a systemic deviation from the unity line such that, on an individual basis, leukocyte telomeres were consistently shorter than those in either skeletal muscle (148 of 153 individuals) or the right atrium (128 of 129 individuals). Thus, the differences in TL between the 2 muscle-rich tissues and leukocytes were not simply a feature of the aggregate data but reflected a consistent within-subject phenomenon.

AGE-DEPENDENCY OF LEUKOCYTE AND SKELETAL MUSCLE TL BUT NOT ATRIAL TL. To gauge telomere shortening in the respective tissues, we examined the relationship between TL and age. Figure 2A shows that leukocyte TL was inversely associated with patient age ($p < 0.001$). Regression analysis revealed that age explained 24% of the variation in leukocyte TL. Interestingly, skeletal muscle TL was also inversely related to age ($p < 0.001$), with age explaining 6% of the variability in skeletal muscle TL (Figure 2B). The derived telomere attrition rates were similar for both tissues (28 and 29 bp/year, respectively). In contrast, there was no detectable association between cardiac atrial TL and patient age ($p = 0.24$) (Figure 2C). These findings revealed differences in telomere dynamics among different muscle-rich tissues, with relative stability of TL in the right atrium.

RIGHT ATRIUM-LEUKOCYTE TL DIFFERENCE IS ASSOCIATED WITH THE RISK OF POST-OPERATIVE EVENTS. Because atrial tissue had long telomeres, with little to no evidence for age-related shortening at a tissue level, atrial cell TL might serve as an autologous reference for gauging leukocyte telomere shortening. This, in turn, might be a risk biomarker. Therefore, we next determined whether the right atrium-leukocyte TL difference (ΔTL^{RA-L}) was associated with post-operative outcomes. There were 123

patients in whom TL measurements were obtained from all 3 compartments (Table 3). Leukocyte TL was not found to confer risk for post-operative complications, nor did the skeletal muscle-leukocyte TL difference (Figure 3). Likewise, skeletal muscle TL and right atrial muscle TL did not confer risk for complications ($p = 0.890$ and $p = 0.090$, respectively). However, $\Delta TL^{RA\text{-}L}$ was significantly associated with the occurrence of a post-operative complication. For each SD increment in $\Delta TL^{RA\text{-}L}$, the risk of sustaining ≥1 post-operative complication increased by 106% (OR: 2.06; 95% confidence interval [CI]: 1.20 to 3.56; $p = 0.009$) (Figure 3). The association between $\Delta TL^{RA\text{-}L}$ and adverse post-operative events persisted after adjusting for diabetes (OR: 2.39; 95% CI: 1.33 to 4.28; $p = 0.003$, with a threshold $p = 0.010$) (Figure 3), which was the covariate selected for multivariate analysis based on its association with complications ($p = 0.014$) on univariate analysis.

RIGHT ATRIUM-LEUKOCYTE TL DIFFERENCE IS ASSOCIATED WITH A PROLONGED ICU STAY. The length of stay in the ICU following cardiac surgery is a powerful determinant of long-term survival and functional capacity (34). We therefore analyzed the potential risk conferred by TL indexes on the length of ICU stay using a Cox proportional hazard model. This revealed that neither leukocyte TL or skeletal muscle-leukocyte TL difference conferred a detectable hazard (Figure 4). Skeletal muscle TL and right atrial muscle TL also did not confer hazard ($p = 0.348$ and 0.133, respectively). However, for each SD increase in $\Delta TL^{RA\text{-}L}$, the risk of remaining in the ICU increased by 31% (95% CI: 1.08 to 1.60; $p = 0.007$). A heightened risk persisted after adjusting for operating room time (hazard ratio: 1.31; 95% CI: 1.07 to 1.59, $p = 0.008$). The latter was selected as covariate for multivariate analysis because it was associated with length of stay in ICU on univariate analysis ($p = 0.018$). Consistent with these findings, among subjects in the upper tertile of $\Delta TL^{RA\text{-}L}$, 24% required at least 5 days of ICU care, whereas only 3% and 2% of patients in each of the middle and lower tertiles required this amount of ICU care, respectively ($p < 0.001$). There were no differences in ICU length of stay among tertiles of leukocyte TL ($p = 0.795$).

DISCUSSION

We quantified TL in leukocytes, skeletal muscle, and cardiac atrial tissue in patients who underwent cardiovascular surgery. We determined that: 1) within an individual, telomeres in skeletal muscle and cardiac atrial cells were substantially longer than those in leukocytes; 2) an inverse relationship between TL and age was evident in leukocytes and skeletal muscle, but right atrium TL was stable; and 3) patients with a relatively wide difference between their atrium TL and leukocyte TL were more likely to have a complex post-operative course. These findings provide evidence that an innately referenced, single time-point assessment of leukocyte telomere shortening might be relevant in the acute clinical care setting.

TABLE 1 Demographic, Clinical, and Operative Characteristics of the Study Population

	All (N = 163, 100%)	Men (n = 130, 80%)	Women (n = 33, 20%)
Demographic and clinical features			
Age, yrs (range)	62 ± 15 (30–89)	62 ± 15 (30–89)	64 ± 16 (30–85)
Caucasian	144 (88)	114 (88)	30 (91)
Obesity (BMI ≥30 kg/m^2)	57 (35)	42 (32)	15 (46)
Smoking	79 (49)	66 (51)	13 (39)
Atrial fibrillation	42 (26)	34 (26)	8 (24)
Diabetes	36 (22)	31 (24)	5 (15)
Comorbidities other than diabetes	68 (42)	56 (43)	12 (36)
Recent (30 days) myocardial infarction	8 (5)	7 (6)	1 (3)
Cerebrovascular disease	16 (10)	14 (11)	2 (6)
Congestive heart failure	14 (9)	12 (9)	2 (6)
Peripheral vascular disease	26 (16)	22 (17)	4 (12)
Chronic obstructive pulmonary disease	23 (14)	20 (15)	3 (9)
Renal failure requiring dialysis	3 (2)	2 (2)	1 (3)
Re-operation	4 (2)	3 (2)	1 (3)
Operative features			
Surgical procedure			
CABG	68 (42)	57 (44)	11 (33)
Valve	25 (15)	17 (13)	8 (24)
CABG and valve	6 (4)	4 (3)	2 (6)
Aortic	64 (39)	52 (40)	12 (36)
Operating room time, h (interquartile range)	4.2 (3.3–6.1)	4.4 (3.3–6.1)	4.1 (3.1–5.8)
Urgent	28 (17)	21 (16)	7 (21)

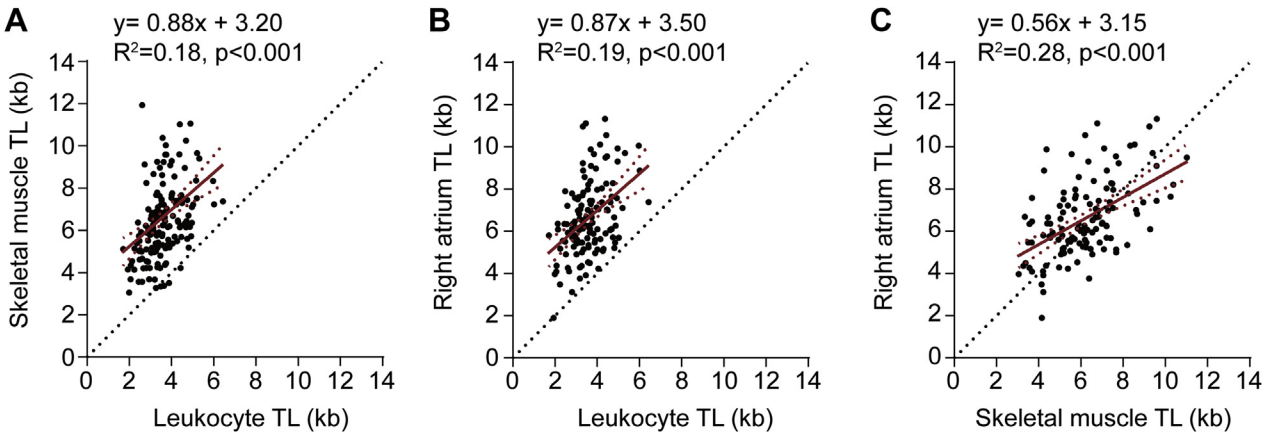
Values are n, mean ± SD (range), or n (%).
BMI = body mass index; CABG = coronary artery bypass grafting.

TABLE 2 Telomere Length Measurements of Study Population

	All (N = 163, 100%)	Men (n = 130, 80%)	Women (n = 33, 20%)
Age, yrs	62 ± 15 (30–89)	62 ± 15 (30–89)	64 ± 16 (30–85)
TL, kb			
Leukocyte	3.58 ± 0.86 (162)	3.61 ± 0.83 (129)	3.46 ± 0.98 (33)
Skeletal muscle	6.35 ± 1.78 (154)	6.35 ± 1.82 (121)	6.37 ± 1.64 (33)
Right atrium	6.62 ± 1.76 (129)	6.56 ± 1.84 (103)	6.86 ± 1.42 (26)
ΔTL, kb			
Skeletal muscle − leukocyte	2.77 ± 1.62 (153)	2.73 ± 1.66 (120)	2.92 ± 1.47 (33)
Right atrium − leukocyte	3.04 ± 1.59 (129)	2.94 ± 1.69 (103)	3.41 ± 1.07 (26)

Values are mean ± SD (range) or mean ± SD (n).
kb = kilobase; TL = telomere length; ΔTL = change in TL.

FIGURE 1 **TL Synchrony Among Leukocytes, Skeletal Muscle, and Right Atrium in Individuals Who Underwent Cardiovascular Surgery**

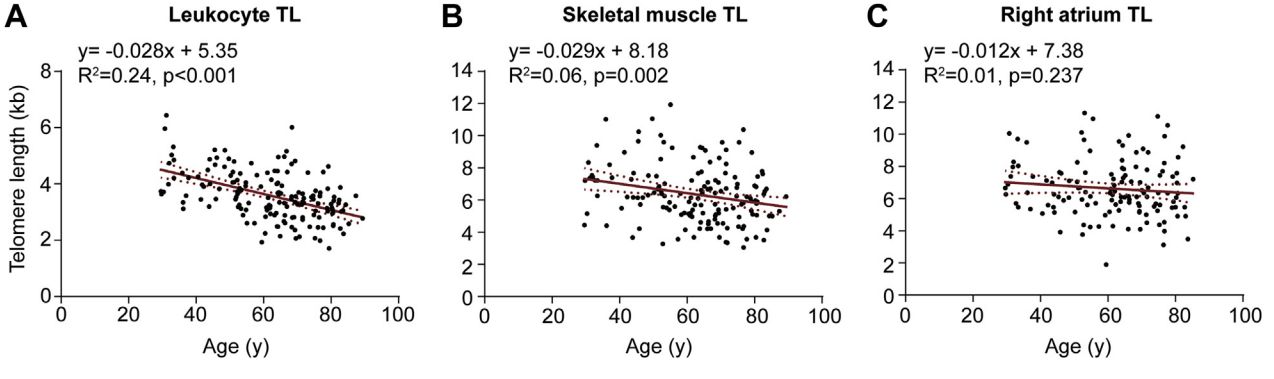


Plots showing the intraindividual relationships between **(A)** skeletal muscle telomere length (TL) and leukocyte TL, **(B)** right atrium TL and leukocyte TL, and **(C)** right atrium TL and skeletal muscle TL in patients who underwent cardiovascular surgery.

The leukocyte telomere attrition rate of ~30 bp/year that we established is in keeping with several large studies (2,13,16). Our finding of similar shortening in skeletal muscle was noteworthy because it supported an emerging reconsideration of telomere dynamics in this "post-mitotic" tissue. Notwithstanding the longer average TL of skeletal muscle, which was likely attributable to developmental program differences with bone marrow cells, the telomere shortening pointed to ongoing skeletal muscle turnover and progenitor cell activity in this adult tissue (20).

In contrast to skeletal muscle, we did not find evidence for age-associated telomere shortening in the cardiac atrium. The explanation for this difference was speculative but might reflect lower progenitor cell activity in adult atria. Recent carbon-14 dating studies showed that cardiomyocyte turnover was substantially less than that of skeletal muscle cells (35,36). It was also possible that emergence of noncardiomyocyte cells with inherently longer telomeres could counterbalance telomere shortening from cell turnover. In this regard, it is noteworthy that 26% of subjects had a history of atrial fibrillation, a condition associated with atrial fibrosis. Although the TL of atrial fibroblasts versus cardiomyocytes is unknown, fibroblast-like cells in liver were found to have longer telomeres than parenchymal cells (37). As well, a recent study of patients with atrial

FIGURE 2 **TL Dynamics in Leukocytes, Skeletal Muscle, and the Right Atrium**



Plots showing a relationship between **(A)** leukocyte TL and age and **(B)** skeletal muscle TL and age, **(C)** but not the right atrium TL and age. Abbreviation as in Figure 1.

fibrillation did not find an association between atrial TL and age (38). Regardless of the exact atrial dynamics, our findings empirically established that the net effect of the cellular events within the adult human atria was a stable TL during 6 decades of life. The present data suggested that this stability, in turn, could provide a framework for internally referencing leukocyte TL, so that that the interindividual variability in TL due to inheritance is partially accounted for.

Recent data from Sabharwal et al. (15) indicated that a difference between skeletal muscle TL and leukocyte TL arises developmentally and in early life (15). A similar situation might exist for cardiac muscle. Accordingly, in the present cohort of patients with cardiovascular disease, the $\Delta TL^{RA\text{-}L}$ might reflect a TL gap in early life plus widening of this gap by ongoing leukocyte TL shortening from replicative and oxidative stresses imposed during adulthood. Thus, assessing the $\Delta TL^{RA\text{-}L}$ might be analogous, albeit not quantitatively identical, to measuring leukocyte telomere shortening from serial TL assessments, a measurement that has been associated with cardiovascular events and mortality (11,12). Importantly, the autologous comparison approach tested here obviated the need for extended-term serial measurements and, as such, might be more salient to clinical care.

Individuals with a wide $\Delta TL^{RA\text{-}L}$ were more likely to have complications following open-heart surgery. This finding constituted the first evidence that a telomere-based measurement was associated with the early response to an intervention. It was noteworthy that adverse post-operative events were not associated with leukocyte TL, which suggested that information conveyed by the atrial-referenced leukocyte TL was different from that conveyed by leukocyte TL itself. An association between the atrial-leukocyte TL difference and clinical care outcomes was further supported by the relationship with length of stay in the ICU. Complications leading to a prolonged stay in the ICU could effectively abrogate the benefits of cardiac surgery, and the ICU length of stay was strongly related to reduced survival and long-term functional decline (34,39). Notably, only the atrium-leukocyte TL difference and the duration of the surgical procedure independently predicted this key endpoint. The latter is a well-recognized predictor of ICU duration and is related to the technically imposed stresses of the prolonged procedures. The telomere shortening index, in contrast, is a measure of previous biologically imposed stresses and thereby captures a different risk dimension.

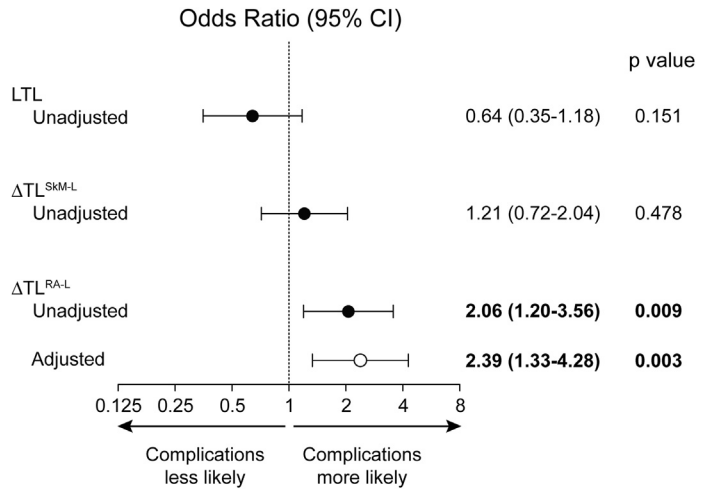
TABLE 3 Adverse Post-Operative Outcomes and Length of Stay in the ICU

	All (N = 123, 100%)	Men (n = 97, 79%)	Women (n = 26, 21%)
Post-operative complications	15 (12)	11 (11)	4 (15)
In-hospital mortality	3 (2)	1 (1)	2 (8)
Cardiac arrest/life-threatening arrhythmia	4 (3)	2 (2)	2 (8)
Intraaortic balloon pump use	1 (1)	1 (1)	0 (0)
Myocardial infarction	1 (1)	1 (1)	0 (0)
Stroke and/or delirium	10 (8)	8 (8)	2 (8)
Reoperation for bleeding	4 (3)	3 (3)	1 (4)
New renal failure requiring dialysis	1 (1)	1 (1)	0 (0)
Respiratory failure	5 (4)	2 (2)	3 (12)
Septicemia	0 (0)	0 (0)	0 (0)
Mediastinitis	2 (2)	1 (1)	1 (4)
Length of stay in ICU, days, median, (interquartile range, min-max)	1 (1–2, 1–32)	1 (1–2, 1–32)	2 (1–3, 1–19)

Values are n (%), unless otherwise noted.
ICU = intensive care unit.

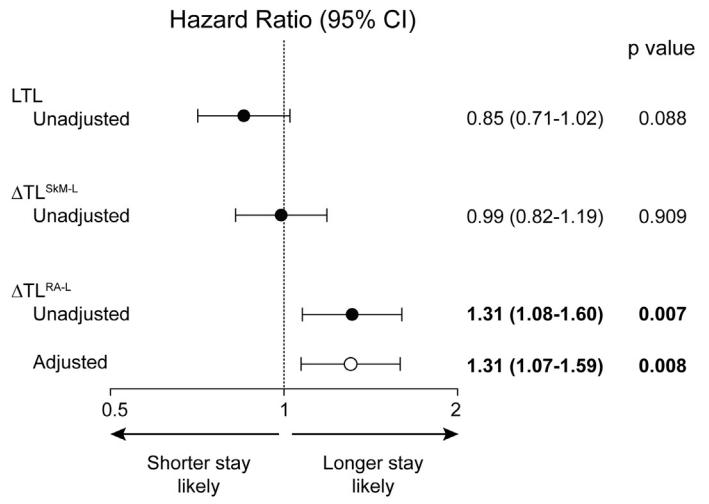
Our report had several strengths. We undertook multitissue TL measurements and were the first to compare TL among different muscle-rich tissues in an individual. The telomere measurement strategy used had a small interassay coefficient of variation, and we used a multiple cell line design that controlled for run-to-run variability. These features

FIGURE 3 Risk of Post-Operative Complications Following Cardiovascular Surgery According to TL and Intraindividual Telomere Length Differences



Odds ratios for developing a post-operative complication per SD in each of leukocyte TL, the difference between skeletal muscle and leukocyte TL ($\Delta TL^{SkM\text{-}L}$), and the difference between right atrium and leukocyte TL ($\Delta TL^{RA\text{-}L}$). **Solid circles** = unadjusted data. **Open circles** = data adjusted for diabetes. The threshold p value was 0.010. CI = confidence interval; LTL = leukocyte TL; other abbreviation as in Figure 1.

FIGURE 4 Risk of Remaining in the ICU Following Cardiovascular Surgery According to TL and Intraindividual TL Differences



Hazard ratios for remaining in the intensive care unit (ICU) for each SD increment in TL or TL (ΔTL). **Solid circles** = unadjusted data. **Open circles** = data adjusted for operating room time. The threshold p value was 0.010. Abbreviations as in **Figures 1 and 3**.

enabled reliable TL comparisons between tissues and across the study population. As well, this was the first report to investigate a linkage between telomere dynamics and acute outcomes in a high-risk population. In this regard, $\Delta TL^{RA\text{-}L}$ might carry conceptually unique risk factor information. There are established predictors of outcomes following cardiovascular surgery, including chronological age, diabetes, and smoking. Although $\Delta TL^{RA\text{-}L}$ did not delineate a specific risk factor, it did report the impact of risk factors, particularly those that imposed replicative and oxidative burdens. In other words, the telomere shortening index afforded by $\Delta TL^{RA\text{-}L}$ might serve as an innate, patient-specific readout of the consequences of risk factor loading, and thus the ability of an individual to withstand interventions or other additional stresses. That is, $\Delta TL^{RA\text{-}L}$ might serve as an indicator of biological aging and thus patient resilience to new insults.

STUDY LIMITATIONS. Our study was a single-site study. Although there are several large biobanks of leukocyte DNA from clinically followed individuals, this is not the case for skeletal muscle or cardiac DNA. Accordingly, this was a proof-of-concept study. It will be important to assess the value of atrial TL referencing in other cohorts and in larger samples with more post-operative events, to ascertain whether it might be a predictive tool with incremental value over current predictive strategies. It is also possible that a larger cohort may uncover some age-associated atrial telomere attrition. However, the fact that telomere attrition was readily identified in skeletal muscle, but not in the atria from the same individuals, points to the relative stability of atrial TL.

Although we did not identify an association between leukocyte TL and post-operative events, this study did not negate the potential value of leukocyte TL as a risk biomarker or as a potentially causal determinant of cardiovascular disease (14,40,41). In this regard, a recent study identified short telomeres, but not telomere attrition, as being associated with carotid atherosclerosis (42). Thus, there may be distinctions between leukocyte TL and telomere attrition with respect to clinical associations. Finally, we recognize that pre-emptively harvesting cardiac tissue is not feasible in most instances. It is nonetheless possible that longer term management of patients who have undergone cardiac surgery may be informed by this index. In this regard, the present cohort will be followed for an extended period. Importantly, the present findings also open the potential for assessing other approaches, such as right ventricular endomyocardial biopsy, which might increase opportunities for clinical usefulness.

CONCLUSIONS

We found that relating leukocyte TL to cardiac atrial TL constituted a biologically rational strategy for gauging leukocyte telomere shortening. The findings raise the possibility of using a personalized index of telomere dynamics to inform care.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Ascertaining biological age is important to cardiac risk assessment. An autologous referencing approach to gauging leukocyte telomere shortening may have an important advantage over assessing only leukocyte TL because it is not confounded by the inherited TL. Moreover, it obviates the need for serial TL measurement, which requires several years. Our study is the first to report that a single-time, referenced assessment of telomere dynamics may be salient to acute care of individuals with advanced cardiovascular disease.

TRANSLATIONAL OUTLOOK: Strategies to evaluate leukocyte telomere attrition by internal referencing may identify high-risk individuals. If similar strategies can be adapted pre-operatively, the approach may allow for identification of risk subgroups to guide cardiac surgical decisions. This proof-of-concept study also sets the stage for ascertaining if long-term adverse effects are predicted by the cardiac leukocyte TL difference.

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