Studying telomeres in a longitudinal population based study

Tim De Meyer1, Ernst R. Rietzschel2, Marc L. De Buyzere2, Wim Van Criekinge1, Sofie Bekaert1

1 Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, 2 Department of Cardiovascular Diseases, Ghent University Hospital, Ghent, Belgium

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1. ABSTRACT

Telomeres, the termini of linear chromosomes, consist of large but variable numbers of DNA oligomer repeats embedded in a nucleoprotein complex. In humans, telomere length (TL) is largely genetically determined but also featured by an age dependent attrition. TL has therefore been put forward as a marker for biological aging and was also reported to be associated with aging diseases such as cardiovascular disease. However it remains unclear whether the biomarker value in a particular disease depends on shorter TL at birth or rather if it’s a mere reflection of an accelerated telomere attrition during lifetime, or else, if it is a combination of both. While the importance of telomere attrition is supported by cross-sectional evidence associating shorter telomeres with oxidative stress and inflammation, longitudinal studies are required to accurately assess telomere attrition and its presumed link with accelerated aging. In this review we present different models for the biomarker value of TL and discuss the theoretical and methodological considerations of studying TL in a longitudinal population study with a special emphasis on cardiovascular disease.

2. INTRODUCTION

Telomeres are the nucleoprotein structures constituting the physical ends of linear chromosomes (Figure 1). Functional telomeres are distinguished from double-strand breaks, thereby playing an important role in the preservation of chromosomal integrity in mammals (1). The concept of telomeres functioning as a biological clock was first acknowledged at the cellular level. Olovnikov’s prediction that the nature of DNA replication implies progressive telomere attrition, the so-called end-replication problem, could be experimentally verified in vitro and provided a cellular mechanism to register the number of divisions (2,3). In normal somatic cells, progressive telomere attrition leads to critically short telomeres and replicative senescence, a state characterized by the absence of replication and biochemical changes (4). This explains the earlier observation that most cultured cells could only undergo a limited number of population doublings, the Hayflick limit (5).

However, the telomere attrition rate is not only determined by the division rate of proliferating cells. The
repair of single strand breaks associated with oxidative stress is less efficient in telomeres than in centromeric DNA, resulting in an incomplete telomeric DNA replication and an accelerated telomere attrition (6,7,8). Thus, telomere attrition is further modulated by both oxidative stress and the cellular antioxidant capacity (9,10), and, most probably, also by other factors such as further degradation of one specific telomere strand (11).

At the organismal level, the impact of TL on the complex aging process, whether or not reciprocal, is primarily assessed through cross-sectional epidemiological studies. In most cases peripheral blood leukocyte (PBL) TL is used as a systemic TL measure, supported by the observation that TL is to a large extent conserved among different tissues (12,13). At first sight, human systemic TL dynamics reflects the underlying processes observed in vitro: in a population there is a clear negative association between TL and the subject’s calendar age (14,15). Furthermore, also in vivo TL appears to be shorter in subjects with increased levels of oxidative stress and inflammation (16,17,18,19,20). These observations and the plenty reported associations between shorter TL and aging diseases led to the presumption that PBL TL is also in vivo a systemic marker of biological aging, above and beyond calendar age.

However, the nature of the biomarker value of TL in systemic aging remains unclear. Where an aging phenotype might be closely associated with the TL of the aging cells in situ, it remains uncertain that these local events are accurately seized by a systemic index of aging as PBL TL. Furthermore, the causal relationship between critically shortened telomeres and replicative senescence at a cellular level does not necessarily hold at the organismal level. It is at least as likely that the biological processes underlying the aging phenotype also affect, as a confounding factor, the systemic TL. Increased levels of oxidative stress and inflammation are most probably prototypes of these confounding factors.

Where a causal relationship suggests that the aging phenotype emerges from a, predominantly genetically determined, baseline TL, the confounding factor theory implies that accelerated aging would be reflected in an increased telomere attrition rate. These two theories are not necessarily mutually exclusive: in the case where the baseline TL is responsible for an aging phenotype, it will be the telomere attrition rate which determines at which point in life the critically short TL will be reached and the phenotype emerges. Thus, measuring telomere attrition rates is absolutely necessary to evaluate the biomarker value of systemic TL per se and to quantify the impact of accelerated telomere shortening.

The assessment of telomere attrition rates and their impact on an aging population requires large-scale longitudinal studies. In this review we will further elaborate the different possible roles of (systemic) TL as a biomarker, the motivation for a longitudinal approach and discuss the theoretical and methodological considerations. We pay special attention to the link between TL and cardiovascular disease (CVD), as this is a typical aging disease in which the biomarker value of TL has been intensively studied. This is also within the scope of the Asklepios study, a large scale longitudinal population study (N>2500) of which the first round has been completed and the first follow-up round is being prepared. The study focuses on the interplay between aging, cardiovascular hemodynamics and inflammation in CVD (21).

In the near future, this study will, as the first/one of the firsts, provide the scientific community with longitudinal telomere attrition rates for a large population (21,20).

**3. TL AS A MITOTIC CLOCK**

The current paradigm of telomere function is that telomeres act as a mitotic clock, counting the number of cell divisions, and initiating replicative senescence when one or few telomeres are critically short after a certain number of cell divisions. As tumor development requires a large series of cell divisions to accumulate the adequate number of relevant mutations or analogous functional alterations (e.g. DNA methylation), replicative senescence has been reported as a putative tumor suppressor mechanism (3,14,22,23).

However, this protective mechanism comes at a cost. From an evolutionary point of view, selection for the ideal tumor suppressor mechanism (i.e. optimal TL) only occurs during, roughly, the reproductive phase in life. As nowadays most humans surpass this point in life, TL could indeed become a limiting factor. Longer telomeres at birth might then indeed increase longevity, but would also increase the risk of tumor development earlier in life (23).

Cells can be rescued from critical telomere shortening, and the natural machinery to avoid senescence and gain virtually eternal life is utilized in normal germ and stem cells, and abused in most tumors: activation of telomerase (24,25,26,27). This telomere elongating...
enzyme, consisting of a protein-RNA complex (25,28), counteracts telomere attrition, but is not or insufficiently expressed in normal somatic cells, resulting in a net TL loss in these cells (26). The pharmacological activation of telomerase could be an appropriate therapy for aging diseases associated with replicative senescence (29). On the other hand, telomerase inhibitors have been proposed as a new option for chemotherapy (30), illustrating the trade-off between accelerated biological aging and increased cancer risk.

4. MAJOR TL DETERMINANTS IN A POPULATION

In humans, TL is to a very large extent genetically determined, with inheritance estimates roughly ranging from 40% up to 80% (31,32,33,34). Several putative loci affecting TL have been identified using quantitative-trait linkage analysis (32,33). While originally X-linked inheritance was proposed (35), current evidence suggests that TL is inherited paternally (34,36).

After birth, baseline TL gradually declines with calendar age (14,15). In children, the telomere attrition rate appears to be higher than in adults, most likely due to a differential regulation of hematopoietic telomere biology (15,37). Although equal at birth (13), adult men generally have shorter telomeres than their female counterparts (38), which can be explained by the observed higher telomere attrition rate in men (20,35,39). Conclusions about telomere attrition rates were however (almost) all inferred from cross-sectional data, which implies that the precision of the measurements was largely affected by the inherited interindividual TL differences. Longitudinal studies will avoid this problem and result in an improved understanding of the age and gender dependency of telomere attrition.

One hypothesis links the gender difference in TL with the gender difference in body size and the gender gap in life expectancy. This hypothesis states that, as men are generally taller than women, more cell divisions are required to obtain and maintain their body size. This results in a shorter TL in men, creating also a possible explanation for the lower life expectancy in men (40). Since telomerase activity is higher and heavily regulated during development (41), particularly postnatal weight gain will be important. This theory complies with the Barker hypothesis, stating that lower birth weight will predispose children to aging diseases and CVD in particular due to the growth catch-up in early childhood (42). While the latter hypothesis appears to be correct (43), the involvement of telomeres is doubtful. Although children aged approximately 5 years old with a short birth weight indeed appear to have shorter telomeres (44), cross-sectional results show no or only limited association between TL and body size later in life, in any case insufficient to explain the difference in TL between men and women or to link this difference with the gender difference in lifespan (20).

Surprisingly, next to inheritance, age and gender, paternal age at birth was recently identified as important TL determinant (34,39), with an even more important effect than gender (45). Children of older fathers appear to have longer telomeres, which is in line with the earlier finding that sperm cells’ TL increases with donor age (4). Interestingly, this paternal age effect is incorporated in the offspring’s genetically determined TL, and will be further transmitted through general inheritance. This might be a major source of the variability in inherited TL (39,45). As paternal age is subject to demographic evolution, TL might fluctuate between different generations, again complicating telomere attrition estimation based on cross-sectional data.

5. TL AS AN AGING BIOMARKER

5.1. Origins of biomarker value

The in vitro results on telomere attrition and cellular senescence and their consequence, namely telomere shortening with increasing calendar age, all support an aging biomarker role for TL. This is further strengthened by the faster shortening in men compared to women, mimicking the gender difference in longevity (46). This has led to numerous studies investigating possible associations between TL and aging diseases.

The role of TL in aging diseases might depend both on baseline TL at birth and on the telomere attrition rate during lifetime. While the first is predominantly genetically determined, the telomere attrition rate most likely depends on genetics, lifestyle and their interplay (e.g. in oxidative stress). This leads to several possible scenarios explaining the biomarker role of systemic TL in aging diseases, with, possibly, different diseases requiring different models. Here it is important to recognize the difference between the organismal or systemic TL, as measured e.g. in PBL, and the focal TL: in the tissue(s) under study.

5.1.1. Telomere attrition

In a first scenario, the aging phenotype is associated, whether or not causally, with oxidative stress, inflammation, or other telomere attrition modulating phenomena, but not with senescence itself. If these processes act on a systemic level with an impact which is large enough, this might result in a shorter systemic TL being associated with the aging phenotype. An observation in favor of this mechanism was proposed to be responsible for the shorter systemic TL found in subjects with osteoarthritis, most probably due to the involvement of oxidative stress and low-level chronic inflammation (47).

5.1.2. Baseline TL

In a second option, short baseline TL at birth results in early replicative senescence, causing an aging phenotype without an altered telomere attrition rate. In this model, subjects are predisposed to an aging disease emerging only later in life. In this case, the high synchrony between the TLs of different tissues at birth (13) suggests a systemic marker as PBL-TL to be a good biomarker for the shorter focal TL.

Although this would reflect a causal relationship between longevity and baseline TL at birth, reports on such cases are rare. For a large part, this can be attributed to the fact that it is practically impossible to exclude the role of
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telomere attrition without longitudinal data. In aging studies, measuring TL at birth will improve our understanding of the impact of baseline TL. Since the aging phenotype under study only appears much later in life, this information is generally not available which in turn complicates the analysis.

One piece of indirect evidence for the occurrence of this mechanism are the shorter telomeres found in women of whom the father had a shorter lifespan (34). If the TL in daughters is predominantly genetically determined (31,32,33), particularly via the father (34,36), the daughters’ TL partially reflects their fathers’ baseline TL. Under the assumption that no confounding factors exist, which is uncertain (e.g. paternal age at birth), this finding implies shorter TL at baseline to be causally associated with lifespan. This interpretation is further complicated by the lack of association for all parent-offspring pairs.

Other evidence for this mechanism is found in dyskeratosis congenita, an inherited bone marrow failure syndrome associated with very short telomeres (48,49,50). In autosomal dominant dyskeratosis congenita, this is caused by a mutation in TERC, the RNA component of telomerase (51). It is proposed that in this subtype, preshortened telomeres are inherited, predisposing the offspring to the development of dyskeratosis congenita (49,52). This is supported by the fact that in dyskeratosis congenita patients no age-dependent telomere attrition is observed (49,50), most probably due to the fact that a critical limit has already been reached and cells with shorter TLs continuously are being selected against (49). Another example where telomerase mutations might play a causal role is pulmonary fibrosis (53).

5.1.3. Baseline TL and TL attrition rate

Finally, the aging phenotype might be associated with both TL at birth and accelerated telomere attrition. As in the previous scenario, this suggests a causal relationship between the aging phenotype and replicative senescence. The TL at birth then composes the buffer capacity and determines, together with the telomere attrition rate (and residual telomerase activity), at which point in lifetime critically short telomeres appear. The relative importance of TL at birth is determined by the rate of the increased telomere attrition.

Causal relationships between systemic PBL-TL shortening and senescence in specific (non PBL) tissues cannot be inferred. However, if a causal relationship between aging phenotypes and focal replicative senescence has been established, systemic TL still can be a valuable biomarker, upon the condition that the systemic TL attrition accurately reflects the telomere attrition of the cells in the tissue under study. No studies performing this kind of analysis have yet been reported, probably due to the complexity of the problem.

Concluding, since an altered telomere attrition rate results in different baseline TLs, only a longitudinal study will be able to attribute the proper importance to baseline TL and/or TL attrition rate, and therefore elucidate the telomere dynamics and consequently the specific biomarker role of TL in the aging phenotype under study. In the next section, we review the association between TL and CVD in the perspective of these models.

5.2. TL as a biomarker in CVD

5.2.1. Cross-sectional evidence associating systemic TL with CVD

A first, clear, indication for the *in vivo* association between systemic TL and CVD was found in small pilot study showing shorter telomeres in subjects with severe coronary artery disease (54). This association was further supported by studies showing shorter systemic telomeres in subjects with myocardial infarction (19,55), chronic heart failure (56), carotid atherosclerosis combined with hypertension (57,58), stroke (19) and degenerative aortic valve stenosis (59).

However, the link between telomeres and CVD appears to be modulated by age, for example, in the elderly (ca. over 70 years old) no significant associations between TL and respectively coronary disease (59), myocardial infarction and stroke (19) were found. On the other hand, heart disease associated survival was poorer in subjects with shorter TL aged above 60 (60) and shorter TL was also associated with left ventricular function in a group of 85 year old (61). Confirmation of the biomarker value of TL in CVD came from a large, prospective study, where shorter telomeres at baseline resulted in an increased risk for coronary heart disease at follow-up in subjects without statin treatment (62).

5.2.2. Focal TL and replicative senescence in CVD

Although this vast body of evidence suggests a biomarker role for systemic TL in CVD, the mechanism underlying this association is still unclear. Evidence for the involvement of focal replicative senescence in atherosclerosis was provided by the observation that replicative senescence was present in a large part of the vascular endothelial cells in the atherosclerotic lesions from coronary (but not mammary) arteries from subjects with ischemic heart diseases (63). This result complies with the shorter telomeres found in coronary endothelial cells from subjects with coronary artery disease compared to normal subjects. Note that the overlap between TL in diseased and non-diseased subjects was minimal. Furthermore, in the coronary artery disease subjects, the telomeres were also distinctly shorter at atherosclerotic sites compared to non-atherosclerotic sites (64).

Endothelial dysfunction has been recognized as a key event in CVD (65), suggesting that critical focal telomere shortening is causally, or at least closely, associated with CVD. This conclusion implies that focal TL, most probably also accurately reflected in systemic TL at birth, might be an important factor in CVD.

5.2.3. Focal telomere attrition in CVD

The arterial endothelial cells can mainly be found in the intima, the inner arterial layer which is in direct contact with the blood flow. In the intima, an age
dependent telomere attrition was observed, which was higher in arterial regions characterized by higher hemodynamic stress and increased cell turnover (66,67). This might also be the case in the media, the tissue underlying the intima (67), although this was not confirmed in another study (66). Vascular smooth muscle cells, characterized by proliferation and migration from the media towards the intima in the atherosclerotic process, exhibited a marked telomere shortening and senescence in atherosclerotic plaques compared to normal vessels (68).

These results suggest that (differential) focal telomere attrition plays a role in the etiology of CVD, affecting the point in life where critical telomere shortening, probably associated with CVD, is encountered. The biomarker value of systemic TL then totally depends on the manner in which it reflects the focal processes. Since TL is most probably synchronous between PBL and the specific vascular tissues at birth (13), this requirement is brought down to the question to which extent systemic TL attrition mimics focal TL attrition.

5.2.4. Systemic TL attrition in CVD

Systemic TL has been associated with many CVD risk factors, for example, shorter telomeres were shown for subjects smoking (17,35), obese individuals (17), subjects with increased blood pressure (16,19,38,69), increased insulin resistance (16,18,19,70,71) and diabetes (19,71,72). Since most of these factors are, at least partially, life style related, this implies altered telomere attrition rates. It has to be stated that several of the associations mentioned could not be validated in other studies, even when sufficiently powered. Since in most cases only cross-sectional data were available, these inconsistencies might be explained by the fact that the effect of telomere attrition was observed, of which the estimation largely depends on the cross-sectional study design (cf infra).

The question arises if the association between systemic TL and CVD might not be attributed to solely these known risk factors, which are then the confounders, instead of mimicking the focal effect of shorter telomeres. This is supported by the observation that the degree by which the telomeres for CVD patients are shorter compared to controls is much smaller for systemic telomeres than for those in the arterial tissues, where a clear distinction was observed, e.g. (63,64).

However, the biomarker value of systemic TL in CVD appears to be, at least partially, independent of CVD risk factors (62). Furthermore, atherosclerosis is considered an inflammatory disease, with invasion of certain peripheral leukocytes into the atherosclerotic lesions (73), physically linking systemic PBL TL with CVD. This is further supported by the fact that inflammatory and the closely associated oxidative stress markers, direct modulators or telomere attrition and CVD risk factors (74), have been associated with shorter systemic telomeres (16,18,19,20). Yet, the oxidative stress and inflammation markers are only point estimates, complicating the associations between oxidative stress/inflammation and respectively CVD and baseline TL. In contrast, telomere attrition might surpass these short-term measurements and reflect the cumulative effect of oxidative stress and inflammation between the TL measurements (20) (Figure 2).

Under the condition that inflammation and/or oxidative stress (possibly combined with other attrition modulating factors) play important roles in both CVD and TL dynamics, systemic TL attrition might become a very valuable biomarker in CVD. Furthermore, since the degree of telomere attrition is probably large and variable between subjects, systemic TL attrition might even play a more important role than systemic baseline TL itself: systemic telomere attrition partially mimics telomere attrition in the vascular tissue, but the discrepancy between both results in an increasing deviation between baseline systemic and baseline focal TL (Figure 3). A full evaluation of the biomarker values of systemic TL and attrition thus clearly awaits longitudinal studies.

6. TL IN LONGITUDINAL STUDIES

6.1. Theoretical considerations

6.1.1. Comparison with cross-sectional studies

Throughout this review, we illustrated the importance of obtaining longitudinal telomere attrition rate...
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Figure 3. Theoretical scheme of systemic (black) and focal (red) TL for a healthy subject (o, −−) and a subject (x, −−) developing atherosclerosis. Telomere attrition is larger for the atherosclerotic subject (both systemic and focal) than for the healthy individual, but the final systemic TL is still shorter in the healthy individual. While the differences between systemic and focal telomere attrition rates remain constant, the discrepancies between systemic and focal baseline TLs increase.

An alternative methodology to increase the power of a telomere attrition study, based on cross-sectional data, is to increase the age-span of the subjects under study (77). Although no large-scale longitudinal TL studies have been reported so far, smaller studies demonstrate the advantages of a longitudinal approach. The major advantage is of course that fewer subjects are necessary to obtain more precise results on telomere attrition rates. For example, in a baboon study, the rapid telomere decline in the year after birth followed by a more moderate telomere shortening could be clearly shown for each of the four animals under study (76). In a cross-sectional study more baboons were required to deduct a similar conclusion since particularly the inherited heterogeneity in TL complicated the analysis (78). Several other studies also demonstrated the increased power of longitudinal TL studies, as they were capable of obtaining firm conclusions using only a limited number of subjects (70,79,80,81). It has been estimated that the sample size required to discriminate between attrition rates is approximately five times smaller for the longitudinal approach than for the cross-sectional analysis (77).

Factors affecting TL during lifetime, e.g. oxidative stress, will have larger, cumulative, effects on TL with increasing age of the subjects, and hence, these effects might be easier to detect in older populations. It is yet worth mentioning that a survival bias might occur as a consequence of the earlier death of subjects with, in the case of the example, increased oxidative stress (77). Indeed, if TL and oxidative stress are both associated with longevity, the surviving subjects under oxidative stress will have longer telomeres than their deceased counterparts, which, as a consequence, decreases the power to detect the impact of oxidative stress on TL.

6.1.2. Theoretical considerations in longitudinal studies

Problems arising in longitudinal TL studies are to a large extent those encountered in longitudinal studies in general. The appropriate study design will ideally result in the proper elimination of the different sources of bias and an increased power compared to cross-sectional studies. However, several issues have to be kept in mind.

In longitudinal studies, one of the major problems is the attrition of the study participants, which might result in a severe source of bias, e.g. a survival bias. If accelerated telomere attrition in se is associated with decreased survival through the aging phenotype under study, its effect will be underestimated in the same way as in cross-sectional studies. The choice of the age-range of the study population will further modulate this possible survival bias.

One critical choice to make in the longitudinal design is the time interval between the different rounds, as the error on an individual’s telomere attrition rate estimate (expressed in standard deviations) is under the appropriate statistical conditions proportional to the multiplicative inverse of the measurement interval (e.g. in years). This implies that an increasing interval will yield a strong decrease in error for relatively short intervals (e.g. <5 years). For longer intervals (e.g. >8 years) this decrease becomes negligible. Therefore it is appropriate to take a measurement interval which is large enough. On the other hand, intervals which are too large will have several disadvantages. The most important drawback is the fact that, for each individual, several variables might vary largely and non-linearly between the measurements, complicating inference from the repeated measurements towards the whole measurement interval.

6.2. Methodological considerations

As the available quantities of DNA are often limited and intended for multiple purposes, the method of analysis should be well considered. Southern blot based analysis (82), characterized by a digestion step by nontelomere cutting restriction enzymes, electrophoretic separation of the fragments on gel, Southern blotting and visualization, still remains the gold standard. Several new methods, particularly quantitative PCR, are emerging. The main reasons for the increasing popularity of quantitative PCR are the reduced cost, quantity of required DNA and the high-throughput approach in which the experiments can be performed (83,84). These advantages are obviously utterly important in large scale studies.

Drawbacks of the PCR method are that only a mean, relative TL value is obtained, instead of a TL distribution as with Southern blot analysis and that a
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calibration curve is required to derive absolute TLs, amplifying measurement errors. Other methods have been developed (85,86), and, as several among those are being further optimized for high-throughput analysis, they might yield more attention in future epidemiological research. Important examples are flow cytometry and (or combined with) quantitative FISH (87,88,89,90).

Independent of the methodology, much attention should be given towards standardization and uniformity. In longitudinal studies, standardization not only implies a fixed, robust protocol, but also a well-considered manner to perform the repeated experiments. In retrospective studies, the repeated measurements can easily be performed in a same batch. In prospective studies, the requirement of fast scientific output will regularly lead to a cross-sectional analysis after the first round has been completed. Ideally, after each subsequent round, the previous measurement should be repeated in a same batch as the corresponding new one to increase the precision of the telomere attrition rate estimate.

Nevertheless, limitation of funding and/or sample quantity (DNA) might make this practically infeasible and result in separate analysis batches of the repeated measurements and consequently an increased error due to the inter-batch variance. Although inferior, standardization can then be performed in the same manner as in cross-sectional analysis. One option is to use control samples, which is regularly done in Southern blot based analysis, for example by using stable cell-lines (91). Another possibility is to include a batch adjustment in the subsequent statistical analysis, e.g. (34). A possible problem with this alternative is that the batch size should be large enough to keep the lost amount of functional variation to a strict minimum. Take into account that, even when the repeated measurements are performed within a same batch, one of these last two options is required when batch effects would occur in the analysis of telomere attrition rates.

Automatisation is another requirement to increase the feasibility of a large-scale longitudinal study. Since the practical implementation is heavily dependent on the methodology, here, we illustrate this with an example from the Asklepios study, using the Southern blot based analysis. In a last step of the analysis, telomere smears are visualized, normally accompanied by several molecular weight markers. We developed a software tool in Matlab to process the more than 200 images from the Asklepios study semi-automatically. Telomere smears and molecular weight markers are automatically detected and can be corrected manually if required. Then the tool fits a normal distribution on each of the profiles, with exclusion of the subtelomeric fragments which are sometimes visible on the lower part of the blot (92). This approach avoids the influence of the subtelomeric fragments on TL estimates and is more robust to small noise fluctuations than the simple use of the maximal intensity point. The mean of each fitted profile is then transformed to the corresponding molecular weight using a molecular weight marker based transformation. This kind of automatisation greatly facilitates the processing of the large quantities of raw experimental data.

7. PERSPECTIVE

In this review, we emphasized the importance of obtaining telomere attrition rate estimates through longitudinal studies. This approach should elucidate if and to what extent the biomarker value of systemic TL is determined by telomere attrition. Several scenarios are possible, all giving more or less attention to the baseline TL and attrition rates, but all are dependent on longitudinal studies.

The first large-scale longitudinal TL studies, such as the Asklepios study (21), will result in telomere attrition values. At that point it will become clear, if, and to what extent, telomere attrition is associated with oxidative stress, inflammation, and novel factors. It will also become possible to link telomere attrition with CVD and other aging phenotypes. This will increase our understanding of aging and its determinants.

Independent of the specific biomarker role of TL in aging diseases, other hypotheses can be tested using longitudinally derived telomere attrition rates. Accurately derived attrition rates might elucidate the origin of the gender difference in telomere attrition. Since telomere attrition indicates cell turnover, it will become possible to monitor this process during the aging process. Finally, these studies will allow us to test the hypothesis that the systemic telomere attrition rate is a biomarker for the rate of systemic, organismal aging.

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Abbreviations: TL: Telomere Length, PBL: Peripheral Blood Leukocytes, CVD: Cardiovascular Disease

Key Words: Telomeres, Telomere Attrition, Longitudinal Studies, Biological Aging, Epidemiology, Cardiovascular Disease, Asklepios Study, Review

Send correspondence to: Tim De Meyer, Dept. Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium, Tel: 32-9-264-9922, Fax: 32-9-264-6219, E-mail: Tim.DeMeyer@UGent.be

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