# Telomere length as a predictive biomarker in osteoporosis (Review)

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**Abstract.** Telomeres are the ends of chromosomes that protect them from DNA damage. There is evidence to suggest that telomere shortening appears with advanced age. Since aging is a significant risk factor for developing age-related complications, it is plausible that telomere shortening may be involved in the development of osteoporosis. The present review summarizes the potential of telomere shortening as a biomarker for detecting the onset of osteoporosis. For the purposes of the present review, the following scientific databases were searched for relevant articles: PubMed/NCBI, Cochrane Library of Systematic Reviews, Scopus, Embase and Google Scholar. The present review includes randomized and non-randomized controlled studies and case series involving humans, irrespective of the time of their publication. In six out of the 11 included studies providing data on humans, there was at least a weak association between telomere length and osteoporosis, with the remaining studies exhibiting no such association. As a result, telomere shortening may be used as a biomarker or as part of a panel of biomarkers for tracking the onset and progression of osteoporosis.

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*Abbreviations:* DDR, DNA damage response; t-loop, telomere-loop; TRF-1, telomeric repeat binding factor 1; TRF-2, telomeric repeat binding factor 2; TIN-2, TRF-1 interacting nuclear protein 2; POT-1, telomeric overhang binding protein 1; TL, telomere length; AFAR, American Federation for Aging Research; U.S.; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; OPG, osteoprotegerin; ROMO, romosozumab; BMD, bone mineral density; LTL, leukocyte telomere length; TRF, telomere restriction fragment; bps, base pairs; HIV, human immunodeficiency virus; OR, odds ratio; HR, hazard ratio; SD, standard deviation; CRP, C-reactive protein; qPCR, quantitative polymerase chain reaction; qFISH, quantitative fluorescence *in situ* hybridization; PNA, peptide nucleic acid; ; WS, Werner syndrome; Terc, telomerase RNA component

*Key words:* osteoporosis, telomere length, predictive biomarker, aging disorders

## Contents



## 1. Introduction

The primary function of telomeres is to prevent the destruction of the genome from the DNA damage response (DDR), thus ensuring genomic stability during DNA replication (1). Structurally, the ends of telomeres are single-stranded, whereas telomeres are double-stranded at all their length (2). The numerous repetitive sequences of the hexanucleotide 5'-TTAGGG-3' are organized in a complex, three-dimensional lariat-like structure known as the telomere-loop (t-loop) (3). When the 3' end of a single DNA strand of telomeres is inserted into a double-stranded duplex of telomeres, the formation of a

D-loop occurs (4). In the t-loop structure, 3' single-stranded G-rich overhangs, known as G-overhangs, protrude from the double-stranded telomeric region (5). The organization of telomeres in forming such looped structures is crucial and protects them from degradation. This t-loop conformation requires the presence of specific telomeric interacting proteins, such as telomeric repeat binding factor 1 (TRF-1), telomeric repeat binding factor 2 (TRF-2), TRF-1 interacting nuclear protein 2 (TIN-2), telomeric overhang binding protein 1 (POT-1), TIN-2 and POT-1 interacting protein 1 and repressor-activator protein 1, that stabilize the t-loop (2). As mentioned earlier, telomere-binding proteins comprise the 'shelterin' protein complex (6). Telomere shortening arises from incomplete lagging strand DNA synthesis, resulting in single-stranded overhangs. During the aging process, telomeres are progressively shortened below a certain threshold due to each cell division, known as the 'end-replication problem' (6). For that reason, it has been demonstrated that telomere shortening is associated with advanced aging (7) (Fig. 1). Accordingly, the genetic rescue of telomerase can compensate for premature aging in telomerase-deficient mice (8). Similarly, nutraceutical supplements can sustain the telomere length (TL) at a greater extent in females than males (9). Additionally, nutraceutical formulations can attenuate aging by increasing the physical action of aged animals (10).

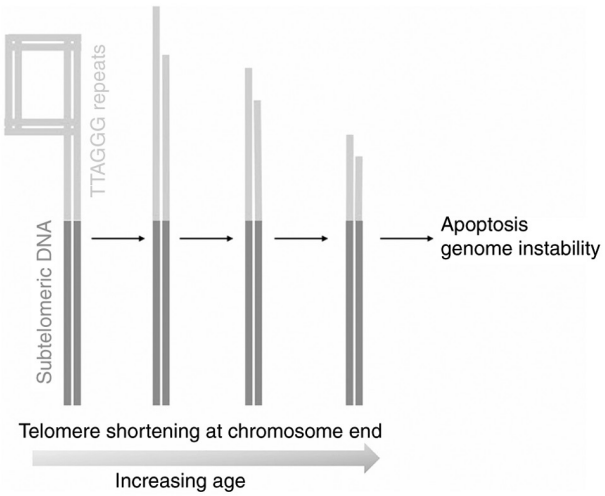


Figure 1. Telomere length and age. In younger somatic cells, telomeres have long tracts of telomere repeats with closed ends. Through cell division, the number of short ends increases and can enhance DNA damage signals, along with apoptosis and genomic instability.

Aging arises from multiple mechanisms, such as cellular senescence, genome instability, metabolic dysfunction, mitochondrial deterioration, microbial dysbiosis and sustained low inflammation, epigenetic alterations including DNA methylation, stem cell exhaustion, disturbed cellular communication, proteasomal degradation or dysregulated autophagy and telomere shortening (11). According to the American Federation for Aging Research (AFAR) (12), the hallmarks of aging have been defined based on the following criteria: i) The prediction of life expectancy than natural chronologic age; ii) be subject to experiments to elucidate characteristics that accelerate aging; iii) be subject to investigations to shed light on mechanisms underlying the prevention of aging; iv) be minimally invasive without harming individuals (13). The compliance of TL to the criteria noted by the AFAR defines telomere shortening as a reliable biomarker of aging (14,15).

Notably, telomere shortening represents an essential hallmark of the aging process, and it is accelerated in age-related disorders (7). For example, short telomeres have been observed in leukocytes of osteoporotic patients compared to long ones of controls, whereas long telomeres have been reported in females with low osteoporosis risk (16). In agreement with the aforementioned findings, it has been demonstrated that aged osteocytes that were senescent through a high p16 expression were associated with bone loss (17).

Osteoporosis is an aging-related disease of bone metabolism, is prevalent among the elderly, and at least 200 million cases worldwide have been reported (18). The characteristic features of osteoporosis are an increased vulnerability to bone fractures and high fragility due to low bone mass and deterioration of bone microstructure (19). Osteoporosis imposes a significant economic burden in the aging society, necessitating the identification of markers that stratify individuals to osteoporosis (20). Post-menopausal women are mainly exposed to bone fractures, such as vertebral and hip fractures (21). In Europe and the USA, >30% of women and 20% of men >50 years of age have osteoporosis (22). At the same time, it is also estimated that >40% of post-menopausal women and 30% of men can experience a fracture related to osteoporosis during their lifetimes (23,24).

A complete understanding of the mechanisms underlying osteoporosis is crucial in order to develop effective therapeutic strategies to attenuate its progression. Among the factors determining bone loss are low estrogen levels in post-menopausal women and low testosterone levels in men, resulting in osteoporosis (25). Estrogen-deprivation-related osteoporosis observed in post-menopausal women appears to differ from age-related osteoporosis (26,27). However, estrogen is not the only factor that contributes to bone loss during aging (28); bone homeostasis can also be affected by parathyroid hormone, vitamin D (cholecalciferol), calcitonin and corticosteroids (29) (Fig. 2).

Bone remodeling has been reported as a key parameter for determining the onset and progression of osteoporosis. In bone remodeling, osteoclasts and osteoblasts are implicated in orchestrating bone architecture through the degradation of old bone (bone resorption) and the formation of new bone (bone formation), respectively (30). Previous research has analyzed the signaling pathways that regulate the balance between osteoclastic bone resorption and osteoblastic bone formation. Such signaling pathways are the following: Receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) and canonical Wnt (31). For example, OPG prevents the binding of RANKL to the RANK receptor, thus preventing osteoclast function (27) (Fig. 3). Currently, several pharmaceutical agents, including bisphosphonates, estrogens, the selective estrogen receptor modulator, raloxifene, the human monoclonal antibody, denosumab, and the recombinant human parathormone, 1-34 teriparatide, are commonly used to treat osteoporosis effectively (18) (Fig. 3). Recently, romosozumab (ROMO), an approved monoclonal antibody directed against sclerostin in several countries for the treatment of osteoporosis in post-menopausal women who are at a

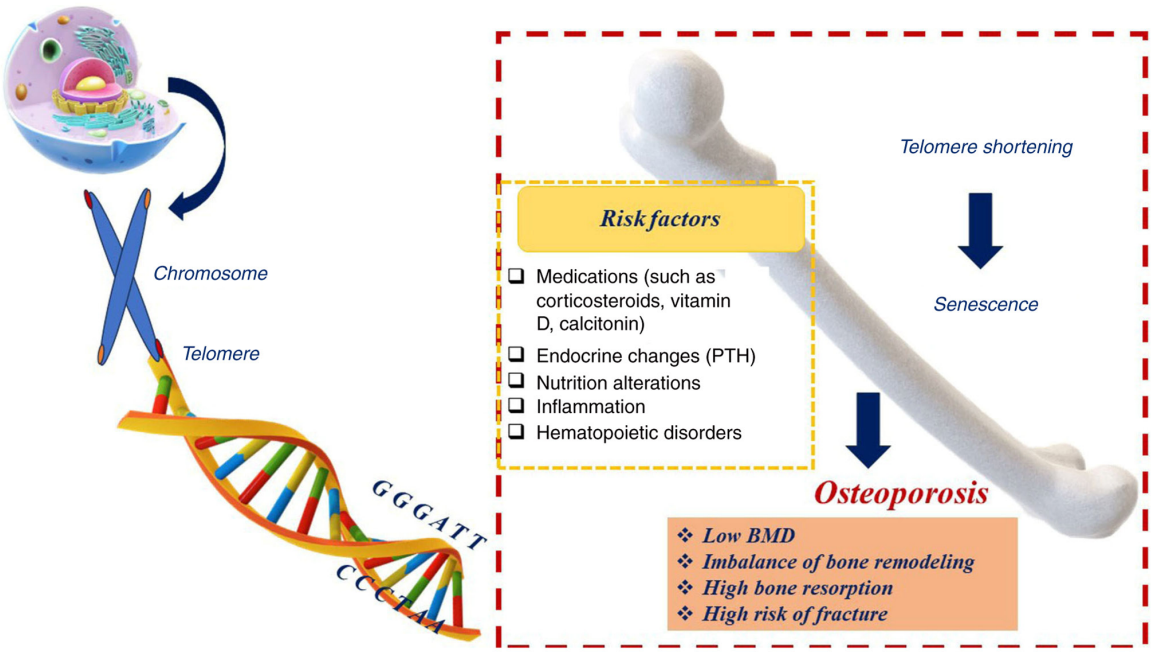


Figure 2. Since osteoporosis is a highly multifactorial entity, it may be related to multiple risk factors. Multiple factors, such as PTH, vitamin D (cholecalciferol), calcitonin, corticosteroids, estrogen, inflammation, lifestyle and hematopoietic alterations promote telomere shortening, which increases the risk of developing osteoporosis. BMD, bone mineral density; PTH, parathyroid hormone.

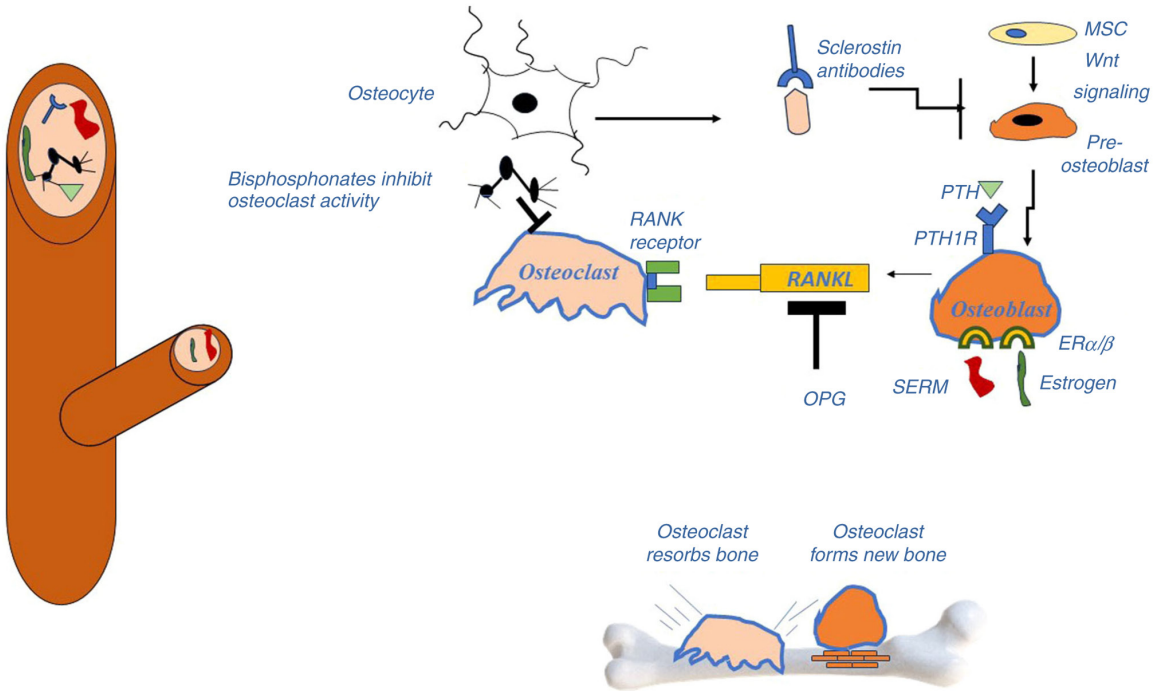


Figure 3. Bone is a tissue that consists of diverse cell types (osteoblasts, osteoclasts) that interact in a complex manner. In particular, bone marrow-derived stem cells differentiate into osteoblasts, which in turn are converted into osteocytes following their incorporation into the mineralized matrix. As cells age, they undergo apoptosis or senescence. An imbalance between osteoclasts and osteoblasts may result in the development of fragile porous bone. RANKL is required for osteoclastogenesis, whereas OPG hinders the osteoclast route. Sclerostin, the Wnt pathway, SERM and estrogen are key factors involved in the differentiation of MSCs into osteoblasts. When sclerostin antibodies are present owing to their production by osteocytes, the Wnt signaling pathway renders osteoblastogenesis inactive. ER, estrogen receptor; MSC, mesenchymal stem cell; OPG, osteoprotegerin; PTH, parathyroid hormone; RANKL, receptor activator of nuclear factor-κB ligand; SERM, selective estrogen receptor modulator.

high risk of fractures, has attracted significant attention owing to its ability to reinforce bone formation. At the same time, it can attenuate bone resorption after its short-term use (32,33). The underlying molecular mechanism of ROMO relies on the induction of the Wnt signaling pathway since the action of ROMO is directed to prevent sclerostin-a glycoprotein,

which exerts inhibitory effects on osteoblasts and further induces bone resorption (32,33). Following the review of three-phase clinical trials, researchers have previously evaluated the superior effect of ROMO compared with teriparatide in post-menopausal women with osteoporosis who were at a high risk of fractures and they were previously treated with bisphosphonate therapy, thereby causing bone remodeling and increasing bone mineral density (BMD) (34). Despite therapeutic options, their clinical efficacy is hindered due to the emerging side-effects after their long-term use, making it urgent to identify novel and effective therapeutic interventions against osteoporosis (35). In this direction, further investigations are required to elucidate the possible cardiovascular events after using ROMO in postmenopausal women with a risk of high fractures (33).

Since telomere shortening is considered a molecular hallmark of aging (11), a summary of the state of the field provides clues that telomere shortening can be a driver for the onset and progression of osteoporosis. In the present review, a discussion was performed of the current research data on the possible association of TL with osteoporosis in humans.

## 2. Clinical studies regarding telomere length and osteoporosis

Initially, it was generally questionable whether TL can contribute independently to aging as a biomarker (36-38). To answer that question, a number of studies have demonstrated that TL can fulfill the main prerequisites defined by the AFAR. Epidemiological and clinical studies have provided evidence that telomere shortening is the most widely and reliable biomarker that characterizes the aging process (15,39). Numerous studies have illustrated a strong association between different biomarkers and the onset of aging; current aging markers are not without drawbacks. Indeed, other features have been employed to evaluate various aspects of the aging process since the mechanism underlying the aging process in humans is incredibly complicated.

Apart from the crucial involvement of telomere shortening in aging, biomarkers of aging are usually used for evaluating the progression of age-related disorders, such as osteoporosis (40). Identifying preventive strategies against osteoporosis requires precise and reliable biomarkers for assessing the rate of osteoporosis. Over the past decades, age-related telomere shortening has attracted increasing attention in research as one of the most promising fields.

The literature search performed herein resulted in the isolation of relevant published articles; there were three non-randomized-controlled studies and eight longitudinal cohort studies. The main findings of the review are summarized in Table I.

Initially, Bekaert *et al* (41) performed an early observational study comprising 352 elderly healthy male subjects (aged 71-86 years). They found that the mean leukocyte TL (LTL) was reduced with an advanced age. The age-corrected TL was positively associated with bone loss, as confirmed by biochemical analysis of bone turnover and BMD at different distal forearm sites, including the mid-region of the forearm, ultra-distal forearm and the total forearm (41). Furthermore, they elucidated the use of mean LTL as a predictive marker for bone loss, according to the sex steroid status of elderly healthy male individuals (41). For that purpose, telomere restriction fragment (TRF) length analysis was performed along with various examinations of testosterone, estradiol and sex hormone-binding globulin in the blood of males (41). The results proved that the short length of telomere restriction fragment determined bone loss at various sites of their radius and ulna ($P<0.05$), which was inversely associated with the age of the participants ($P=0.049$), irrespective of the hormonal status of healthy older adults (41). Furthermore, aged individuals with bone loss had a mean TRF length 423 base pairs (bps), shorter than that of age-matched controls without bone loss, given that the telomere erosion rate in leukocytes appears to be shortened by 23 bps per year (42).

Based on experiments revealing a tight association between telomere shortening and the senescence of osteoblasts *in vitro* (43,44), a large population-based study was performed in a series of 2,150 female twins (aged 18-80 years), where the association between LTL and BMD was evaluated. A positive association between TL with BMD of the spine and distal forearm (but not with the femoral neck) was revealed, and longer telomeres were associated with a reduced risk of developing osteoporosis at two or more sites in women >50 years of age (16). In a clinical setting, women with osteoporosis had a shorter TL by 117 bps than their matched controls, implying accelerating skeletal aging due to TL. As a result, it was proposed that the LTL could be used as a marker for skeletal aging (16).

In the same year, Tamayo *et al* (45), in a non-randomized controlled study of 35 elderly patients (aged 59-95 years) with osteoporosis and 130 healthy individuals, found that LTL was statistically significantly shorter ($P=0.001$) in the osteoporosis group than that in the control group. Nielsen *et al* (46), in a longitudinal cohort study involving 420 women (mean age, 64 years; range, 25-93 years), did not find a statistically significant association between LTL and BMD; nevertheless, a statistically significant association was indeed observed between LTL and age as well as between body mass index-adjusted age and BMD (46). It was also increasingly apparent that accelerating aging was related to human immunodeficiency virus (HIV) infection, which in turn accounted for telomere damage and mitochondrial DNA damage (47,48). In this context, in a non-randomized-controlled study involving 73 women (mean age, 43 years) living with HIV aged >50 years, it was highlighted that the observed shortening of LTL was statistically significantly related to a lower BMD at the lumbar spine [mean difference, -0.39; 95% confidence interval (CI), -0.61, -0.17] and total hip (mean difference, -0.29; 95% CI, -0.52, -0.07), suggesting that LTL was negatively associated with a low bone mass in women with HIV, and that this connection may be related to the pathophysiology of premature aging in HIV-infected women (49). The aforementioned results were important, given that aged individuals living with HIV had a higher prevalence for osteoporosis, as shown by a pooled odds ratio (OR) at 3.7 compared to their age-matched controls, following a meta-analysis of 20 studies (50).

In agreement with the aforementioned findings, Tao *et al* (51) conducted a cohort study of 1,017 elderly Chinese adults (mean age, 66.4 years), from whom 584 were older women at the post-menopausal stage and 433 were older

Table I. The main findings of the published articles.

| Authors, year of publication, country | Participants | Outcome measures | Main findings | (Refs.) |
|---|---|---|---|---|
| Kveiborg *et al*, 1999, Denmark | Young (aged 20-26 years), elderly (aged 48-85 years, and patients with osteoporosis (aged 52-81 years) | TL in peripheral blood leukocytes | No significant changes were observed between patients with osteoporosis and the age-matched controls | (60) |
| Bekaert *et al*, 2005, Belgium | 352 Healthy males, 71-86 years of age | TL in the leucocytes of peripheral blood | Age-corrected mean telomere restriction fragment length was associated with longitudinal bone loss at the total forearm, particularly at the mid-region of the forearm and at the ultra-distal forearm | (41) |
| Valdes *et al,* 2007, UK | 2,150 Healthy and osteoporotic women, 18-79 years of age | Telomere leukocyte length, bone mineral density, and osteoporosis | TL positively related to bone mineral density of both the forearm and the spinal column | (16) |
| Sanders *et al*, 2009, USA | 2,750 Healthy and osteoporotic adults, 71-79 years of age | Telomere leukocyte length, bone mineral density, and osteoporosis | TL was not related to bone mineral density, osteoporosis, and risk of fractures | (65) |
| Tang *et al*, 2010, China | 1,867 Elderly adults (mean age, 72 years) | Telomere leukocyte length and hip bone mineral density | No association was found between TL and baseline bone mineral density or bone loss over a 4-year period | (61) |
| Tamayo *et al*, 2010, Spain | 35 Adults aged >40 years with osteoporosis vs. 130 healthy individuals | Telomere leukocyte length and osteoporosis | Telomere leukocyte length was statistically significantly shorter in patients with osteoporosis | (45) |
| Nielsen *et al*, 2015, Denmark | 420 Women (mean age, 63.9 years; range, 25-93 years) | Telomere leukocyte length and bone mineral density | No statistically significant associations were found between leukocyte TL and bone mineral density | (46) |
| Kalyan *et al,* 2018, Canada | 73 Women with HIV (mean age, 43 years) | Telomere leukocyte length and bone mineral density | Reduction of leukocyte TL was statistically significantly related to lower bone mineral density | (49) |
| Tao *et al*, 2019, China | 1,017 Elderly Chinese adults (mean age, 66.4 years) from whom 433 were males and 584 cases were females at the post-menopausal stage and probably osteoporotic | Telomere leukocyte length and bone mineral density | Leukocyte TL was found to be associated with a lower bone mineral density and osteoporosis in elderly women, but not in the male population | (51) |
| Kirk *et al*, 2022, Australia | 20,400 Elderly adults with osteosarcopenia (n=205) compared to the matched controls in the UK Biobank (mean age 67.8 years, 53% male) | Telomere leukocyte length and osteosarcopenia | No association was found between leukocyte TL and osteosarcopenia or femoral neck bone mineral density | (62) |
| Curtis *et al*, 2022, UK | 111,395 Adults in the UK (mean age, 56.7 years) | Telomere leukocyte length and risk of fractures | Weak association in females, even weaker in males | (53) |

HIV, human immunodeficiency virus; TL, telomere length.

men, providing substantial evidence that there was a positive association between LTL and BMD through analysis of the results with multiple linear and ordinal logistic regressions, thereby increasing risk of developing osteoporosis in women

at post-menopausal stage (51). Importantly, that positive association of LTL and BMD reduced as women aged. On the contrary, no association was observed between the two parameters mentioned above in older males. As a result, it was concluded that the predictive value of LTL in osteoporosis was sex-dependent (51).

A significant association between short TLs and a low telomerase activity has also been shown to be associated with skeletal pathologies, such as osteoporosis and osteoarthritis, in which the dysregulated restitution of the subchondral bone occurs in the elderly (52).

Recently, Curtis *et al* (53) analyzed the positive association between LTL and the risk of fractures in a longitudinal cohort study. The authors of that study examined a population of 51,900 males and 59,500 females from the UK Biobank (mean age, 56.7 years). They showed that there was a weak association between a longer LTL and a reduced risk of any fracture in women [hazard ratio (HR)/standard deviation (SD), 0.96], with less evidence found in males (HR/SD, 0.98) (53). According to that study, this was the most extensive relevant study, showing only a weak association between LTL and the risk of fractures (53).

The majority of studies have reported a positive association between telomere shortening and the pathological characteristics of osteoporosis. In particular, telomere shortening is strongly associated with a low BMD (13,52). The mechanism by which telomere shortening causes osteoporosis has come to light through experiments using mice or individuals harboring germline mutations in genes implicated in telomere maintenance (15,40,44,45,48,50,52-59). However, only a limited number of studies contradicted the positive association between telomere shortening and the aggravation of skeletal pathology (60-63).

Initially, Kveiborg *et al* (60) investigated the LTL derived from 49 healthy women aged 20-26 and 48-85 years, and compared the value of LTL to that of osteoporotic women of 52-81 years of age. They did not observe any marked differences among groups (60). Moreover, that study had no statistical power due to its small study group (<30 individuals), given that the analysis of the results is affected by sample size (64). Similar results were obtained from a longitudinal cohort study in which 2,750 elderly adults (aged 71-79 years) were enrolled (65). The results revealed no association between LTL and BMD at the total hip or femoral neck, changes in BMD over time, the presence of osteoporosis and the risk of fractures in this population of elderly males and females (65). Apart from the negative association between TL and osteoporosis, it was reported that the TL was independent of the following factors: weight, fasting insulin, and fasting glucose in elderly males and females (65). Since systemic inflammation is regarded as the primary mechanism underlying the association between telomere shortening and osteoporosis, and women with osteoporosis are characterized by higher serum levels of C-reactive protein (CRP) than the controls (16,65,66), Sanders *et al* substantiated the inverse correlation of TL with serum levels of CRP and IL-6 (65). Compared to the results from the study by Valdes *et al* (16), the association of TL with BMD was sex- and age-dependent (16,65). In another longitudinal cohort study of 1,867 elderly Chinese adults (mean age, 72 years), Tang *et al* (61) found no statistically significant association between TL and baseline BMD at the total hip and femoral neck in both males and females (61).

Recently, Kirk *et al* (62) provided insight into the association between LTL and the incidence of osteosarcopenia in a population of 20,400 elderly adults (mean age, 68 years; 47% females) from the UK Biobank (62). Osteosarcopenia, according to the World Health Organization (WHO) criteria, is defined as follows: i) Bone density of the femoral neck T-score ≤-1; along with ii) a low appendicular lean mass as calculated from the relation mass/height$^2$ or low grip strength; and iii) a slow walking pace (63). One of the results of that study was that LTL was not associated either with osteosarcopenia or the low bone density of the femoral neck. The authors suggest it may be worth studying the same outcome measures in an older population (>74 years) (62).

### 3. Challenges of studying telomere length in osteoporosis

The inconsistency among studies presented herein may be related to variability in the population of studies, the methodology of studies and the size of the groups, establishing different aspects of statistical analysis. Furthermore, a discrepancy can arise through measuring TL in leukocytes and not in bone cells like osteoblasts.

After adjusting for age, two epidemiological research studies have reported a statistically significant association between LTL and BMD, though measuring LTL via Southern blot analysis (16,41). On the contrary, when LTL was evaluated through quantitative polymerase chain reaction (qPCR), no link was found between LTL and BMD (61,65). It is generally accepted that the qPCR is more accurate and reliable than Southern blot analysis, due to its high-throughput nature and sensitivity (67); however, this comparison appears to be underestimated as no report comparing the two methodologies has been performed to date, at least to the best of our knowledge (64).

Indeed, the high inconsistency of results may be attributed to different methods used to measure TL. In this direction, several reliable methods exist to evaluate TL with various forms of sensitivity, either at the population or at the single-cell level. Initially, studies used Southern blot analysis to evaluate the length distribution of the terminal restriction fragments in cells. Then, other studies performed qPCR to measure the copy number of telomere repeats in homogenized cells, obtaining a general image of the enrichment of telomere ends in cells (14). Significant advances in the telomere field have been noted, using telomere shortest length assay (TeSLA) . In the former technique, the length of all telomeres can be evaluated. In the TeSLA technique, PCR amplifies telomeres at the single-chromosome level, and gel electrophoresis then enables the evaluation of their length (14,68). Recently, the most precise method to evaluate TL at a single-cell level and at a single-chromosome level is quantitative fluorescence *in situ* hybridization (qFISH) with the use of telomere peptide nucleic acid (PNA) probe (14). Epidemiologists isolating peripheral blood (PB) usually follow the latter. Among all these techniques, only qFISH allows the measurement of individual TLs at single cell level (14). Despite the superiority of qFISH compared to other techniques, the qFISH can recognize the telomeric repeats that the PNA probe defines.

However, DNA methylation-based methods have been used to evaluate the TL (69). The sensitivity and the reproducibility of the aforementioned innovative methods regarding TL have been enhanced at a single-cell level; however, they are not considered complete. The inconsistency of DNA methylation-based methods arises from differences among techniques (70). It should also be taken into consideration that the reproducibility of TL is hampered by other parameters involving the processing and storage of samples (71). Accordingly, the differences among studies can arise from the different statistical methods used and intrinsic variations among the study groups of each study. The mechanisms underlying age-related telomere shortening remain incompletely understood, since the phenomenon is complex and multifaceted. In addition to the above, the inter-individual variability of samples themselves can complicate the TL measurements to be precise (72), rendering it very difficult to use telomere shortening as a single routine biomarker in clinical practice (73). Those limitations need to be addressed before establishing telomere shortening as a biomarker of osteoporosis in clinical settings.

## 4. Role of telomere shortening in premature aging disorders associated with osteoporosis

Clues of aging mechanisms arise from segmental progeroid disorders in humans. Apart from the direct significance of telomere shortening in osteoporosis, dyskeratosis congenita and Werner syndrome (WS) constitute two genetic diseases that are characterized by common signs of telomere shortening and premature osteoporosis (74-76). Notably, the standard features of the two diseases are premature osteoporosis and telomere shortening owing to the loss of function mutations in maintaining TL homeostasis (77). In the aforementioned genetic disorders, osteoporosis is accomplished to a greater extent than that achieved with natural aging, and the distribution of included osteoporosis in WS appears at the limbs more than the axial skeleton (78,79). In both diseases, increased systemic inflammation and impaired immune system are implicated in their pathophysiology, as represented by telomere shortening in their lymphocytes (80).

Since Wrn helicase plays a crucial role in sustaining TL (81), it is apparent that dysfunctional telomeres are accelerated in double mutant $Wrn^{-/-}$ telomerase RNA component $(Terc)^{-/-}$ mice. Double-deficient mice have been shown to exhibit an abnormal proliferation of their bone-forming cells, and they are characterized by bone loss and age-related osteoporosis owing to the disrupted differentiation of osteoblasts, accompanied by advanced senescence observed in mesenchymal stem cells and the normal differentiation of osteoclasts (82). As a result, the osteoporosis observed in double-deficient mice appears to arise from damage in the distribution and the functionality of osteoblasts (57), suggesting the significance of a short TL in the risk of fractures. Consistent with the aforementioned findings, the transfection of telomerase reverse transcriptase (TERT) into osteoblasts that are implanted into $Wrn^{-/-}$ $Terc^{-/-}$ mice has been shown to restore bone homeostasis (57).

## 5. Conclusions and future perspectives

The present review discussed different results regarding the usefulness of TL for identifying individuals with a higher susceptibility for developing osteoporosis and also highlighted the significance of TL in patients with osteoporosis. Indeed, the present review proposes that TL shortening may be used as a prognostic or predictive marker to evaluate the onset and progression of osteoporosis. However, further large and well-characterized cohorts with large sample sizes and proper design are urgently required to gain new insight into the mechanisms through which telomere shortening leads to the development of osteoporosis and to encourage the use of telomere shortening as a predictive tool for the onset and progression of osteoporosis.

Thus, it remains questionable whether telomere shortening can be a reliable biomarker of osteoporosis or comprising a part of a composite panel of biomarkers for osteoporosis. Moreover, a combination of panel markers can exert greater predictive value in evaluating the progression of osteoporosis than single measures. In parallel, telomere shortening can serve as a predictive tool for assessing the risk of developing osteoporosis.

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## Authors' contributions

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## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

DAS is the editor-in-chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision for this article. The other authors declare that they have no competing interests.

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