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Fracture prediction, imaging and screening in osteoporosis

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Abstract | Osteoporosis is associated with increased fragility of bone and a subsequent increased risk of fracture. The diagnosis of osteoporosis is intimately linked with the imaging and quantification of bone and BMD. Scanning modalities, such as dual-energy X-ray absorptiometry or quantitative CT, have been developed and honed over the past half century to provide measures of BMD and bone microarchitecture for the purposes of clinical practice and research. Combined with fracture prediction tools such as Fracture Risk Assessment Tool (FRAX) (which use a combination of clinical risk factors for fracture to provide a measure of risk), these elements have led to a paradigm shift in the ability to diagnose osteoporosis and predict individuals who are at risk of fragility fracture. Despite these developments, a treatment gap exists between individuals who are at risk of osteoporotic fracture and those who are receiving therapy. In this Review, we summarize the epidemiology of osteoporosis, the history of scanning modalities, fracture prediction tools and future directions, including the most recent developments in prediction of fractures.

Major osteoporotic fractures
Fractures attributable to osteoporosis including hip, forearm, humerus or clinically presenting vertebral fractures.

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Osteoporosis is a disorder associated with a decrease in BMD, low bone mass and increased bone fragility; individuals with osteoporosis are at an increased risk of fragility fractures. The economic and societal burden of fragility fractures is massive, previously estimated at 37 billion euros per year in 27 European countries alone[1], and is set to rise owing to an increasing skew towards an older population[2,3]. Importantly, the ability to predict those at risk has developed enormously over the past 20 years through the use of fracture prediction tools and an increasing understanding of scanning modalities, such as dual-energy X-ray absorptiometry (DXA). Despite this, a treatment gap exists between those at risk of fracture and those receiving treatment for the prevention of fragility fractures.

In this Review, we expand on the current epidemiology of fragility fractures, provide an up-to-date definition of osteoporosis and discuss the widening gap in treatment for those at risk. We also highlight the development of fracture prediction tools and the benefits they have brought in identifying those at risk, with particular focus on the recent Screening of Older Women for the Prevention of Fractures (SCOOP) trial. We then discuss the role of DXA in enhancing the identification of individuals at risk of fracture and examine more recent imaging modalities and analyses.

The epidemiology of fractures

Fractures are a major concern for the health of individuals and the general population at large, with common fragility fracture sites being found in the hip, spine and wrist. In 2010 in Europe, there were 22 million women and 5.5 million men with osteoporosis, accounting for 2% of the overall burden of non-communicable diseases[1]. This population experienced an estimated 3.5 million fragility fractures, with 610,000 hip fractures, 520,000 vertebral fractures, 560,000 forearm fractures and 1.8 million 'other fractures' (comprising fractures of the pelvis, rib, humerus, tibia, fibula, clavicle, scapula and sternum and other femoral fractures)[1]. In the USA, one in two women experience osteoporosis-related fractures after menopause[4]. In the UK, there are an estimated 200,000 osteoporosis-related fractures per year[5]. These fractures severely effect quality of life, with 50% of patients with hip fracture losing the ability to live independently[6]. In the USA, fragility fractures are responsible for >432,000 admissions to hospital and 180,000 admissions to nursing homes each year[7].

Incidence, mortality and economic cost. The incidences of age-specific vertebral, forearm and hip fractures are increasing owing to the elderly population being the fastest growing age demographic[1,8,9]. Although the incidence of fragility fractures continues to rise in transitioning populations, notably, the rate of hip fracture has stabilized in many resource-rich countries[10] (FIG. 1), and wide global variation exists by geography[11], ethnicity and socio-economic status[12].

The mortality associated with major osteoporotic fractures is substantial, with 20% mortality from hip fractures within the first year[13,14]. Moreover, hip fractures

Key points

- The WHO defines osteoporosis as a measurement of BMD that is at least 2.5 standard deviations less than the mean BMD for a 30-year-old man or woman.
- Dual-energy X-ray absorptiometry provides a measure of BMD that can be used to diagnose osteoporosis.
- Central and peripheral quantitative CT can be used to provide measures of bone microarchitecture within a research setting.
- BMD, combined with clinical risk scores, including Fracture Risk Assessment Tool (FRAX), can be used to predict which individuals are at high risk of fracture.
- A gap exists between individuals who are at risk of fracture and those who are receiving treatment and requires closing as a matter of paramount importance.

result in 20% of orthopaedic bed occupancy in the UK, and the mean in-hospital stay is 27 days. The annual economic cost of fragility fractures in Europe was estimated at €37 billion[1], with 66% of the cost attributable to incident fractures, 29% to prevalent fractures and 5% to associated pharmacological costs. In the USA alone, the cost of fragility fractures in 2005 was estimated to be $17 billion, with a subsequent increase to $25.3 billion estimated by 2025 (REF.[15]). A shift in the demographic landscape of fractures has occurred, which is associated with the increasingly elderly skew of the population[16]; therefore, the above costs might increase further. As such, more recent health economic analyses are required to elucidate the modern day financial impact of fragility fractures.

Medical interventions for osteoporosis. The past 20 years have seen marked developments in medical interventions for osteoporosis including calcium and vitamin D supplementation, hormonal replacement therapy and bisphosphonates[1,17]. These pharmaceutical therapies reduce the incidence of osteoporotic fractures[18–22]. For example, bisphosphonates decrease all fractures by 35%, non-vertebral fractures by ~25% and vertebral fractures by 50%[20,23]. In addition, denosumab has been shown in a trial to reduce fracture rates after 10 years of treatment[24]. Notably, in this trial, vertebral fracture yearly incidence in the denosumab treatment group (females aged 60–90 years) remained at a similar rate during the trial extension (to 10 years) to that seen in the original trial (3 years): vertebral fractures at 0.9–1.9% and non-vertebral fractures at 0.8–2.6%. This yearly incidence was lower than that observed in a virtual placebo group, and both efficacy and safety surveillance are ongoing. Since 2015, bone-forming agents such as teriparatide[25,26] and abaloparatide[27,28] have shown good efficacy in randomized controlled trials.

The osteoporosis treatment gap. A major concern in the management of osteoporosis is that a minority of patients receive treatment[1,29]. This untreated population is referred to as the osteoporosis treatment gap[30], which refers to the difference between the number of individuals who are at high risk of fracture and the proportion of these people who receive fracture preventive interventions. An unfortunate reality of the treatment gap in osteoporosis is that, of the individuals who sustain a fragility fracture, <20% receive secondary preventive treatment, with this proportion being even lower in older females and patients in long-term care. Fracture assessment tools, which utilize clinical variables to provide a measure of fracture risk, have therefore been developed and will be discussed later[31,32].

Globally, marked variation exists in the use of fracture assessment tools, with 1,000-fold variation in tool use worldwide despite far lower variation in fracture rates[33]. This paucity of tool use could be attributable to a lack of coherent local guidelines or difficulty accessing the tools online or even in paper format[34]. Beyond the variation in assessment of fracture risk, some resource-rich countries, including the UK and USA, have shown a concerning downward trend in treatment of osteoporosis[35,36]. In the USA, this observation might be due to changes in the provision of medical reimbursement. Moreover, in the UK and USA, increasing concern exists regarding adverse events related to bisphosphonates, which have been hyped in the lay media[37]. This concern is countered by a Danish study, which demonstrated that, even in individuals who were overtreated with 10 years of the bisphosphonate alendronate, the fracture risk was lower than in age-matched controls[38,39].

Progress in the effective identification of high-risk individuals has depended upon the definition of osteoporosis, the development of fracture risk prediction tools and an understanding of imaging modalities for assessing bone parameters, all of which are discussed below.

Diagnosis of osteoporosis

A step-change in the assessment of bone fragility occurred with the advent of non-invasive methods for determining BMD, the most prominent of which is DXA[40]. Historically, the lumbar spine and proximal femur were sites that were considered; however, since 2013, the femoral neck has been recognized as the reference site for epidemiological studies[41].

DXA provided a homogenized, widely utilizable method for calculating BMD as a T score that measures the number of standard deviations from the mean BMD for a 30-year-old man or woman. As a result, in 1994, the WHO produced an operational definition of osteoporosis based on a BMD T score of −2.5 or lower[42]. This score has since become the diagnostic criterion for osteoporosis. A 2006 study showed that there is a 1.5–2.5-fold increase in fracture risk per standard deviation decrease in BMD[21]. Thus, BMD is a good predictive measure of future fractures. By contrast, another measure with equal predictive power is blood pressure as a predictor of future stroke. Both measures have more predictive power than serum cholesterol levels have for cardiovascular disease risk[43].

Although BMD is a good specific predictive measure, the sensitivity for BMD alone in predicting future fractures is <50%, and those in an osteopenic range (T score between −1.0 and −2.5) are still at risk of fracture. The Rotterdam study[44] demonstrated that 44% of women with non-vertebral fractures over a follow-up of 6.8 years had a BMD T score between 0 and −2.5 and that 12% had a completely normal BMD. Another study from the USA showed that only 46% of women who sustained a hip fracture during a 5-year follow-up period had a T score of −2.5 or less for BMD at baseline[45].

Denosumab
A fully humanized monoclonal antibody that binds to the receptor activator of RANK ligand, thus blocking the action of RANK ligand. It is delivered via subcutaneous injection as an anti-resorptive agent for the treatment of osteoporosis.

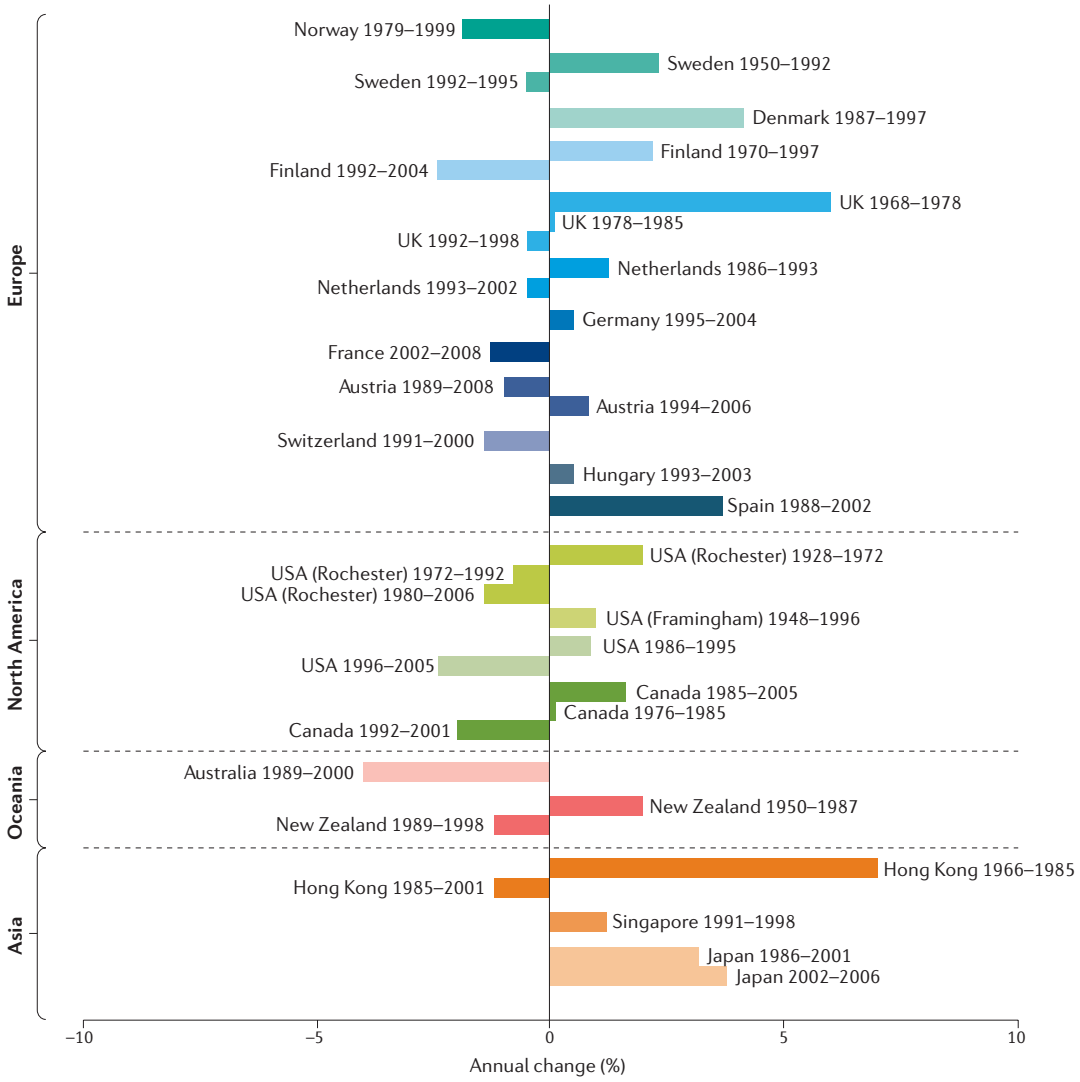

Fig. 1 | **Secular changes in hip fracture worldwide.** This figure demonstrates the secular trends in hip fracture across the globe. Geographical regions are divided by dotted lines, and countries are shown in differing bars with labels including the country name and the years between which the secular trend was measured. Annual percentage change is shown on the *x* axis, with a percentage rise being positive and a percentage decrease being negative. The general trend for European, Australasian and North American countries is an increase in hip fracture rates in the last quarter of the 20th century, with a plateau as they near the year 2000. In Asia, the general trend is towards a continued increase in rates approaching the year 2000 threshold. Figure reproduced from REF.[10], Springer Nature limited.

For the above reasons, 2019 European clinical guidelines have repeated the recommendation of using fracture risk factors (such as fall risk and age[46]) for identifying those at risk of fracture, as well as a thoracic kyphosis and loss of height of >4 cm (to identify subclinical vertebral fractures)[47]. The low sensitivity of BMD is of high clinical importance and emphasizes that BMD does not take non-skeletal determinants of fracture risk into account. As such, assessment modalities and particularly DXA have a crucial role in identifying at-risk individuals.

Thoracic kyphosis
An S-shaped deformity of the spine that can be precipitated by osteoporotic vertebral fractures.

Dual energy X-ray absorptiometry

Absorptiometry generally relies upon an energy source (for example, photon or X-ray) passing through a test material and a detector that is used to measure the degree of attenuation. This measurement can be compared with a standard control material to calculate the density of the test material. Depending on the properties of a tissue, it will attenuate radiation differently, which allows quantification and separation of different tissues (for example, fat, muscle or bone) from one another. DXA was first described by Mazess in 1981 (REF.[48]). This method uses two different energy sources (at ~40 KeV and >70 KeV) to allow discrimination between soft tissues and bone and provides increased resolution and precision[49] and a shorter scan time than previous modalities.

DXA has since become the gold-standard measure for BMD owing to the scientific demonstration of a strong correlation with biomechanical bone strength via finite element analysis[50], a correlation with the clinical outcome of fracture risk[51] and the relatively low radiation burden[52]. Moreover, DXA is also a viable measure for

muscle mass in the assessment of sarcopenia[53]. Notably, DXA measurements alone may be more advantageous than using clinical risk factors (and the related prediction tools) alone when identifying individuals with rare conditions that increase the risk of fracture. However, DXA can be used in conjunction with clinical risk tools (for example, Fracture Risk Assessment Tool (FRAX), which we describe later) in order to more accurately identify those at risk of future fracture.

Quantitative measures derived from 2D densitometry. DXA is a form of 2D densitometry, and the quantitative measurements that can be derived from this method include bone area (cm^2), bone mineral content (grams) and areal BMD (grams per cm^2). Areal BMD is calculated using pixel by pixel attenuation values of a test material (in this case bone) against a control phantom[54]. Bone area is calculated by summing the pixels that lie within the bone edges, and bone mineral content (in grams) is calculated by multiplying areal BMD and bone area. In 2001, a model was proposed to enhance the comprehension and interpretation of bone densitometry measures in children and adolescents[55], although this model can also be employed in the understanding of adult bone physiology. The model focuses on three key areas: material BMD, compartmental BMD and total BMD.

Material BMD refers to the mineralization of a small volume of organic bone matrix. A small volume is necessary to exclude marrow, lacunae, canaliculi and osteonic canals from the sample. This measurement can be performed invasively via bone biopsy, or, since 2000 (for cortical bone), via a virtual bone biopsy afforded by high-resolution peripheral quantitative CT (HRpQCT)[56], which is described later. Compartmental BMD refers to the amount of mineral in the cortical and trabecular compartments and can be assessed by quantitative CT (QCT) methods (central, peripheral and HRpQCT). DXA does not provide measures of compartment BMD, as it is a 2D method. Instead, DXA provides an integrated measure of total BMD, which refers to the entire density of the material within the periosteal envelope.

A limitation of DXA is the aforementioned 2D image that it provides, which limits the ability to measure density (mass per volume), as the depth of the bone cannot be accounted for. This limitation creates a size dependence to measurements, which is problematic in children. Several methods exist to account for these inaccuracies in the use of DXA in children. The methods include calculation of a size-corrected total BMD from the DXA image, bone mineral apparent density[57,58] and regression methods to take into account the size of the child[59,60].

Over the past decade, developments in DXA scanning include vertebral fracture assessment using lateral views of the thoracolumbar spine[61]; hip structural analysis, which utilizes hip cross sections to ascertain bone strength; and trabecular bone score (TBS), which provides a measure of bone quality (rather than the quantity supplied by BMD) and is a surrogate of bone microarchitecture.

Trabecular bone score. TBS is an analytical tool that is used on data acquired using DXA to provide a surrogate measure of bone microarchitecture, providing information on bone structure above and beyond areal BMD[62]. The tool uses a sequence of experimental variograms to quantify variation in grey-level texture between pixels[63] and generate a value that is strongly related to experimental trabecular separation, trabecular number and connective density[64,65]. The region of interest is usually an anterior–posterior view of the lumbar spine on DXA; a higher TBS is consistent with fracture-resistant bone, and a lower score is consistent with weaker bone[66]. Seemingly, there is an age-dependent variation in TBS, with a relative plateau in mid-life (aged 30–45 years) and a gradual reduction with age[67].

A point of interest is whether the TBS provides any information for the effective prediction of fractures independently of clinical risk factors and areal BMD. A study in a cohort from Manitoba attempted to address this question in women[68] and men[69] and found that, in women, TBS predicted incident fractures (HR 1.36, 95% CI 1.30–1.42; $P < 0.001$). After adjustment for clinical risk factors and femoral neck areal BMD, the associations were attenuated, although a hazard ratio of 1.18 (95% CI 1.12–1.23) remained for a major osteoporotic fracture[68]. In males, the area under the curve for the prediction of incident major osteoporotic, hip and clinical vertebral fractures was better than that expected by chance alone[69].

In 2016, a meta-analysis of 14 cohorts was performed to assess the triangular relationship between clinical risk factors, TBS and areal BMD with regard to fracture prediction. The authors found that TBS was partially independently predictive of future major osteoporotic and hip fractures and concluded that the score can have some utility in clinical practice[70]. Through this analysis, a low risk of fracture was defined as a lumbar TBS > 1.31, and a high risk of fracture was defined as a TBS < 1.23.

Although TBS has been demonstrated to respond to fracture prevention therapy, including bisphosphonates and raloxifene, the percentage change is generally less than that observed in areal BMD[71,72]. An advantage of TBS is that the tool can be applied to DXA, radiographs, CT and QCT and at any skeletal site, although DXA of the lumbar spine is the most common modality. A potential disadvantage of areal BMD is the artefact caused by degenerative disease (particularly in the spine), which leads to falsely raised BMD levels. However, owing to the methodology, TBS is not affected by degeneration or osteophytes[73]. In addition, TBS is available as a modifier to FRAX online. Potential clinical and technical issues with the accuracy of TBS exist, including artefact generated from image resolution, noise and soft tissues, including adipose tissues[74]. As such, the most accurate measures of TBS will be obtained from individuals with a BMI between 15 and 37 kg/m^2.

In summary, TBS provides additional information regarding bone quality beyond the quantitative measures provided by 2D densitometry. Although DXA is the clinical leader in the image assessment of bone, other scanning modalities have been developed (BOXES 1,2) and are used in the research context.

Periosteal envelope
The membrane of connective tissue that surrounds bone. It has two layers, an outer fibrous layer and an inner layer, which plays a crucial role in osteogenesis.

Bone mineral apparent density
An estimated volumetric bone density. Volume is calculated from the dual-energy X-ray absorptiometry (DXA)-assessed bone area by assuming the vertebrae are either a cube or a cylinder. It is a method of reducing the size dependence of DXA measurements and is particularly useful in children.

Box 1 | **Microindentation**

Stiffness is the ability of a structure to resist deformation. When subjected to a particular load, a stiffer bone will deform less than a softer, more compliant bone. The increasing porosity of bone with age leads to reduced stiffness and, thus, increased risk of fracture[115]. Measurements of BMD do not capture bone porosity, and so other methods have been developed to assess this element of fracture risk.

Microindentation is a novel methodology that involves inserting a probe through the skin and down onto the bone surface at a particular anatomical site (for example, the anterior midtibia) to measure the stiffness and toughness of a bone. Bone Material Strength Index (BMSi) is measured by comparing the indentation distance of the bone with a reference standard calibration material. On the surface of the bone, the probe induces microfractures; the weaker the bone is, the greater the distance that the probe extends into the bone and the lower the BMSi[116].

Microindentation has been shown to distinguish between degrees of fracture risk[116–118], between individuals with osteoporosis and controls[119] and between individuals at risk of atypical femoral fracture and controls[120]. However, the technique has also been associated with areal BMD in isolation without associations with fracture risk[121].

This method has limitations, as it tests the tibia, which is an uncommon site of fragility fracture. Moreover, the invasive nature of microindentation means that it might be less likely to be tolerated in clinical practice than imaging modalities. Regarding the latter, a 2018 study has sought to address this in a large cohort of 345 Australian males, in whom the procedure was well tolerated, although 56 individuals were contraindicated by the presence of excessive soft tissues in the midtibial zone[122]. Overall, it is estimated that ~1,500 individuals have had the procedure globally, with 2 reported episodes of adverse events; 1 was a mild skin infection and 1 was a reaction to local anaesthetic[122].

In summary, microindentation is a technique that shows promise and seeks to identify individuals who are at risk of fracture owing to bone weakness, who are potentially missed by measurement of BMD alone[123].

Research scanning modalities

As previously attested, DXA is the current gold standard for predicting those at high risk of fragility fractures, although this method does have some issues and limitations. These issues include the lack of estimates of compartmental and material BMD, the fact that BMD measurements are size-dependent (as they are calculated using a 2D projection of a 3D structure with no adjustments for object depth) and the fact that the measures of BMD are susceptible to changes in body composition[75].

In order to counter these issues and to provide additional measures of bone structure, morphometry and biomechanics, other, non-DXA scanning techniques have developed and are employed, largely in the research setting. Indeed, elements of bone microarchitecture have been shown to be predictive of incident fracture independent of BMD[76]. These scanning modalities include central QCT, peripheral QCT and HRpQCT.

Central quantitative CT. Central QCT was first developed in the 1970s[77] but came to wider usage in the 1980s[78]. Central QCT is a modality that uses multiple 2D slices, and the central description of the modality refers to the fact that the regions of interest are the lumbar spine (particularly the L1–L3 vertebrae), the proximal femur and peripheral sites, and central QCT also provides a measure of muscle mass[79]. The advantages of central QCT over DXA include the ascertainment of mean volumetric BMD (measured in mg/cm^3). This measurement has the advantages of being less sensitive to changes in bone size than areal BMD, and assesses compartmental BMD, bone geometry and biomechanical measures of bone strength[79]. Compared with DXA, the main disadvantages of central QCT are the increased burden of ionizing radiation[80] and the potential issues that exist with confounding by changes in bone marrow fat owing to the majority of scanners being single-energy devices[81].

Peripheral quantitative CT. The next scanning modality in the QCT family is peripheral QCT, which became commercially available in the 1990s[82], with the most common model being the XCT 2000 (Stratec, Pforzheim, Germany). This method takes 2D slices (1–2 mm thick) of the radius and tibia, which (owing to the very low radiation burden) can be performed at multiple sites along the bone. This modality provides not only valuable data on volumetric BMD, compartmental BMD, bone geometry and bone strength but also muscle measures including cross-sectional area and muscle density[83]. Measurements of muscle provide the opportunity to calculate a bone to muscle ratio, which is relevant when considering some hypotheses for bone strength and loading (for example, the mechanostat theory[84]).

High-resolution peripheral quantitative CT. The most recently developed QCT scanning modality is HRpQCT (XtremeCT, Scanco Medical, Bruttisellen, Switzerland), which allows multiple 2D slices (most commonly of the radius or tibia) to be recreated into a 3D virtual bone biopsy. The enhanced spatial resolution afforded by this modality is in excess of that provided by standard peripheral QCT, QCT or MRI[85]. HRpQCT imparts a low dose of radiation (<3 μSv), and owing to semi-automated contouring and segmentation of tissue, this method provides data on densitometry, morphometry, microarchitecture and biomechanical measures (including stiffness and elastic modulus) through finite element analysis[86,87].

Non-dual-energy X-ray absorptiometry scanning in clinical practice. A recent prospective study by the Bone Microarchitecture Consortium found that HRpQCT measurements (particularly peripheral skeleton failure load, which is the prediction of the external force required to cause failure of the bone) were statistically significantly associated with future risk of fracture over ~4.5-year follow-up after adjustment for BMD[76]. However, it should be emphasized that, although the above non-DXA scanning modalities provide valuable data to drive forward densitometric research, they are not currently used in clinical practice owing to a lack of routine accessibility. Whilst quantitative ultrasonography was used extensively in research studies, particularly in the 1990s, the practical limitations of this technology and inferior ability to predict individual fracture status (compared with DXA) led to diminishing use and application. Quantitative ultrasonography also lacks a coherent standardization across different models and instruments of algorithmic data resolution and resultant reported parameters[88]. This method does, however, have a potential utility in low-resource settings where DXA is unavailable. Interestingly, MRI has also been used to assess bone densitometry and has future potential in terms of usage in the clinical or research settings[89].

Box 2 | **Biochemical markers of bone turnover**

Bone turnover is characterized by bone formation and bone resorption. Biochemical bone turnover markers (BTMs) have been discovered and developed to capture measurements of these two activities. International expert groups in the fields of clinical chemistry and osteoporosis have come to a consensus that the amino-terminal propeptide of type I procollagen (PINP) and the carboxy-terminal telopeptide of type I collagen (CTX-I) should be the markers for bone formation and bone resorption, respectively[124].

CTX-I is a product of the breakdown of type I collagen and has a strong circadian rhythm, which necessitates early morning blood collection. PINP is formed from the post-translational cleavage of type 1 procollagen and has no circadian rhythm; however, owing to obvious practicalities, it is usually collected contemporaneously with CTX-I (unpublished observations; N.R.F., E.M.C., K.W., N.C.H., E.M.D. and C.C.).

Pre-analytical variability of BTMs is an important consideration, with circadian variation, seasonal variation, physical activity and food intake being examples of modifiable determinants[124]. The last of these is due to the intestinal induction of glucagon-like peptide 2, which stimulates a post-prandial decrease in CTX-I. Less modifiable determinants include age, sex, hormone levels (including menopausal changes and endocrine disorders) and the effect of certain medications (for example, corticosteroids, anti-epileptics and heparin)[124].

BTMs appear to be predictive of fracture (independently of age, BMD and prior fracture) in particular demographic groups, including postmenopausal women and elderly women[125–127]. Moreover, they are associated largely with major osteoporotic fractures and can be predictive over a relatively short follow-up period (<5 years) (as opposed to 10-year prediction probabilities by Fracture Risk Assessment Tool (FRAX)). The association with future fractures is probably due to the link between a high turnover state and lower BMD and poor quality bone microarchitecture[128].

BTMs can be employed to monitor the response to fracture-protective therapies and are often used in the context of bone-forming agents such as teriparatide[27]. 2019 guidelines have also noted their potential utility in predicting fractures when BMD is unavailable[47].

In conclusion, BTMs appear to be a useful adjunct to traditional methods of identifying those at risk of fracture (in particular, postmenopausal women) and may have an additional role in monitoring response to treatments.

Fracture prediction tools

In clinical practice, imaging (particularly DXA) is used not in isolation but together with clinical risk factors for fractures[47]. These risk factors can each be assessed in isolation but have also been incorporated into usable tools for assessing fracture risk.

Fracture Risk Assessment Tool. The WHO definition of osteoporosis was used to determine the threshold for treatment, but, although the definition held at a population level, many individuals sustain fractures with BMD T scores that are closer to 0. This observation has led to the development of fracture risk prediction tools, including FRAX, QFracture and Garvan.

The first clinical risk score was developed as a proof of concept in 2006 (REF.[90]). This algorithm was produced from data on the General Practitioner Research Database and provided a measure of future fracture risk. However, there are two important limitations in the use of this tool that are both based around the absence of BMD from the algorithm (owing to the primary care nature of the data collected). The first limitation is that it seemed counter-intuitive to exclude BMD as an important parameter in the prediction of fracture. The second limitation is that the medical trials designed to prevent fragility fractures had been performed in individuals with low BMD and, thus, it seemed a non-sequitur to ask clinicians to base their decision to treat on an alternative yard stick.

The next (and now most widely adopted) of the fracture prediction tools was FRAX, which was published in 2008 (REF.[91]). The FRAX tool was developed via systematic meta-analyses of primary data from 9 global, geographically spread cohort studies and then validated on data from a further 11 cohort studies. Key principles in the development of the FRAX tool were that any variable included in the algorithm (and thus the clinical tool) should be intuitively linked to fracture, readily clinically available, at least partly independent of BMD and associated with a fracture risk that might be reversible through pharmacological treatment[92].

The clinical parameters incorporated into the FRAX tool include age, sex, weight, height, previous fracture, parental hip fracture, current smoking status, glucocorticoid usage, the presence of rheumatoid arthritis, secondary causes of osteoporosis, alcohol consumption and BMD (though the last can be excluded in under-resourced settings, which preclude the use of DXA). These clinical parameters are used to provide a separate 10-year probability of any osteoporotic fracture and hip fracture. The tool, which is available in over 30 languages, has been made freely available via the FRAX website and is used for an estimated 225,000 calculations per month[93], although paper formats are available in under-resourced settings. Fracture incidence is known to differ across the globe[11], and FRAX has the ability to adjust according to global region; in 2006, 80% of the global population was covered by the FRAX tool[93].

The limitations of FRAX include the unquantified glucocorticoid exposure, which is recorded as a binary yes–no, and the omission of lumbar spine BMD, TBS, hip axis length and falls history. Methods to account for some of these considerations have now been documented or implemented through adjunctive algorithms or national guidelines[93]. For example, diabetes mellitus increases the risk of fracture but is not directly included in the FRAX tool. Different approaches have been used to circumvent the limitations of the tool including incorporation of TBS, ticking the rheumatoid arthritis button (on the FRAX website), increasing the age input by 10 years and reducing femoral neck BMD T score by 0.5 standard deviations (for example, a T score of −1.75 became −2.25)[94].

A further example of FRAX tool refinement is that of spine–hip discordance, which uses the difference between lumbar spine and femoral neck BMD T scores to improve fracture prediction by using the following rule: "increase or decrease the FRAX estimate for major osteoporotic fracture by one-tenth for each rounded BMD T score difference between lumbar spine and femoral neck"[95].

QFracture. The QFracture tool was published in 2009 (REF.[96]). This tool was derived using Cox proportional hazards models on the data of 2 million individuals aged between 30 and 85 years in the General Practitioner Research Database in the UK. The same data set was then used to validate the tool. Consequently, QFracture is primarily applicable to the UK population, and although it is only calibrated on hip fracture, the tool does provide estimated incidences of hip, forearm, spinal

and shoulder fracture. As in the 2006 tool, BMD is not included, and QFracture is therefore subject to the same limitations. The number of risk factors was extended in 2012, on the basis of National Institute for Health and Clinical Excellence guidance on the risk assessment for osteoporosis, to include history of previous fracture, presence of epilepsy (or anticonvulsant use), ethnicity and the presence of type 1 diabetes mellitus.

The current list of clinical parameters included in the QFracture tool includes age, sex, ethnicity, smoking status, alcohol use, type 1 or type 2 diabetes mellitus, parental history of hip fracture and/or osteoporosis, nursing or care home residence, history of prior osteoporotic (wrist, spine, hip or shoulder) fracture, history of falls, dementia, cancer, obstructive airways disease (asthma or chronic obstructive pulmonary disease), cardiovascular disease, chronic liver disease; chronic kidney disease, Parkinson disease, rheumatoid arthritis or systemic lupus erythematosus, gastrointestinal malabsorption, epilepsy (or use of anticonvulsants), use of antidepressants, use of corticosteroids, and BMI. The following additional factors are used for only women: oestrogen-only hormone replacement therapy and endocrine problems (including thyrotoxicosis, primary or secondary hyperparathyroidism and Cushing syndrome).

Garvan. The Garvan fracture prediction tool was developed on the basis of ~2,500 members of the Australian Dubbo Osteoporosis Epidemiology Study (DOES)[97]. This tool does not include rheumatoid arthritis, secondary osteoporosis, steroid use, smoking, alcohol, parental hip fracture or secondary osteoporosis in the parameters that are entered into the risk calculation. However, the Garvan tool does provide a novel angle through the inclusion of the number of fractures since the age of 50 years and the number of falls in the previous year.

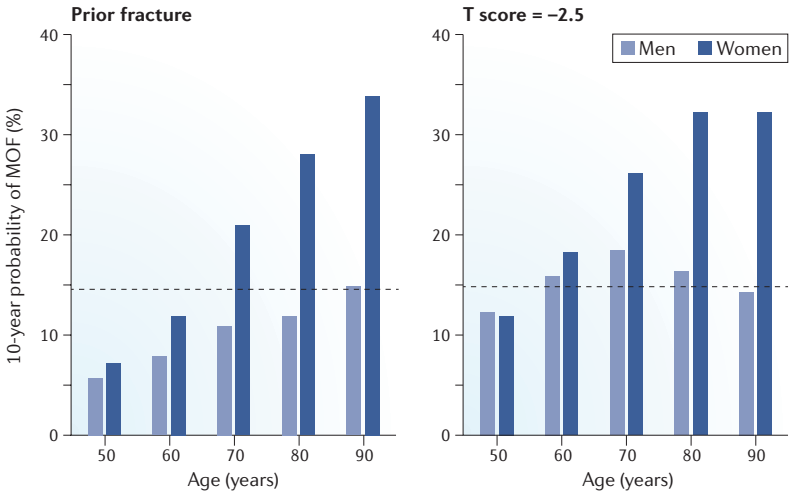

Fig. 2 | **The pitfalls of a fixed Fracture Risk Assessment Tool threshold for intervention.** The graph plots 10-year probability of major osteoporotic fracture (MOF; calculated using Fracture Risk Assessment Tool (FRAX)) against age. The dotted line depicts the treatment threshold on the basis of history of prior fracture (left panel) and a BMD T score of −2.5 (right panel). A substantially undertreated population is evident, as only those individuals whose 10-year probability of MOF falls above the line receive treatment. Figure reproduced from REF.[93], Springer Nature Limited.

The tool previously provided a risk of fracture at a large number of sites (including distal femur, pelvis, patella, proximal and distal tibia and fibula, ribs and sternum, hands and feet) but has now focused down to a 5-year and 10-year percentage risk of hip fracture and any osteoporotic and/or fragility fracture[97]. The potential disadvantages of the Garvan risk score are that it is based on a single Australia cohort (which could limit its wider applicability) and it does not take the competing hazard of death into account.

Prediction tools worldwide. FRAX has been more widely adopted globally than QFracture or Garvan. In 2016, FRAX had been incorporated into 120 guidelines worldwide, and it is widely incorporated into DXA software and primary care computer systems[98]. When incorporated into these recommendations, FRAX is either used with a fixed FRAX intervention threshold (with or without BMD) or as a gateway to an assessment that includes age-dependent intervention thresholds[93].

The use of fixed thresholds for intervention is usually incorporated with a measure of BMD and a history of prior fragility fracture and is very simple to use in a clinical setting. However, the simplicity of the use of fixed thresholds for intervention masks the issue demonstrated in FIG. 2, which depicts the FRAX percentage 10-year risk of major osteoporotic fracture against age for men and women with a history of prior fragility fracture and individuals with a BMD T score of −2.5. Notably, the fixed threshold for BMD T score results in a minimal proportion of women aged between 80 and 90 years being treated and in undertreatment of the whole population[93].

The above observation is clearly unsatisfactory and counterintuitive to good clinical practice. For this reason, in the UK, the National Osteoporosis Group Guidelines employs a combination of age-dependent and fixed thresholds to guide further investigation (via DXA) and intervention[99,100]. To expand on this, the intervention thresholds for the initiation of pharmacological therapy are, for women with a history of prior fragility fracture (with no requirement for further assessment), age-dependent thresholds until the age of 70 years and fixed thresholds thereafter.

The National Osteoporosis Foundation guidelines in the USA suggest that pharmacological therapy should be initiated in those with a prior history of hip or vertebral fracture and in individuals with a T score of −2.5 or less[101]. Additionally, postmenopausal women and men ≥50 years with T scores in an osteopenic range (that is, −2.5 to −1.0) and a US-adapted FRAX score of ≥3% risk of hip fracture and ≥20% risk of major osteoporotic fracture should receive treatment. Here, the reference to a US-adapted FRAX score indicates that National Osteoporosis Foundation guidelines have been calibrated according to USA fracture rates and mortality. The American College of Rheumatology (ACR)[102] and Scottish Intercollegiate Guidelines Network (SIGN) guidance use FRAX risk to direct BMD screening and intervention thresholds[103]. Notably, the SIGN guidelines use fracture clinical risk factors as an initial assessment, followed by BMD assessment; a BMD T score of −2.5 or

Box 3 | **Osteoporosis as a case study of the Wilson–Jungner criteria**

In order to contextualize the developments in osteoporosis and fragility fracture, it is interesting to review the condition as a case study in light of the Wilson–Jungner criteria for the validity of screening[129]. The criteria address the condition itself, the test for that condition, subsequent treatment and feasibility of screening. Osteoporosis amply satisfies the considerations with regard to the condition, being common, well-defined and with its epidemiology, natural history and costs thoroughly characterized[1,13].

Osteoporosis is readily detected through the use of a validated, reliable and widely available technique, dual-energy X-ray absorptiometry, satisfying the second criterion. There is a wide range of effective treatments for osteoporosis, which have been shown to reduce fracture risk, and given the inevitable decline in BMD with age, earlier intervention before fractures have occurred, is fully justified. Historically, the field has focused on an opportunistic case-find approach to ascertainment of disease, but with the results of the Screening of Older Women for the Prevention of Fractures (SCOOP) trial, the last point considered by Wilson and Jungner (the use of a population-screening approach) is now supported.

less is the gateway to treatment, which is an approach that could potentially widen the treatment gap[103].

European guidance regarding thresholds for pharmacological intervention in postmenopausal women recommend the use of a FRAX-based approach to clinical decision-making and that women over the age of 65 years with a history of prior fragility fracture are considered for treatment without any further assessment[47]. Younger postmenopausal women should undergo an additional assessment of BMD. This recent guideline also recommended that age-dependent thresholds are clinically appropriate and cost-effective in their identification of those requiring treatment[47].

After FRAX was devised and validated, it was important to examine whether the test had a discernible effect on fracture rates within the context of randomized controlled trials, which are described below.

The SCOOP trial

The WHO recommendations for screening (BOX 3) for fragility fractures include the assessment of fracture risk into high-risk, medium-risk or low-risk groups; high-risk individuals are considered for treatment, low-risk individuals are not recommended for treatment, and medium-risk individuals are further assessed with a measurement of BMD[104].

The Screening of Older Women for the Prevention of Fractures (SCOOP) trial was designed as a pragmatic, unblinded, randomized controlled trial of women aged 70–85 years. It was based in seven centres in the UK including Birmingham, Bristol, Manchester, Norwich, Sheffield, Southampton and York, from which 12,483 participants were recruited.

Aims and rationale. The aim of the study was to examine the effectiveness and cost-effectiveness of a community-based screening programme to decrease fragility fractures in older women and thereby address the aforementioned treatment gap in this population. The structure of the study is depicted in FIG. 3.

Previous trials of population screening for osteoporosis have been undertaken, including one based in a population of postmenopausal women, which was started in the 1990s and reported in 2010 (REF.[105]) and reported that screening marginally increased the usage

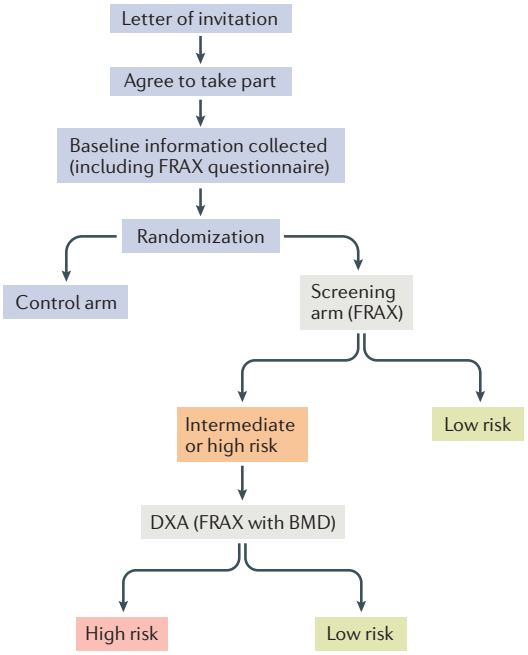

Fig. 3 | **Participant flow for the SCOOP study.** The inclusion criteria for the Screening of Older Women for the Prevention of Fractures (SCOOP) trial were female sex, age 70–85 years and the ability to provide informed consent. The exclusion criteria were individuals on osteoporosis treatment, individuals with substantial comorbidity and other factors (for example, recent bereavement). After completing a Fracture Risk Assessment Tool (FRAX) questionnaire, participants underwent block randomization stratified by age (70–74, 75–79 and 80–85 years) and general practice. Owing to the pragmatic study nature, double-blinding was not feasible; however, research staff acquiring hospital fracture data were blinded to the participant study arm. A total of 12,483 participants were randomized to either the control arm or the screening arm, constituting 59,401 person-years of observation. The control arm comprised individuals receiving usual care (provided in primary care); individuals in the screening arm had their 10-year probability of fracture calculated using FRAX. Those at moderate to high risk underwent dual-energy X-ray absorptiometry (DXA) to calculate BMD. Treatment decisions were made in primary care on the basis of the above findings. In SCOOP, the primary outcome measure was the proportion of individuals sustaining fragility fractures (that is, not excluding fractures of the skull, hand, foot and nose) in each group. Secondary outcomes included the proportions of all fractures, hip fracture rate, cost-effectiveness, mortality and EQ-5D (a health-care quality assessment tool) in each group and a qualitative evaluation of participant acceptability. Effectiveness data analysis was performed using Cox's proportional hazards models. Linear models were used for quality of life analyses, and all relevant analyses were performed on an intention to treat basis. Economic analyses were obtained from a tax payer's perspective according to the costs to the National Health Service (NHS). A qualitative exploration of acceptability and adherence was performed. Data in FIG. 3 were first presented in REF.[130].

of osteoporosis treatments and reduced fracture incidence. In addition, a more recent randomized controlled trial of primary care-based screening was reported in 2012 (REF.[106]), which found that screening for osteoporosis increased prescription of osteoporosis medication at 6 months (OR 2.24, 95% CI 1.16–4.33). The primary difference between these studies and SCOOP is that, with SCOOP, the primary outcome was fracture incidence and not treatment uptake.

Results. The study population comprised women aged between 70 and 85 years, who were assigned to either a screening arm (those found to have moderate or high risk of fracture by FRAX underwent further assessment of BMD) or a control arm (receiving usual care, provided in a primary care setting; FIG. 3). The key effectiveness findings of the SCOOP study were published in 2018 (REF.[107]), although there were no significant differences in the primary outcome measure of all osteoporosis-related fractures between the screening arm and control arm (HR 0.94, 95% CI 0.85–1.03; $P = 0.178$) or the rate of all clinical fractures (HR 0.94, 95% CI 0.86–1.03; $P = 0.83$), as shown in TABLE 1. However, in a pre-specified analysis, the rate of hip fracture was statistically significantly lower in the screening arm (HR 0.72, 95% CI 0.59–0.89; $P = 0.002$).

In terms of numbers needed to treat, the absolute size in hip fracture rate reduction was 0.9%, which means that 111 women aged 70–85 years would need to be screened in order to avert a single hip fracture. Notably, the reduced risks that were observed in SCOOP were strongly affected by the efficacy of the currently available treatments, and as the efficacy of treatments rise, the risk of fracture will probably reduce. Osteoporosis medication use was higher in the screening group than in controls at the end of year 1 (15% versus 4%), with medication use being particularly high in the high-risk group at the 6-month time point (78%). There was no difference in mortality, anxiety or quality of life outcomes between the two groups.

Of the 6,233 participants randomized to the screening arm, 3,049 (49%) reached criteria for subsequent DXA assessment of BMD and 898 (14%) received treatment with osteoporosis medication by 6 months. At 1 year, 953 (15%) of individuals in the screening arm had received at least one treatment with osteoporosis medication, and this proportion remained relatively stable, between 13% and 15%, over the course of the 4 years of follow-up. In the control arm, 264 (4%) received an osteoporosis medication by 1 year, but this number steadily rose to 633 (10%) at 4 years. In terms of the fractures, across both arms, there were 1,975 fragility fractures, which affected 1,657 participants (13% of those randomized). The most common sites were distal forearm and hip. The qualitative work performed as part of the SCOOP study demonstrated that the screening was acceptable to women[108].

Trial limitations. The limitations of the SCOOP study include the fact that, of the eligible population, only one-third of individuals participated and that there appeared to be selection bias towards healthy individuals, with mortality lower than expected (9% observed versus 19% expected), and higher educational and socio-economic status. Relatively few participants were at high risk of fractures (14% observed versus 20–40% expected); however, the rates of fracture were higher than predicted. It is also possible that general practitioners may have been more likely to treat individuals in the control arm owing to the contamination of their involvement in an osteoporosis-related study.

Whether this model of population screening is eligible for national roll-out depends not only on efficacy but also on cost-effectiveness and the feasibility within the constraints of the public purse.

Cost-effectiveness analysis. Since the advent of the SCOOP study, there have been two helpful systematic reviews of cost-effectiveness in the field of fragility fractures. The first found that health economic models

Table 1 | **Efficacy outcomes for the SCOOP study**

Outcome	Control arm ($n = 6,250$)	Screening arm ($n = 6,233$)	HR (95% CI)	*P* value
Osteoporosis-related fractures				
No fracture	5,398 (86.4%)	5,428 (87.1%)	NA	NA
Fracture	852 (13.6%)	805 (12.9%)	0.94 (0.85–1.03)	0.178
Hip fractures				
No fracture	6,032 (96.5%)	6,069 (97.4%)	NA	NA
Fracture	218 (3.5%)	164 (2.6%)	0.72 (0.59–0.89)	0.002
All clinical fractures				
No fracture	5,248 (84.0%)	5,282 (84.7%)	NA	NA
Fracture	1,002 (16.0%)	951 (15.3%)	0.94 (0.86–1.03)	0.183
Mortality				
Survived	5,725 (91.6%)	5,683 (91.2%)	NA	NA
Died	525 (8.4%)	550 (8.8%)	1.05 (0.93–1.19)	0.436

Data in TABLE 1 were first published in REF.[130]. NA, not applicable; SCOOP, Screening of Older Women for Prevention of Fracture.

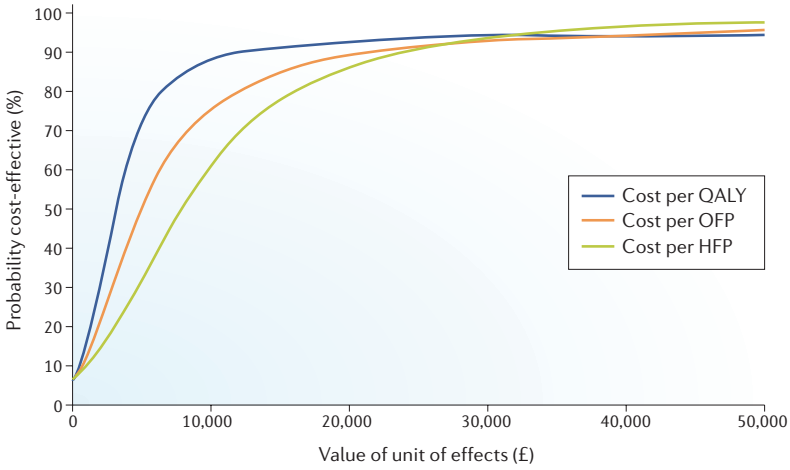

Fig. 4 | **Cost-effectiveness acceptability curves from the SCOOP study.** The graph depicts cost-effectiveness acceptability curves for cost per quality-adjusted life-year (QALY; blue line), per osteoporotic fracture prevented (OFP; orange line) and per hip fracture prevented (HFP; green line) from the Screening of Older Women for Prevention of Fracture (SCOOP) study. Figure reproduced from REF.[113], CC BY 4.0.

have recently evolved in terms of their complexity and emphasis[109], whereas the second purports to the cost-effectiveness of drug therapy for osteoporosis in post-menopausal women[110]. The latter review found that osteoporosis medications were cost-effective in women aged 60 years and over, particularly if additional risk factors for fracture were present[110].

Given that the SCOOP study was performed in the UK, the subsequent health economic analyses were performed according to this geography. A three-level EQ-SD (an instrument used to assess health-related quality of life) assessment provides a measure of quality-adjusted life-years (QALYs)[111]. The costs of DXA scans, clinical review, primary care consultations and written notifications in SCOOP were calculated locally through dialogue with the general practitioners involved. Inpatient, outpatient and emergency department data sets were run though a Healthcare Resource Group 4+reference costs grouper[112].

The key heath economic finding from the SCOOP study was that the screening model trialled was cost-effective. There was an increase of 0.0237 QALYs for participants in the active arm of the trial, with an £2,772 incremental cost per QALY in the screening arm versus the control arm[113]. The screening intervention also reduced fractures; the cost per osteoporosis-related fracture prevented was £4,478, and the cost per hip fracture prevented via the screening programme was £7,694. The cost-effectiveness acceptability curves suggested that there was a 93% probability of the screening intervention being cost-effective, at a value of >£20,000 per QALY, concluding that the screening programme was a highly cost-effective strategy[113] (FIG. 4).

A post hoc analysis focusing on those who are at high risk of fracture was published in 2018 (REF.[114]), which aimed to examine possible interactions between screening efficacy and baseline FRAX 10-year risk of fracture and fracture outcomes. Interactions were observed between history of prior fracture, parental fracture history, smoking and the efficacy of screening[114]. Importantly, in individuals at highest risk of fracture, the estimated reduction in hip fracture risk was >50%[114] (FIG. 5). Despite the limitation that not all participants included in the SCOOP trial had BMD measurements at baseline or during follow-up, the conclusion of the post hoc analysis was that those women who are at high risk of hip fracture on the basis of FRAX probability are responsive to appropriate osteoporosis management[114]. The greater reduction in hip fracture risk in those who had higher baseline risk strongly suggests that treatment rather than other factors explained the observed effect.

The effect of screening was greatest in those with the risk factors of prior history of fracture and parental history of fracture[114]. Of note, prior history of fracture and parental history of fracture respresent the most relevant clinical risk factors. These factors might have had some bearing on persistence and uptake of medications by study participants. The presence of these two factors might also have driven increased treatment rates in the screening arm.

In summary, if the SCOOP screening strategy is adopted in the UK for 70–85-year-old women (assuming the size of this population is similar to that estimated in 2016 (3.7 million)), it could prevent 8,000 hip fractures each year, would be cost-effective in doing so and would result in considerably better treatment adherence at 5 years of follow-up.

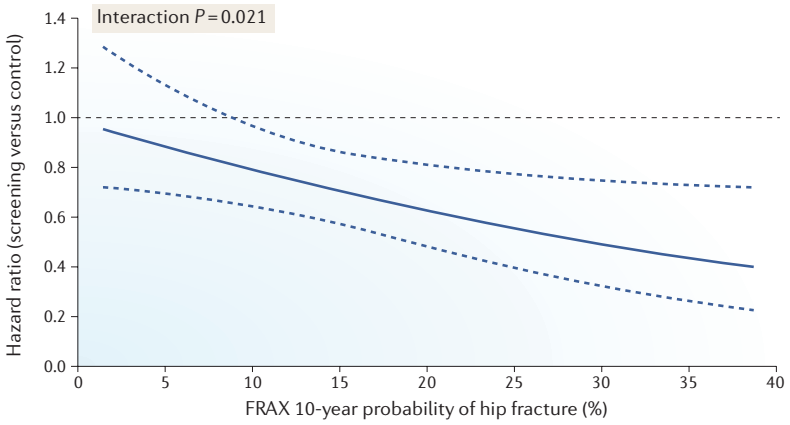

Fig. 5 | **The effect of screening on hip fracture rates in the SCOOP study.** The graph shows the effect of screening on hip fracture risk compared with the control arm, expressed as a hazard ratio, across a range of Fracture Risk Assessment Tool (FRAX) 10-year hip fracture probabilities calculated at baseline without BMD. An interaction is observed between effectiveness of screening and baseline probability of fracture ($P = 0.021$)[114]. Figure reproduced with permission from REF.[114], Wiley-VCH.

Conclusions

The past 20 years has seen a concerted shift from the definition of osteoporosis based on BMD to the effective identification (and therefore treatment) of individuals at risk of fracture. Fracture prediction algorithms such as FRAX and imaging modalities such as DXA present usable and highly effective tools to identify individuals at risk. Moreover, developments in research scanning have enhanced our scientific understanding of bone microarchitecture. As recent trial evidence clearly shows,

primary prevention of osteoporotic fragility fractures is not only effective but also cost-effective[107,113,114]. Despite this, there is still a concerning majority of at-risk individuals who are missed through a lack of assessment, and there must therefore be a concerted effort to address this issue if we are to close the ever-widening treatment gap. In the future, novel methods of fragility assessment (BOXES 1,2) might go some way to address this need.

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Garvan fracture prediction tool: https://www.garvan.org.au/promotions/bone-fracture-risk/calculator/
QFracture: https://www.qfracture.org/

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ORIGINAL RESEARCH

Vitamin D and Bone Mineral Density in HIV Newly Diagnosed Therapy-Naive Patients Without Any Secondary Causes of Osteoporosis

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Abstract
Bone loss and vitamin D deficiency are common in HIV patients. However, bone health status in newly diagnosed HIV patients has not been thoroughly described. Our aim was to assess the bone mineral density (BMD), bone resorption and vitamin D status in newly diagnosed HIV patients. A prospective observational study in HIV newly diagnosed therapy-naive persons. Patients with secondary causes of osteoporosis were excluded. Bone densitometry (DXA), a bone resorption marker (CTx), 25-hydroxyvitamin D (25OHD), CD4 count and HIV viral load (VL) were done in 70 patients. Vitamin D results were compared with a group of healthy volunteers. All patients were men, mean age 31 years (19–50). Low BMD (Z score ≤ 2.0) was found in 13%, all of them in lumbar spine, and in only one patient also in femoral neck. Bone resorption was high in 16%. One out of four participants had low BMD or high bone resorption. Vitamin D deficiency (25OHD < 20 ng/mL) was found in 66%. Mean 25OHD in patients was significantly lower than in healthy volunteers ($p = 0.04$). No associations were found between BMD, CTx, 25OHD and VL or CD4 count. We hypothesize that HIV infection negatively affects bone health based on the results we found among newly diagnosed, therapy-naive, HIV-infected patients, without any known secondary causes of osteoporosis. Low BMD or high bone resorption, are significantly prevalent in these patients. HIV-infected patients had a higher prevalence of vitamin D deficiency than controls, which was not correlated with CD4 count or VL.

Keywords HIV/AIDS · Bone density · Bone mineral density · Vitamin D · 25OHD

Introduction

As of 2016, 36.7 million people were living with HIV/AIDS worldwide and 61,000 cases of HIV or AIDS were notified in Chile [1]. With the development of highly active antiretroviral therapy (HAART), life expectancy has improved considerably among HIV-infected patients [2]. Currently, as mortality decreases, premature aging and non-infectious diseases, such as cardiovascular and bone metabolic diseases have emerged [3].

Bone loss is common in HIV patients. A 6.4 times greater risk of osteopenia and 3.7 times greater risk of osteoporosis have been reported in this population compared to general population [4]. Increased risk of fragility fractures has also been associated with HIV infection when compared to non-HIV infected subjects [5, 6]. Vitamin D deficiency is very common worldwide [7] and highly prevalent in the HIV-infected population [8–10]. Vitamin D deficiency is a classic risk factor for bone metabolic diseases such as osteomalacia and osteoporosis. Evidence is still inconclusive regarding vitamin D deficiency's role in predisposing HIV patients to other non-skeletal diseases, such as cardiovascular disease, diabetes and certain malignancies [11]. Nutritional compromise (including vitamin D deficiency), comorbidities, direct effect of HIV on bone, and effects of antiretroviral therapy (ART) may explain bone compromise in HIV patients. However, the risk of HIV on bone health in newly diagnosed, therapy-naive, HIV-infected patients without any secondary causes of osteoporosis has not been thoroughly investigated.

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This article presents baseline results from an ongoing, prospective, observational study investigating the risk of osteoporosis and levels of vitamin D in HIV patients after initiation of ART. Our aim for this basal stage was to assess the bone mineral density (BMD), bone resorption, and vitamin D status in newly diagnosed, therapy-naive, HIV-infected patients without any secondary causes of osteoporosis.

Methods

Study Population

Newly diagnosed, therapy-naive, HIV-infected subjects, men or women, who received medical attention at hospitals and outpatient clinics of our institution were recruited between June 2014 and June 2016. Newly diagnosed was defined as having an HIV serology confirmation up to 1 year prior to recruitment. Eligible patients for the study were invited to participate if their attending physicians estimated that they would start therapy no longer than 6 months after enrollment (according to the national treatment recommendations at that time) [12].

Exclusion criteria included: <18 years of age or >50 years, history of fragility bone fractures [13] or bone metabolic disease, severe low BMD at baseline (defined as Z score ≤ 3.0 at DXA) or abnormalities on biochemical parameters such as: calcium disorder (calcium corrected by albumin <8.5 mg/dL or >10.5 mg/dL), TSH <0.3 μUI/mL or >10 μUI/mL, in men, testosterone <200 ng/dL or <249 ng/dL with calculated bioavailable testosterone $<35\%$, and estimated glomerular filtration rate less than 60 mL/min based on serum creatinine, according to the Cockcroft method [14]. Women with absence of menses for the past 3 months or any patient with past medical history of diabetes mellitus, celiac disease, inflammatory bowel disease, gastrectomy, bariatric surgery, malignancies, an event of symptomatic nephrolithiasis documented in the year prior to recruitment or those using drugs that affect bone metabolism as bisphosphonates, calcitonin, denosumab, teriparatide, strontium, calcium, estrogen, testosterone, analogs of vitamin D, aromatase inhibitors, thiazolidinediones, anticonvulsants, corticosteroids and loop diuretics were also excluded [15–17].

Signed informed consent was obtained from each patient and the study was approved by the Ethics Review Committee of the School of Medicine of Pontificia Universidad Católica de Chile.

Data Collection

Clinical Data

Demographic information, medical history, including physical examination and risk factors for osteoporosis (i.e., alcohol abuse, present or past use of tobacco, sunlight exposure and estimation of daily calcium intake) were recorded. Alcohol abuse was defined as drinking more than three glasses of beer or wine/day or more than 40 g/day or if drinking habit brings social/labor problems or if a medical diagnosis of alcoholism is done. Sunlight exposure and daily calcium intake were estimated as previously reported [18].

Biochemical Assessment

A fasting serum sample was obtained to measure: calcium, albumin, phosphorus, total alkaline phosphatases, creatinine by autoanalyzer, TSH, testosterone, intact PTH, carboxy telopeptide (CTx), by electrochemiluminescence immunoassay, 25-hydroxyvitamin D (25OHD2 + 25OHD3) by liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) (AB Sciex QTrap® 4500), lymphocyte T CD4 count by Flow Cytometry (FACSCalibur) and VL by real-time PCR Ampliprep/cobasTaqman. Undetectable VL for our laboratory is <20 copies/mL. Normal values for PTH were 15–65 pg/mL and 0.016–0.584 ng/mL for CTx. Vitamin D deficiency was defined as 25OHD below 20 ng/mL. Corrected Ca was calculated by subtracting albumin from 4.0 and multiplying the difference by 0.8; then, the product was added to the measured calcium level [19]. All the assays were done at the Central Laboratory of our institution, which is affiliated with an external quality control program from the College of American Pathologists (USA), and the main methods are published previously [20].

BMD Assessment

BMD was measured at the lumbar spine (L2–L4), total hip and femoral neck. DXA used was from GE Medical Systems Lunar DPX, Madison, WI, USA. The same device was used in all patients and results were analyzed by the same radiologist. Quality controls were performed on DXA to verify the validity of imaging procedures, and DXA analysis was done according to the last ISCD recommendations. Z score was used for DXA analysis (subjects younger than 50 years), and low BMD was defined as Z score ≤ 2.0. Reference database provided by the manufacturer was used to derive Z score [21].

Control Group for Vitamin D Levels

For the comparison of vitamin D levels of HIV patients, a contemporary control group of 21 healthy volunteers was included. Control group was selected among blood donors of our institution and it was paired by sex, age, season of sampling, and residence in the same geographic area. Volunteers with autoimmune diseases, malabsorption syndrome, neoplasms, active infection, renal or hepatic diseases, and

subjects who received vitamin D greater than 50,000 UI up to 6 months prior to recruitment were excluded.

Statistical Analysis

Sample size was calculated to identify BMD potential loss in lumbar spine, changes in levels of 25OHD and CTx from baseline through week 52 after initiation of ART, to obtain a power of 80% and significance of 5% and assuming 10% lost to follow-up. Calculated sample size was 61 patients.

Lymphocyte T CD4 data is shown as median and IQR. Fisher's exact test was used to evaluate association between two categorical variables. Student's *t* test for equal or unequal variances was used to compare two means, as appropriate. Pearson's correlation coefficient assessed correlation between two continuous variables. Confidence interval was 95%. *P* value < 0.05 was considered statistically significant. Statistical analysis was performed on STATA SE 12.0.

Results

A total of 139 patients were invited to participate in this study; 43 of the 139 did not meet the inclusion criteria (26 received HIV serology confirmation more than 1 year before recruitment, 17 had already initiated antiretroviral therapy). Exclusion criteria eliminated five patients (three older than 50 years, and two due to diseases that may affect bone metabolism). Twenty-one additional patients met inclusion criteria but declined to participate. Thus, a total of 70 patients were finally recruited from June 2014 to June 2016 (Fig. 1) Given the anticipated 10% of patients lost to follow-up, we recruited an excess of nine patients over the estimated sample size (61% in autumn/winter season). Mean time between HIV diagnosis and study recruitment was 116 days (range 14–364). Table 1 shows patients' general characteristics. Although both male and female patients were invited to participate, only men were recruited, most of them were CDC Stage A.

Three out of 70 patients (4%) had comorbidities, including controlled high blood pressure (1) and congenital hypothyroidism (1), and one case of Von Willbrand's disease. One patient had Hepatitis B co-infection. No patients had Hepatitis C.

Biochemical and BMD Assessment

Biochemical findings are shown in Table 2. According to exclusion criteria of the study, all patients had normal serum calcium levels; 17.1% had hypophosphatemia (1.7–2.5 mg/dL), 7.1% had high levels of total alkaline phosphatases (116–217 U/L), and one case had concomitant high SGOT. Only two patients (2.86%) were diagnosed as having

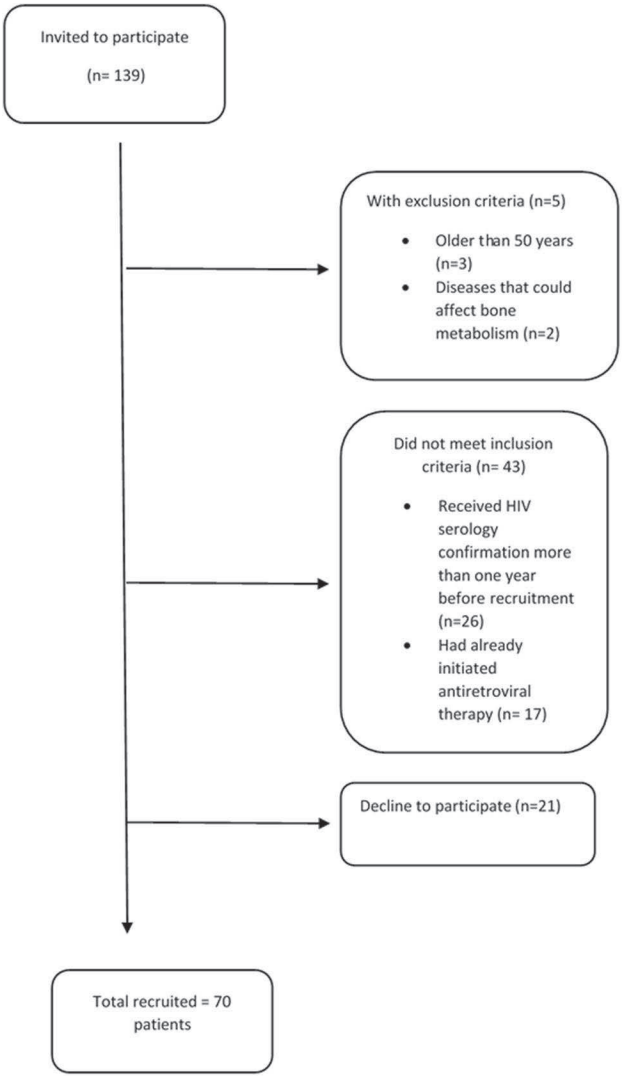

Fig. 1 Patients screened

secondary hyperparathyroidism (increased levels of PTH with normal serum calcium), both with severe vitamin D deficiency (25OHD 6.7 and 11.9 ng/mL).

Vitamin D deficiency was found in 66% of patients and in 48% in control group. The quartil distribution of 25OHD values in patients was as follows: < 10 ng/mL (5.7%), 10–19.99 (60%), 20–29.99 (29%), 30–39.99 (5.7%). There were no patients with vitamin D ≥ 40 ng/mL. Mean 25OHD in patients was 17.7 ng/mL (SD 6.6). Mean 25OHD in control group was 20.8 ng/mL (SD 6.6). Comparing patients versus controls, mean 25OHD was significantly lower in patients ($p = 0.04$) (Fig. 2). In control group, there was a higher BMI ($p = 0.009$) and sunlight exposure ($p = 0.016$) than patients; whereas sunblock use ($p < 0.001$) and smoking ($p = 0.006$) was lower (Table 3). In all subjects, measured 25OHD corresponded exclusively to 25OHD3, and 25OHD2 was always lower

Table 1 General characteristics of HIV patients

Characteristic	Result
Patients, *N*	70
Male, %	100
Sexual transmission of HIV, %	100
Mean age, years (range)	31 (19–50)
Mean BMI, kg/m^2 (SD)	24 (3.0)
Underweight (BMI < 18.5), %	1.4
Current smoker, %	74.3
Heavy alcohol drinkers, *n*	0
Illicit drugs, %	39
Mild or moderate exercise (≥ 15 min/twice a week), %	49
Normal sun exposure (score ≥ 3), %	51.4
Sunblock use, %	35.7
Mean daily calcium intake, mg/day (SD)	617 (193)
HIV CDC classification, stage A %	84
Median lymphocytes CD4 count, cells/mL (IQR)	370 (217)
High viral load (≥ 10^5 copies/mL), %	36

CDC Centers for Disease Control and Prevention, *SD* standard deviation, *IQR* interquartile range

Table 2 Biochemical findings in HIV patients

Serum	Reference value	Result
Calcium corrected by albumin	8.5–10.5 mg/dL	9.35 (0.35)
Phosphate	2.6–4.5 mg/dL	3.14 (0.57)
Total alkaline phosphatase	45–115 U/L	83 (23)
PTH	15–65 pg/mL	35.54 (17.19)
25OHD	20–60 ng/mL	17.7 (6.64)
Vitamin D deficiency, %	25OHD < 20 ng/mL	66
CTx	0.016–0.584 ng/mL	0.46 (0.18)
High bone resorption, %	CTx > 0.584 ng/mL	16

than sensitivity limit of the assay. Among the eleven patients with CTx over the upper reference value (16%), only one patient had low BMD. Exclusion criteria such as biochemical measurements were not included in these results.

BMD was low in nine patients (13%) (*Z* score ≤ 2.0). In all of them, BMD of the lumbar spine was compromised, and only one patient had concomitant involvement in mean femoral neck (Table 4). One out of four participants had low BMD or high bone resorption (Fig. 3).

Figure 3 shows a pie plot for combined categories of low BMD and high bone turnover in 70 VIH patients. Overall, 13% of patients had low BMD, 16% had high bone turnover and 27% had low BMD or high bone turnover.

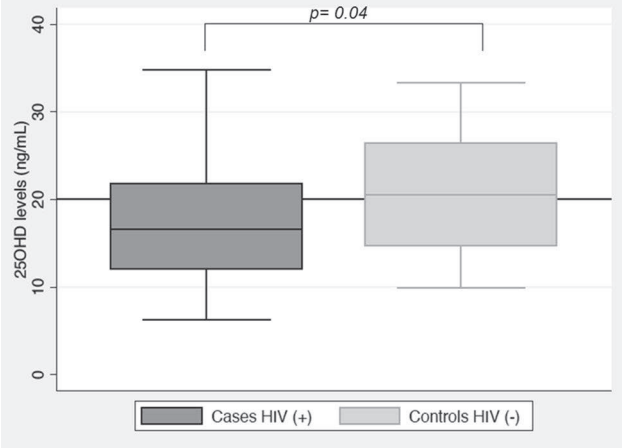

Fig. 2 Mean 25OHD levels in patients compared with controls

Bivariate Analysis

In the bivariate analysis, a comparison was made between media 25OHD, vitamin D deficiency, BMD with other variables such as age, BMI, season sampling, sunlight exposure, smoking, drug use, dairy intake, CD4 count, VL, CDC stage and CTx. There was a statistically significant association between levels of vitamin D and season sampling (16.0 ± 5.4 in winter/autumn vs. 20.5 ± 7.6 in spring/summer; $p = 0.009$). No other significant association was found. Moreover, no association was seen between 25OHD and BMD.

Discussion

This study shows that in newly diagnosed, therapy-naive, HIV-infected patients, without any known secondary causes of osteoporosis, HIV infection negatively affects bone health. In fact, nearly one out of four of these patients had low BMD or high bone resorption, both conditions well-known risk factors for osteoporotic fractures [16]. HIV-infected patients additionally had a higher prevalence of vitamin D deficiency when compared to controls.

A high prevalence of low bone mass and osteoporosis has been reported in multiple studies of HIV-infected patients [4, 22–27]. Several cohort studies have shown increased rates of bone fracture among HIV-infected patients compared to uninfected controls [5, 6, 28, 29]. In the HIV Outpatient Study (HOPS), a large and diverse cohort of 5,826 HIV-infected US adults in treatment, the age-adjusted fracture rates were 1.98–3.69 times higher than rates in the general population [28]. However, given that these studies involved patients enrolled in ART, they cannot conclude that higher risk of bone fracture is because of the HIV infection by itself, the ART, or other comorbidities frequently experienced by

Table 3 Contributing factors for Vitamin D levels in HIV patients and controls

	Patients $n=70$	Healthy volunteers $n=21$	p value
Sex (male), %	100	100	
Age (years)	31.2 (8.0)	33.3 (8.2)	0.311
BMI (kg/m^2)	24.0 (3.0)	25.9 (2.7)	**0.009**
Season of the year, autumn/winter, %	61.4	52.4	0.459
Normal sun exposure (score ≥ 3), %	51.4	81.0	**0.016**
Sunblock use, %	35.7	4.8	**0.006**
Current smoker, %	74.3	14.3	**< 0.001**
Vitamin D levels (ng/mL)	17.7 (6.6)	20.8 (7.0)	0.04

Statistically significant p values are highlighted in bold

Results are expressed as mean (SD)

n Number of patients or number of healthy volunteers

Table 4 Bone densitometry findings in HIV patients

Bone densitometry findings	Result
Lumbar spine (L2–L4)	
BMD (g/cm^2)	1.172 (0.142)
Z score	−0.3 (1.2)
Low BMD, n (%)	9 (13)
Mean femoral neck	
BMD (g/cm^2)	1.047 (0.142)
Z score	0.0 (1.1)
Low BMD, n (%)	1 (1)
Mean total hip	
BMD (g/cm^2)	1.041 (0.134)
Z score	−0.2 (0.9)
Low BMD, n (%)	0 (0)

Results are expressed as mean (SD)

HIV-positive patients, such as hepatitis C virus co-infection, hypogonadism or low body mass index.

There is limited data that estimates the relative contribution of untreated HIV on bone loss in HIV-infected patients naive to ART. Reported prevalence of low BMD in this group of patients is diverse. A study by Masyeni et al. [30] showed that almost 90% of participants showed any grade of low bone mass and approximately 10% had osteoporosis. Previous to the aforementioned study, in a group of 44 patients, aged between 26 and 30 years, the prevalence of osteopenia was 26% [31]. The ACTG 5260 trial was a substudy of the ACTG A5257 randomized clinical trial, which determined cardiovascular and metabolic analysis. This substudy included 331 patients, 10% with low BMD [32]. Recently, the START BMD substudy, involving 424 ART-naive participants, showed a 1.9% prevalence of osteoporosis and 35.1% of low BMD. These findings were associated with traditional risk factors but not with CD4 cell count or viral load [33]. In a large clinical trial of vitamin D and calcium

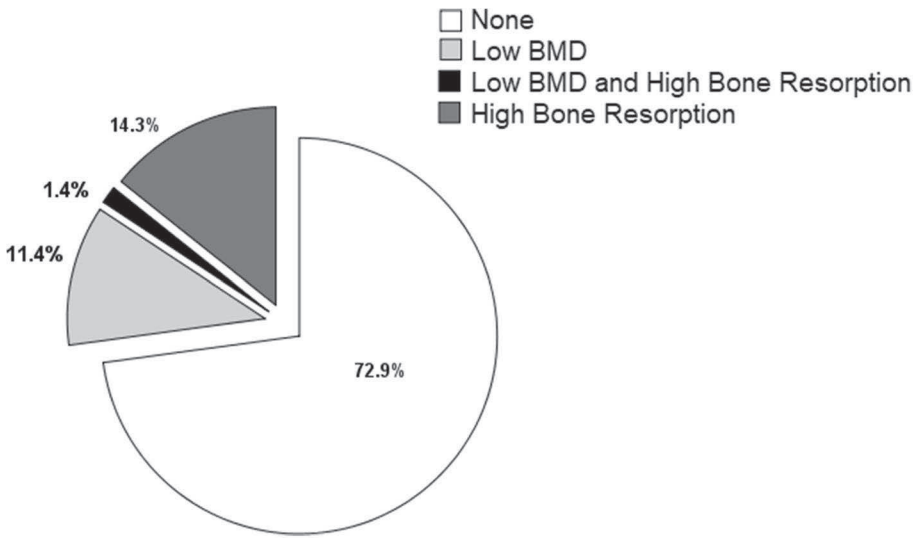

Fig. 3 Percentage of low BMD and/or high bone resorption

supplementation to prevent bone loss in HIV naive-infected individuals, baseline BMD *Z* score was below − 2.0 at lumbar spine in 9% of the intervention group and 10% of the placebo group, showing similar results to our study [34]. A recent study of zoledronic acid infusion in HIV-infected patients administered at ART initiation showed baseline prevalence of osteopenia was 35.4% in the placebo group and 21.9% in the zoledronic acid group [35]. It is important to note that only a few of these studies have exhaustively excluded secondary causes of osteoporosis or included bone turnover markers [32, 34, 35], both of which were performed in our study.

Carrying HIV is a state that has been associated with bone loss by several mechanisms. Certain viral proteins on bone cells or HIV-associated inflammation may directly affect bone health [34]. In addition, there are other multiple risk factors related to the host, such as age, smoking, alcohol use, Hepatitis C virus co-infection, hypogonadism, low body mass index, antiretroviral therapy, among others that influence in bone loss [4, 5, 36, 37]. We did not find any association between BMD or CTx and HIV viral load, age, tobacco, physical activity, calcium daily intake or vitamin D levels. We believe the small sample size, strict criteria of enrollment, and the high percentage of the patients that were in the initial stage of HIV may explain this lack of association.

This study, performed in patients without any known secondary causes of osteoporosis, showed a high prevalence of vitamin D deficiency (66%) in HIV antiretroviral-naive patients, significantly higher than healthy volunteers paired by sex, age, season of sampling and residence in the same geographic area. However, both groups were not matched by some contributing factors for vitamin D levels, such as sunlight exposure, sunblock use and smoking, which could determinate lower levels of vitamin D in HIV patients. On the other hand, a higher BMI in control group could determinate a lower level of vitamin D in this group. High rates of vitamin D deficiency have been described worldwide and also in our country [7, 18]. In HIV-infected patients, vitamin D deficiency has also been found to be highly prevalent. However, most of this data came from patients on ART that had either complications or drugs that could affect vitamin D metabolism. Therefore, it is not clear if HIV, in itself, caused vitamin D deficiency and if this deficiency is truly higher than levels in the general population. Data of vitamin D status in antiretroviral-naive patients are limited, and most of them do not include a control group or exhaustively excluded factors that could affect vitamin D metabolism [8, 9, 38]. Although the difference found in vitamin D levels between patients and healthy volunteers might be explained by several factors, as we mentioned before, we can not rule out that HIV infection itself could induce vitamin D deficiency.

HIV viral load and proinflammatory state could have a role in vitamin D catabolism. As Bearden et al. have mentioned, vitamin D levels could be low in ART-naive individuals newly diagnosed with HIV, since the hormone is depleted as response to viral triggering 1,25(OH)2D-dependent immune defense mechanisms. Alternatively, renal conversion of 25(OH)D to 1,25(OH)2D may be inhibited and the 25OHD being catabolized to inactive metabolites [39].

Another perspective is perhaps these low levels of vitamin D could have a role in a greater HIV replication, as it is shown by Coussens et al. This study showed that patients with lower levels of 1,25-dihydroxyvitamin D resulted in greater productive HIV-1 infection and a high dosage of vitamin D supplementation reversed serum 25OHD deficiency and attenuated the seasonal increase in ex vivo HIV replication [40]. Fabre-Mersseman et al. strengthens this hypothesis when they supplemented with vitamin D patients with severe 25OHD deficiency, showing a reduction in immune activation levels [41].

Vitamin D deficiency progressively leads to low intestinal calcium absorption, secondary hyperparathyroidism, high bone turnover, mineralization defects and bone loss. It also induces myopathy and falls. Moreover, vitamin D deficiency increases the risk of osteoporotic fractures [42]. Vitamin D may play a role in several other non-skeletal functions such as immune response, cellular growth, blood pressure control, and insulin sensitivity. Several studies have described an association between vitamin D deficiency and infections, cancer, and autoimmune and cardiovascular diseases. Therefore, we see increasing evidence in vitamin D's role in the human immune response to HIV. Conversion of vitamin D to its active form ($1,25[OH]_2D$) generates inhibition of HIV replication in macrophages [39]. We did not find an association between vitamin D levels and HIV viral load. An association between higher viral load and vitamin D deficiency is still unclear from previous studies. Recently, Cervero et al. showed that lack of viral suppression was associated with a 3.5 times higher risk of vitamin D deficiency [43]. Another study showed a nonlinear relationship (U-shaped) between viral load and $1,25(OH)_2D$ [39], whereas in others, no association was shown [8]. Given the sample size of our study, we were unable to explore this association; however, our data did not support the relationship between HIV viral load and vitamin D deficiency.

Our study has some limitations. Recruitment of patients at a single site with specific geographic and social characteristics may limit external validity. Additionally, the absence of women in the study is concordant with the minor presence of HIV-infected women treated at our institution [44]. Due to budget limitations, our study protocol did not include other vitamin D metabolites (e.g., 1,25OHD; 24,25OHD), proinflammatory cytokines, or calcium and phosphorus urine excretion that would provide a more comprehensive analysis of possible changes

in vitamin D and mineral metabolism associated with HIV infection. BMD and CTx were not measured in the control group to compare with the study group. However, we did utilize internationally accepted criteria to interpret such results.

Despite these limitations, our study has important strengths, such as a rigorous protocol of exclusion of secondary causes of osteoporosis, the use of CTx as a bone resorption marker in addition to DXA to estimate the risk of osteoporotic fractures, and the inclusion of a control group to compare vitamin D levels, which were measured using one the best reference methods (LC-MS/MS) to measure 25OHD [45].

In summary, HIV infection negatively affects bone health based on the results we found among newly diagnosed, therapy-naive, HIV-infected patients, without any known secondary causes of osteoporosis. These patients also experience a higher prevalence of vitamin D deficiency than controls. Our results emphasize the importance of assessing bone health in therapy-naive, HIV-infected patients, and underline the role of HIV infection itself as a risk factor for osteoporosis.

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Author Contributions MEC originally designed, conducted the study and prepared the first draft of the paper. CC contributed to the experimental work. JJ and AD were responsible for statistical analysis of the data. GG helped in the design of the study and made corrections to the paper. He is the guarantor. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

Compliance with Ethical Standards

Conflict of interest María Elena Ceballos, Camila Carvajal, Javier Jaramillo, Angelica Dominguez, and Gilberto González declare that they have no conflicts of interest.

Ethical Approval All procedures performed in this study were in accordance with the standards of Ethics Review Committee of the School of Medicine of our institution (Pontificia Universidad Católica de Chile) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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Case report

Severe osteoporosis with multiple spontaneous vertebral fractures in a young male carrying triple polymorphisms in the vitamin D receptor, collagen type 1, and low-density lipoprotein receptor-related peptide 5 genes

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ABSTRACT

Osteoporosis is a common disease with a strong genetic component. Several studies have reported the vitamin D receptor (VDR), collagen type I (COL1A1), and LDL receptor-related protein 5 (LRP5) genes as the most likely candidates. However, most of the studies have been carried out in postmenopausal women and older men and show inconsistent results. CASE PRESENTATION: We report a case of a 26-year old male who presented with severe back pain of acute onset, unrelated to any kind of trauma, and diffuse myalgia. Imaging of the lumbar and the thoracic spine revealed two Grade 3, according to Genant's semiquantitative method, vertebral fractures in T10 and T11 and multiple Grade 1 and 2 fractures from T8 to L2. Measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy) showed severe osteoporosis of the lumbar spine (Z-score=-3.0, BMD = 0.866 gr/cm^2). A complete laboratory and biochemical work-up was performed to exclude secondary causes of osteoporosis. Total genomic DNA was extracted from peripheral blood and was used as a template for genotype analysis. The patient was heterozygous for the p.V667M mutation of the LRP5 gene and for the BsmI [g.63980 G→A, rs1544410] and Sp1 polymorphisms [g.6252 G→T, rs1800012] of the VDR and COL1A1 genes, respectively. Further genotype analysis excluded types of osteogenesis imperfecta associated with mutations in the COL1A1 and COL1A2 genes. CONCLUSION: We herein show that the co-existence of three polymorphic sites in the VDR,

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COL1A1, and LPR-5 genes in a young male adult caused severe osteoporosis with multiple fractures, suggesting a combined effect and/or interaction between these genes.

Key words: COL1A1 polymorphism, Idiopathic osteoporosis, LRP-5 mutation, Multiple fractures, VDR polymorphism, Young adult

INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone mass and abnormal bone microarchitecture leading to increased fracture risk.[1] In postmenopausal women and men aged 50 and above, estrogen deficiency, vitamin D deficiency, secondary hyperparathyroidism, and age-related changes in bone tissue contribute to accelerated bone loss and increased bone fragility.[2-4]

Low bone mass in children and young adults does not necessarily imply skeletal fragility unless the patient sustains low trauma or atraumatic fractures.[5] Low bone mass in young adults could represent either attainment of low peak bone mass in relation to their body size, pubertal timing, and environment during growth,[6,7] a pathological condition with bone fragility due to chronic diseases and secondary causes of osteoporosis, or a genetically predisposed/idiopathic condition.[8] Osteoporosis is considered to be a disease with a strong genetic component of about 40-60%. Polymorphisms and/or mutations in vitamin D receptor (*VDR*), the collagen type I alpha1 (COL1A1), and the low-density lipoprotein receptor related-protein 5 (*LRP5*) genes have independently shown significant associations with bone mineral density and increased fracture risk. In addition, in genome-wide association studies, several single nucleotide polymorphisms (SNPs) have been identified as being associated with bone density or fracture risk at the genome-wide significance level.[9-11] However, most of these studies concern postmenopausal women and men aged above 50 years, while data on younger individuals are scarce. We present a case of a young male adult with severe osteoporosis and multiple atraumatic vertebral fractures in whom, after exclusion of a chronic disease and other secondary causes of osteoporosis, a genetic background of polymorphisms and mutation in all three *VDR*, *COL1A1*, and *LRP5* genes was revealed.

CASE PRESENTATION

A 26-year old male was referred to our center due to severe back pain of acute onset, unrelated to any kind of trauma, and diffuse myalgia. During the past 6 months, the patient had visited the outpatient clinics of the Rheumatology and the Orthopedic departments several times complaining of diffuse pain of moderate intensity along the spine, which was aggravated when lying in bed or sitting. At that time he was diagnosed with seronegative spondyloarthritis and was prescribed non-steroidal anti-inflammatory drugs and methotrexate. His clinical condition, however, was not improved. His medical record was free of any kind of systemic disease, as was also his family medical record. More specifically, there was no family history of frequent fractures, childhood or adolescent osteoporosis, osteogenesis imperfecta, gross skeletal anomalies, rickets, discoloured sclera, or early onset of hearing loss. Clinical examination revealed no other abnormalities.

X-rays and magnetic resonance imaging (MRI) of the lumbar and the thoracic spine revealed two Grade 3, according to Genant's semiquantitative method, vertebral fractures in Th10 and Th11 and multiple Grade 1 (T12, L1) and Grade 2 (T8, T9, L2) fractures (Figure 1). Measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy) showed severe osteoporosis of the lumbar spine (Z-score=-3.0, BMD=0.866 gr/cm^2) (Figure 2). A complete laboratory and biochemical work-up was performed to exclude secondary causes of osteoporosis (Table 1). The patient was found to have low-normal levels of 25-OH-vitamin D and was prescribed cholecalciferol 2200units/d per os.

GENETIC ANALYSIS

Total genomic DNA was extracted from peripheral blood and was used as a template for genotype analysis.

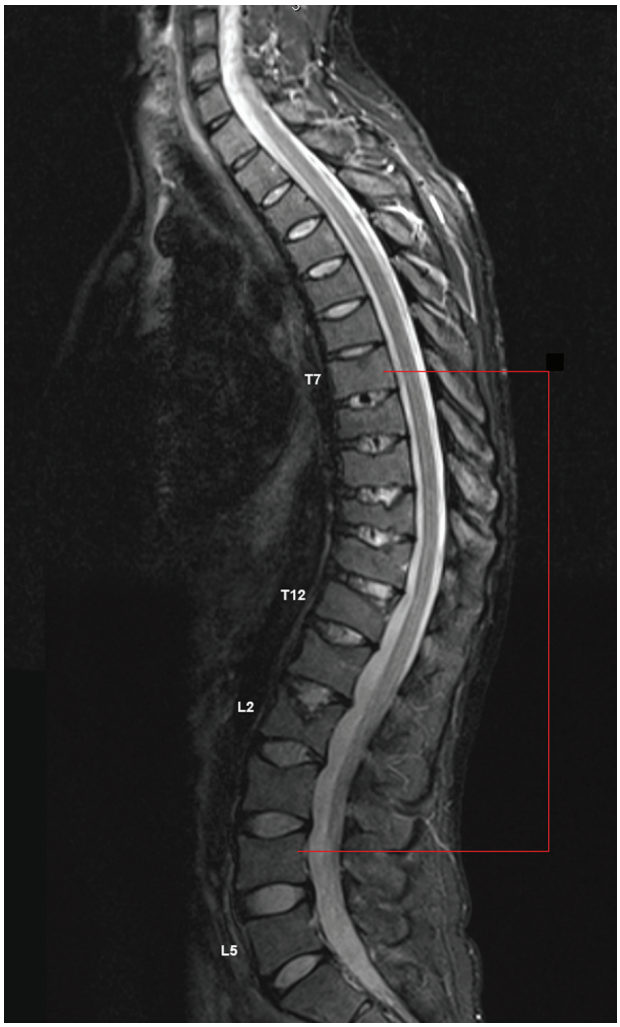

Figure 1. MRI of the thoracic and lumbar spine.

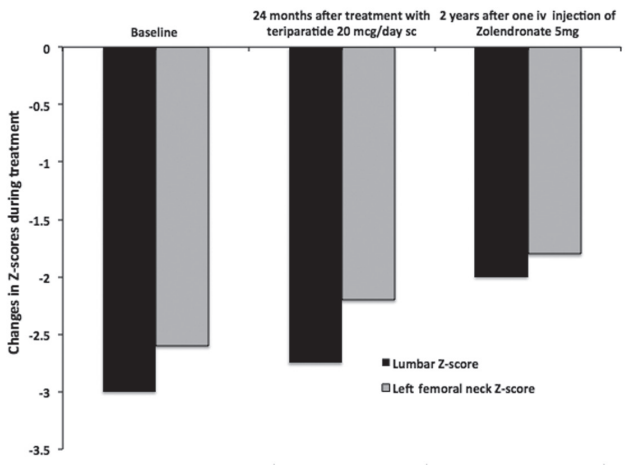

Figure 2. Changes in Z-scores of the lumbar spine and left femoral neck during treatment with teriparatide (20mcg/day sc) and zolendronate (5mg single iv injection).

Table 1. Laboratory work up and baseline hormone profile at the day of admission

Assay	Normal Range	Value
Hematocrit (Hct)	38.8-50.0%	42%
Fasting blood glucose	3.9-5.5 mmol/L	4.2
Alanine aminotransferase (ALT)	7-56 U/L	34
Aspartate aminotransferase (AST)	10-40 U/L	22
Alkaline phosphatase (ALP)	25-100 U/L	44
Serum creatinine levels	80-110 μmol/L	92
Ionized calcium	1.1-1.35 mmol/L	1.2
Phosphate	0.8-1.5 mmol/L	0.92
Parathyroid hormone (PTH) (pg/ml)	10-65	35
25-OH-vitamin D levels	75–250 nmol/L	77
24-Hour urine calcium	15–20 mmol/24h	18.7
Magnesium	1.5-2 mEq/L	1.75
Thyroid-stimulating hormone (TSH) level	0.5-5 mIU/L	3.2
Ferritin	33-450 pmol/L	104
Serum iron	10.7-26.9 pmol/L	15
Testosterone levels	10-25 nmol/L	20
24-Hour urine free cortisol	9.66-124.2 nmol/24h	32
Morning serum Cortisol	7-28 μg/dL	16
Serum protein electrophoresis		
albumin	38–50 g/L	40
alpha-1 globulin	1–3 g/L	2.2
alpha-2 globulin	6–10 g/L	7.5
beta globulin	7–14 g/L	8.1
gamma globulin	7–16 g/L	8.9
Antigliadin antibodies (IGA/IGG)		Not detected
Antiendomysial antibodies		Not detected
Serum tryptase	<11.4 ng/mL	3.2
Rheumatoid factor	<25 IU/ml	17

Types of osteogenesis imperfecta associated with mutations in the COL1A1 and COL1A2 genes were excluded.

We subsequently performed genotype analysis for genes known to correlate with the genetic background of osteoporosis, such as *LRP5, VDR* and *COL1A1,* using a novel Real-Time PCR assay based on SimpleProbe®melting curve analysis. The patient was found to be heterozygous for the p.V667M mutation of the *LRP5* gene and for the BsmI [g.63980 G→A, rs1544410] and Sp1 polymorphisms [g.6252 G→T, rs1800012] of the *VDR* and *COL1A1* genes, respectively.

TREATMENT AND FOLLOW-UP

The patient was treated with daily s.c injections of recombinant teriparatide for 24 months and supplementation with calcium (1000 mg/daily) and vitamin D (800 IU/daily). During the first 6 months of treatment, his back pain and diffuse myalgia improved significantly and the patient did not sustain a new vertebral or non-vertebral fracture. After the completion of the 24-month teriparatide-treatment the patient showed significant gains in the bone mass of the lumbar spine (Z-score=-2.2, BMD=0.970 gr/cm^2) and received an i.v injection of zoledronate 5 mg. Two years later the patient was free of symptoms: he had a BMD measurement in the osteopenic range in the lumbar spine and left femoral neck (Figure 2), there was no history of new fractures, and there were no new morphometric fractures in the thoracic and lumbar spine based on new X-rays. Serum levels of bone formation and bone resorption markers remained in the lower quartiles (Figure 3). In addition to anti-osteoporotic treatment, the patient was subjected to a rehabilitation program because of his long-lasting immobilization due to pain and fear of falling. The program included strengthening in joint mobility, clearance in the extremities and back muscles, posture exercises, and walking training. At the end of treatment, significant improvements were observed in walking, lower and upper extremity muscle strength levels, and posture.

DISCUSSION

The *BsmI* polymorphic site of the *VDR* gene, as well as the polymorphic Sp1 binding site of the *COL1A1* gene, and the *V667M* mutation of the LRP5 gene have been independently associated with osteoporosis and fracture risk. We herein show that the co-existence

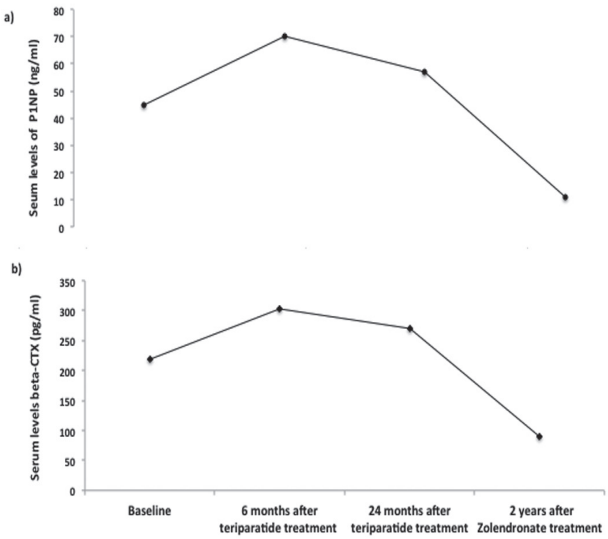

Figure 3. Changes in serum levels of the bone formation markers P1NP (a) and the bone resorption marker beta-CTX (b) during treatment with teriparatide (20mcg/day sc) and zolendronate (5mg single iv injection).

of these three genetic changes in a young male adult caused severe osteoporosis with multiple fractures, suggesting a combined effect and/or interaction between these genes.

The *VDR* gene was among the first candidate genes studied for association with osteoporosis.[12] The active metabolite of vitamin D (1,25 OH D3) acts through its specific receptor, VDR, which is a nuclear transcription factor regulating the expression of the target genes through binding to vitamin D responsive elements.[13] Mutations at the key sites of the *VDR* gene have been reported to cause vitamin D deficiency even when vitamin D itself is supplemented adequately.[14] This was a critical finding since insufficient serum levels of 25-OH-vitamin D are a well-established risk factor for osteoporosis and increased fracture risk.[15] In our case, the initial symptoms of diffuse myalgia, which could be attributed to low vitamin D levels, were successfully treated with vitamin D supplementation. The most frequently studied polymorphisms of the *VDR* gene in association with osteoporosis include BsmI, ApaI, TaqI, FokI,[16] and Cdx2.[17,18] The effect of the VDR genotype on BMD has been found to be stronger in premenopausal women and to decrease with age,[19] but several studies have produced inconsistent results with some showing positive[20-23] or no association between VDR gene polymorphisms

and BMD values.[24-26] Association of fracture risk with *VDR* polymorphisms has also been a matter of debate, with one showing significant association of BsmI and fracture risk and another no association between any genotype of VDR polymorphism and fractures.[27,28] Most of the studies, however, concerned postmenopausal women and older men and thus data regarding the association of the BsmI polymorphism with osteoporosis and fracture risk among the young are scarce.

The *COL1A1* and *COL1A2* genes encode the two alpha chains (alpha 1 and alpha 2, respectively) of collagen type 1 that trimerize to form the procollagen 1 molecule,[29] the main component of bone matrix. Mutations in these genes account for approximately 90% of osteogenesis imperfecta (OI), which is a form of inherited osteoporosis in children characterized by low bone mass, fragile bone with increased fracture risk, blue sclerae, and in some cases impaired odontogenesis. Mild cases of OI can result in the diagnosis of osteoporosis in adulthood or at an advanced age[30] and, at present, almost 17 genetic causes of OI have been identified.[31] Several large-scale studies have demonstrated an association between the polymorphism affecting the Sp1 binding site of the COL1A1 gene and low bone mass and increased fracture risk in the general population.[32-34]

The LRP5 gene encodes for the LRP5 co-receptor of the Wnt signaling pathway, which is of critical importance in bone metabolism regulating osteoblastogenesis and bone formation.[35] Wnt ligands bind to the Frizzled-LRP 5/6 receptor complex in the cell membrane and activate intracellular Wnt signaling. Activation is mediated either through translocation of the cytoplasmic protein beta-catenin to the nucleus, where it acts as a transcription factor activating the transcription of the target genes (canonical pathway), or via calcium and cAMP signals (non-canonical pathway). Loss-of-function mutations in the *LRP5* gene cause the osteoporosis-pseudoglioma syndrome,[36] an autosomal recessive disorder characterized by severe juvenile-onset osteoporosis and congenital or juvenile-onset blindness.[37] Activating mutations, on the other hand, are responsible for the autosomal dominant high bone mass trait.[38] In a recent multicenter study of 37,534 participants, it was demonstrated that the V667M (in exon 9) and A1330V (in exon 18) polymorphisms of the *LRP5* gene are associated with low BMD values and increased risk of fractures.[39]

Our patient suffered from unspecific musculoskeletal symptoms, such as arthralgia and diffuse myalgia, which were initially attributed to seronegative spondyloarthritis, thus delaying the final diagnosis. The lack of evidence of a systemic chronic disease and secondary osteoporosis and the severity of his bone disease with multiple spontaneous vertebral fractures led us to look for a genetic background whereby we were able to further exclude inherited forms of juvenile osteoporosis, such as OI. The co-existence of mutations in more than one of the genes related to osteoporosis in young adults has not been evaluated so far and larger studies are needed to elucidate a possible interaction and synergistic effect between the implicated genes. Administration of teriparatide, as the only bone anabolic agent currently available, followed by an antiresorptive agent and calcium plus vitamin D supplementation proved very effective in our patient, who continues to remain free of symptoms and with no new fractures over a long period of time.

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