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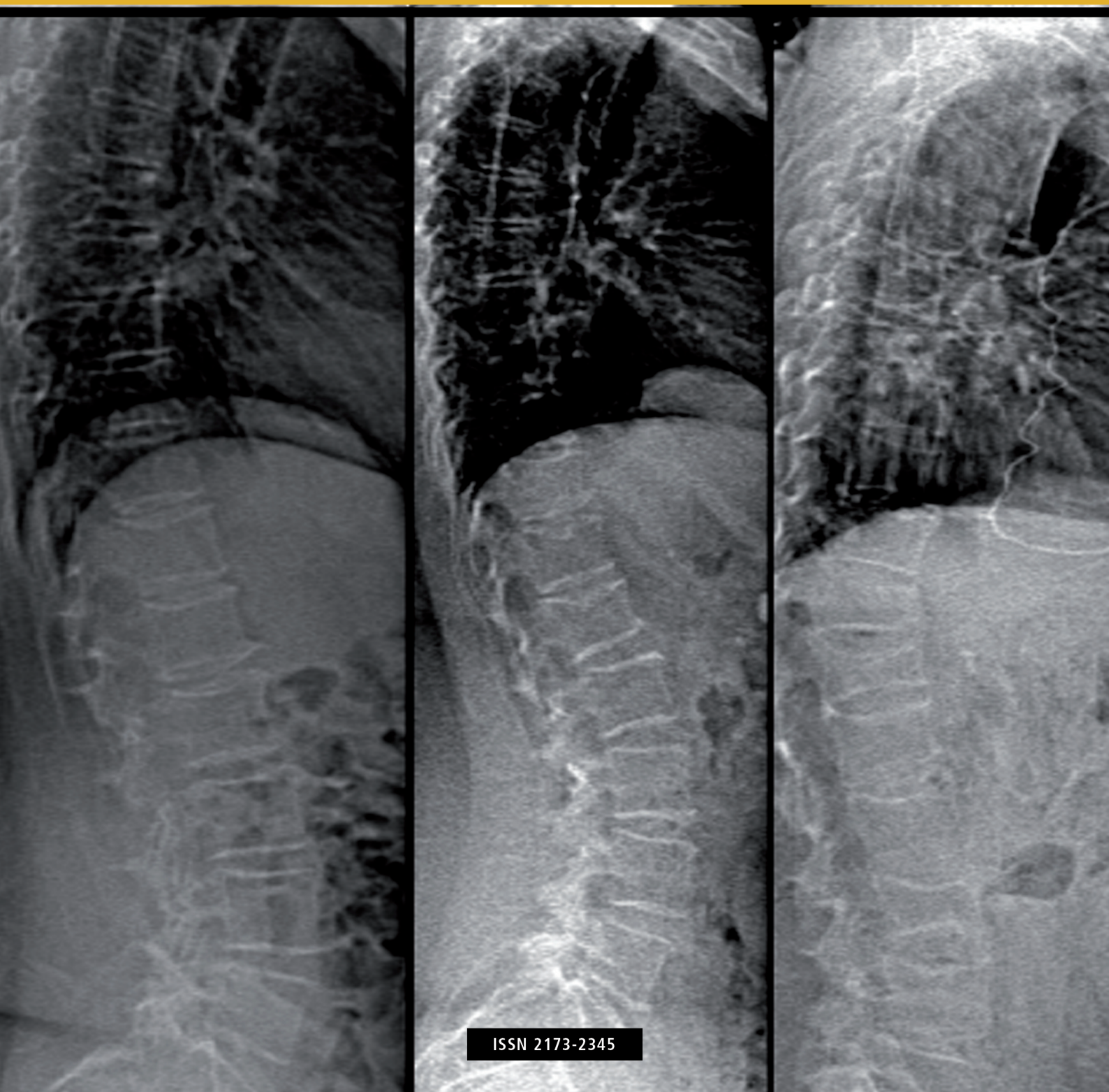

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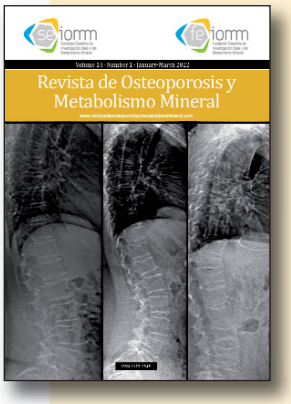

Our cover: Vertebral morphometry images by X-ray absorptiometry, more commonly known by its English nomenclature Vertebral Fracture Assessment (VFA).
Authorship: Unidad de Metabolismo Óseo. Hospital Universitario Marqués de Valdecilla. Santander.

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Towards an individualised approach to management of osteoporosis

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The treatment and management of osteoporosis, like any other disease, should be evidence-based in order to give the patients the best chance of limiting the consequences of the disease. Osteoporosis is a very common condition, affecting more women than men, and often overlooked and undertreated. The updated clinical practice guideline on postmenopausal, glucocorticoid induced, and male osteoporosis from the Spanish Society for Bone and Mineral Metabolism Investigation (SEIOMM)[1] is an important tool for clinicians with respect to diagnosis, future fracture risk assessment, and treatment of osteoporosis.

The diagnostic criteria are based on DXA and the presence of fractures, the criteria are not new, but the emphasis on recent fractures is new and worth noticing. A patient with a prior major osteoporotic fracture has a higher risk of fracture than a person at the same age without a fracture for up to 10 years following the first fracture, however, the risk in the 2 years immediately following the fracture is several times higher[2]. Therefore, the period following a fracture is a window of opportunity for prevention of the next fracture. The Fracture Liaison Service concept was developed to reduce the worldwide gap in fracture patients being assessed for osteoporosis.

The concept was developed more than 2 decades ago and although being implemented at a variable rate around the world, more and more evidence seems to suggest that the concept of systematically investigating fracture patients for osteoporosis is a cost-effective approach by reducing the risk of the next fracture[3].

The guideline divides postmenopausal women with osteoporosis into three risk categories based on a combination of prevalence of fractures, BMD and clinical risk factors. The three risk categories are well defined and leave room for an individualized assessment of fracture risk, however, the important concept of imminent fracture risk is not incorporated in the algorithm. There is always a balance between keeping such algorithms simple and providing the needed information, but in this case an arrow from the high risk group to the very high risk group in the case of a recent fracture could easily have indicated this association between a recent fracture and a higher fracture risk.

There is an increasing amount of evidence supporting the recommendation of using bone anabolic treatments; teriparatide or romosozumab in women at high risk of fracture. The VERO trial clearly showed that teriparatide is superior to risedronate in preventing vertebral and clinical fractures in women at high risk of fracture[4]. Similarly, the ARCH trial demonstrated that romosozumab for 12 months followed by alendronate is superior to alendronate in preventing vertebral, clinical, non-vertebral and hip fractures in women with severe osteoporosis[5]. In addition, there is also evidence to suggest a greater benefit on BMD improvement when using bone anabolic treatment before an antiresorptive, compared to the reverse sequence[6,7]. Although the discussion about a treatment target in the individual patient is still ongoing, the work of the FNIH Bone Quality working group has clearly shown that BMD and increase in BMD in response to treatment are important predictors of future fracture risk[8]. It is therefore important to improve BMD as much as possible, especially, in patients at high or very high risk of fracture.

The moderate risk group comprises the largest number of patients and considering the low grade of evidence for an anti-fracture effect of the SERMs it is somewhat surprising to see SERMs being the first choice of treatment in this group of patients. The evidence from the clinical trials investigating the more potent bisphosphonates; alendronate, risedronate and zoledronate and denosumab have demonstrated that these treatments are effective and reduce vertebral as well as non-vertebral fractures in postmenopausal women with osteoporosis. In addition, these treatments will increase BMD more than the SERMs and for the bisphosphonates allow for periods of treatment interruption.

This updated guideline leaves behind the strategy that one treatment, typically oral bisphosphonate and one regimen, typically oral bisphosphonate for 5 years are the best for all patients. The updated guideline has a very clear individualized approach to the choice of initial treatment as well as long-term management of osteoporosis. The long-term management algorithm is less evidence based due to the lack of well conducted clinical trials investigating the long-term management of osteoporosis. The recommendations for treatment duration of SERMs and denosumab are based solely on the duration of the clinical trials performed and the lack of information about beneficial effects and adverse effects thereafter. The suggested treatment durations for bisphosphonates are based on small studies on treatment discontinuation. This approach to defining treatment duration is clearly different from most other medical diseases and treatments. Although most studies of treatment of hypertension and diabetes have a duration of a few years, it is not recommended to discontinue these treatments after a few years in clinical practice. The increasing risk of rare adverse effects like osteonecrosis of the jaw and atypical femur fracture with increasing treatment duration should be taken into account and the benefit-risk balance considered individually in every patient; however, the benefit-risk balance was very clearly positive after 10 years with denosumab in the FREEDOM trial[9] as the incidence of these rare adverse effects was very low. It is difficult to imagine that the benefit-risk balance would change dramatically in the following years, if the patient is still at risk of fractures.

Bisphosphonates are the exception among the available osteoporosis treatments as bisphosphonates are accumulated in bone during treatment and therefore the anti-fracture effect seems to be preserved with respect to non-vertebral fractures if the patient is at low-to-moderate risk of fracture determined by a combination of treatment duration, fracture history and BMD.

The difficult aspect of treatment interruption is not determining which patients fulfill the criteria developed on the basis of the FLEX and the HORIZON trials, but how to monitor and manage the patients interrupting treatments. It is also not clear if temporary treatment interruption of 1-2 years followed by reinitiating of the treatment affects the risk of the rare adverse effects long term.

The updated guideline recommends treatment specific fixed periods of interruption. This seems to be a good approach as it has been demonstrated that the response in terms of change in bone turnover markers and BMD after discontinuation is highly variable[10]. However, this strategy also raises some questions that are currently unanswered; first, does this strategy of short term interruption of treatment leads to more than a temporary reduction in the risk of the rare adverse effects; second, how many patients and doctors lose track of the treatment strategy and treatment is therefore not reinitiated, and third, some patients seem to have stable BMD and low levels of bone turnover markers for years after treatment interruption, do they need reinitiating or could they stay without treatment for a longer period of time?

One aspect of osteoporosis management that is not mentioned in the summary of the updated guideline is patient education, engagement and empowerment. This is an important aspect of long-term management of osteoporosis treatment. Patients who understand what osteoporosis is, how osteoporosis affects their future risk of fractures, and how this risk can be reduced by medical treatment, physical activity and training, and a healthy lifestyle are more likely to remain compliant with treatment and be able cope with having a chronic disease that may imply changes to daily living and activities[11].

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Executive summary clinical practice guideline of postmenopausal, glucocorticoid-induced, and male osteoporosis (2022 update)*

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Summary
This updated version of the SEIOMM (Spanish Society for Research in Osteoporosis and Mineral Metabolism) osteoporosis guideline incorporates the most relevant information published in the last 7 years (since the 2015 guide) with imaging studies such as vertebral fracture assessment and trabecular bone score analysis. Therapeutic advances include new anabolic agents, comparative studies of drug efficacy, and sequential and combined therapy. Against this background, therapeutic algorithms were updated.

Key words: osteoporosis, fractures, densitometry, anabolic agents, antiresorptive drugs.

1. Introduction

Seven years have passed since the most recent version of the Osteoporosis Guidelines of the Spanish Society for Bone Research and Mineral Metabolism (SEIOMM) was drawn up, using the standard methodology of evidence-based medicine[1]. This update incorporates information released since then. The full text is available in the Guide.

2. Methods

A group of experts (see annexe) reviewed each section to incorporate the new findings published in recent years. The new text was disseminated to other interested entities (including SEIOMM partners, patient associations, the Spanish Agency for Medicines and Health Products, and pharmaceutical industries) to provide input to the document, which was subsequently analysed by the group of experts. Osteoporosis in postmenopausal women was analysed first, followed by osteoporosis in men and glucocorticoid-induced osteoporosis.

ASSESSMENT OF PATIENTS AT RISK OF OSTEOPOROSIS

1. Clinical risk factors for fracture

The main risk factors are shown in table 1. After suffering a first fracture, the greatest risk of suffering a new fracture occurs in the subsequent two years, especially if the first fracture was vertebral[2]. This phenomenon led to formulating the concept of "imminent risk" of fracture.

2. Bone densitometry and imaging techniques

X-ray absorptiometry (DXA), which quantifies bone mineral density (BMD), is commonly used to estimate fracture risk. The diagnosis of osteoporosis is established with a T score <-2.5 in any of the following locations: lumbar spine, total hip, or femoral neck (table 2). In premenopausal women and men under 50 years, the use of Z scores is recommended, with Z ≤-2.0 considered "low BMD for chronological age." The trabecular bone score (TBS) may improve the prediction of fracture risk.

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In general, DXA is recommended when risk factors are strongly associated with osteoporosis or fractures (table 1). Radiography is essential for identifying fractures. In the case of the vertebralfractures, the diagnosis requires a decrease of at least 20-25% in height. In some cases, imaging based on DXA (i.e., vertebral fracture assessment, VFA) may be an alternative.

3. Study protocol. Bone turnover markers

A complete blood count and biochemical analysis should be carried out (kidney and liver function, calcium, albumin, phosphorus, alkaline phosphatase, thyrotropin, 25-hydroxyvitamin D [25OHD], proteinogram and calciuria). The suitability of determining parathyroid hormone (PTH) and bone turnover markers (BTM) is a subject of debate. Other studies should be performed in young patients to rule out secondary causes of osteoporosis (e.g., hypercortisolism, celiac disease, and systemic mastocytosis). DXA and evaluation of possible vertebral fractures will almost always be necessary.

Together with other risk factors, BTMs can aid in identifying patients with a higher risk of fracture and, (above all) they help early assessment of the response to treatment. The most widely used are the carboxyterminal telopeptides of type I collagen (s-CTX, Serum C-telopeptide cross-link type 1 collagen) and the amino-terminal peptides of type I procollagen (procollagen type 1 N-terminal propeptide).

4. Risk prediction tools

A combination of clinical data and DXA is useful to assess fracture risk. Several instruments have been developed for this purpose, including FRAX, the Garvan Medical Research Institute scale, and the QFracture Index. They have a similar discriminatory capacity and are only moderately efficient. FRAX is the most widespread. Unfortunately, its adaptation to the epidemiology of fractures in Spain has been inadequate and underestimates the risk of major osteoporotic fractures.

AVAILABLE TREATMENTS FOR POSTMENOPAUSAL OSTEOPOROSIS

1. Non-pharmacological interventions

A balanced diet should be maintained, with a contribution of 1-1.5 g/kg of protein, regular physical exercise, and avoiding tobacco and excessive alcohol consumption. Fall prevention programmes and hip protectors may be helpful in some cases.

2. Calcium and vitamin D

Patients treated with drugs for osteoporosis should have an adequate intake of calcium and vitamin D[3,4] to attain serum levels of 25OHD>25-30 ng/mL. The generally recommended dose of vitamin D is 800-1200 IU/d (or weekly or monthly equivalent). If calcifediol is used, 0.266 micrograms are given every 15-30 days. Calcium intake should be 1000-1200 mg/day, preferably through diet and supplements if needed.

3. Drugs not indicated in osteoporosis

Calcitonin, strontium ranelate, PTH 1-84, isoflavones, phytoestrogens, and tibolone are not indicated for the treatment of osteoporosis. Thiazides can be used to control hypercalciuria.

4. Oestrogen therapy

Although oestrogen therapy effectively prevents fractures, its possible side effects have prevented it from being recommended as an osteoporosis treatment, except in cases of early menopause or when other alternatives are not available.

5. Selective oestrogen receptor modulators

Selective oestrogen receptor modulators (SERMs) increase spinal BMD. Raloxifene and bazedoxifene reduce vertebral fracture risk by 40% but do not influence nonvertebral fractures[5]. Its main complication is an increased risk of venous thromboembolic disease.

6. Bisphosphonates

6.1. Alendronate

Alendronate at 70 mg/week reduces vertebral, nonvertebral, and hip fractures by around 45%, 25–30%, and 45–55%, respectively[6]. Most clinical trials have included a treatment period of 3–5 years. However, a more prolonged administration may sometimes be recommended.

6.2. Risedronate

According to recent meta-analyses, risedronate reduces the risk of all fractures (vertebral 39%, hip 27% and non-vertebral 22%)[5]. It is administered in doses of 35 mg weekly or 75 mg two consecutive days per month. A weekly gastro-resistant formulation does not require administration on an empty stomach.

6.3 Ibandronate

This agent is less effective than other bisphosphonates (BPs) and does not appear to reduce nonvertebral fractures.

6.4. Zoledronate

Zoledronate at 5 mg/year intravenously reduces vertebral, non-vertebral and hip fractures by 70%, 25%, and 40%, respectively[7]. A network meta-analysis found no differences between the BPs in terms of fracture prevention, while in another two, zoledronate was more effective than other BPs.

6.5. Adverse effects of bisphosphonates

BPs are generally well tolerated. In some patients, oral BPs can cause esophagitis. They should be avoided in patients with difficulty swallowing or Barrett's oesophagus. Acute-phase reaction or self-limited flu-like symptoms are common after the first dose of zoledronate. BPs are not recommended in patients with a glomerular filtration rate (GFR) ≤30 mL/min. Intravenous BPs can cause hypocalcaemia, especially in patients with renal failure or insufficient intake of vitamin D or calcium.

Osteonecrosis of the jaws (ONJ) is rare but potentially severe. The risk in patients treated with BP for osteoporosis is very low (1/1,500-1/100,000 patient-years). It is related to the state of oral health (periodontitis) and dental procedures.

Atypical fractures of the femur (AFF) occur in 1-2 cases per 10,000 patients treated with BP. The risk increases with exposure time; however, this risk is very low compared to the risk of osteoporotic fractures. For each AFF that could appear, some 270 clinical fragility fractures are prevented, including 70 hip fractures[8].

7. Denosumab

Denosumab reduces the risk of vertebral, non-vertebral and hip fractures by around 70%, 20%, and 40%, respectively[9]. It is generally well tolerated. The risks of AFF and ONJ are very low, around 1/10,000 and 1/2,000 patients/year, respectively. Denosumab can be used in pa-

tients with kidney failure, even those on dialysis. An adequate supply of calcium and vitamin D must be ensured to avoid hypocalcaemia.

After discontinuation, an increase in bone turnover markers (BTM) and a loss of BMD gained are observed. In some patients, this phenomenon is associated with multiple vertebral fractures.

8. PTH 1-34 (teriparatide)

Teriparatide exerts a bone-forming effect and reduces vertebral fracture risk by 65% and non-vertebral fractures by 50%. A meta-analysis did not show a significant reduction in hip fractures, but another three concluded that it reduced these fractures by 56–65%. It was shown to be more effective than risedronate in women with severe osteoporosis[10]. Several biological analogues and biosimilars are marketed.

9. Abaloparatide

Abaloparatide reduces vertebral and non-vertebral fractures. It is approved in the US but not in Europe.

10. Romosozumab

Romosozumab is a sclerostin-neutralising antibody with dual anabolic and antiresorptive effects.

According to several meta-analyses[5,11], this agent reduces vertebral (66–73%), non-vertebral (33%), and hip (56%) fractures. In women with severe osteoporosis, a cycle of romosozumab provided additional benefits to alendronate[12].

Romosozumab is generally well tolerated; however, in some studies, a small increase in cardiovascular events was described (1.3% vs 0.9%); therefore, it is contraindicated in patients with a history of myocardial infarction or cerebrovascular accident and should be considered carefully in those with multiple cardiovascular risk factors.

Table 1. Osteoporosis risk factors

1. Factors clearly associated with osteoporosis
   - Advanced age
   - Female sex
   - Personal history of fracture
   - Family history of hip fracture
   - Increased risk of falls
   - Diseases
     - Hypogonadism
     - Early menopause, amenorrhea
     - Anorexia nervosa
     - Malabsorption
     - Rheumatoid arthritis
     - Diabetes (particularly type 1)
     - Immobilization
     - Cushing's disease
   - Treatments
     - Glucocorticoids
     - Aromatase inhibitors
     - Gonadotropin-releasing hormone agonists (and other androgen deprivation treatments in men)

2. Other factors associated with less consistency
   - Hyperparathyroidism. hyperthyroidism
   - Calcium deficiency
   - Vitamin D deficiency
   - Drugs and toxic
     - Selective serotonin reuptake inhibitors
     - Proton-pump inhibitor
     - Anticonvulsants
     - Antiretrovirals
     - Alcohol, tobacco

Table 2. Diagnostic criteria for osteoporosis

- Normal: BMD T ≥−1
- Osteopenia or low bone mineral density: BMD T <−1 and >−2.49
- Osteoporosis: BMD T ≤−2.5
- Severe osteoporosis: BMD T ≤−2.5 + fracture

BMD: bone mineral density; T (T-score or T index): comparison with the BMD value reached in a young reference population.

11. Vertebroplasty and kyphoplasty

Although many noncontrolled studies have shown a marked analgesic effect, randomised clinical trials have provided conflicting results for vertebroplasty and kyphoplasty. Thus, they are not routinely recommended. They can be considered in patients with fractures less than 6 weeks old and severe pain despite medical treatment and in patients with fractures from 6 weeks to a year of evolution and persistent pain that responds poorly to analgesics if they show signs of oedema on MRI.

START AND FOLLOW-UP OF TREATMENT

1. Decision to commence treatment

In general, patients with some of these characteristics should be treated:

1. 1. One or more fragility fractures, especially the vertebrae, hip, humerus, and pelvis (regardless of BMD).
2. 2. BMD <-2.5 T score in the lumbar spine, femoral neck, or total hip.
3. 3. BMD in the “osteopenia” range (particularly if T is <-2.0) together with factors strongly associated with fracture risk (e.g., hypogonadism or early menopause, treatment with glucocorticoids or antiestrogens).

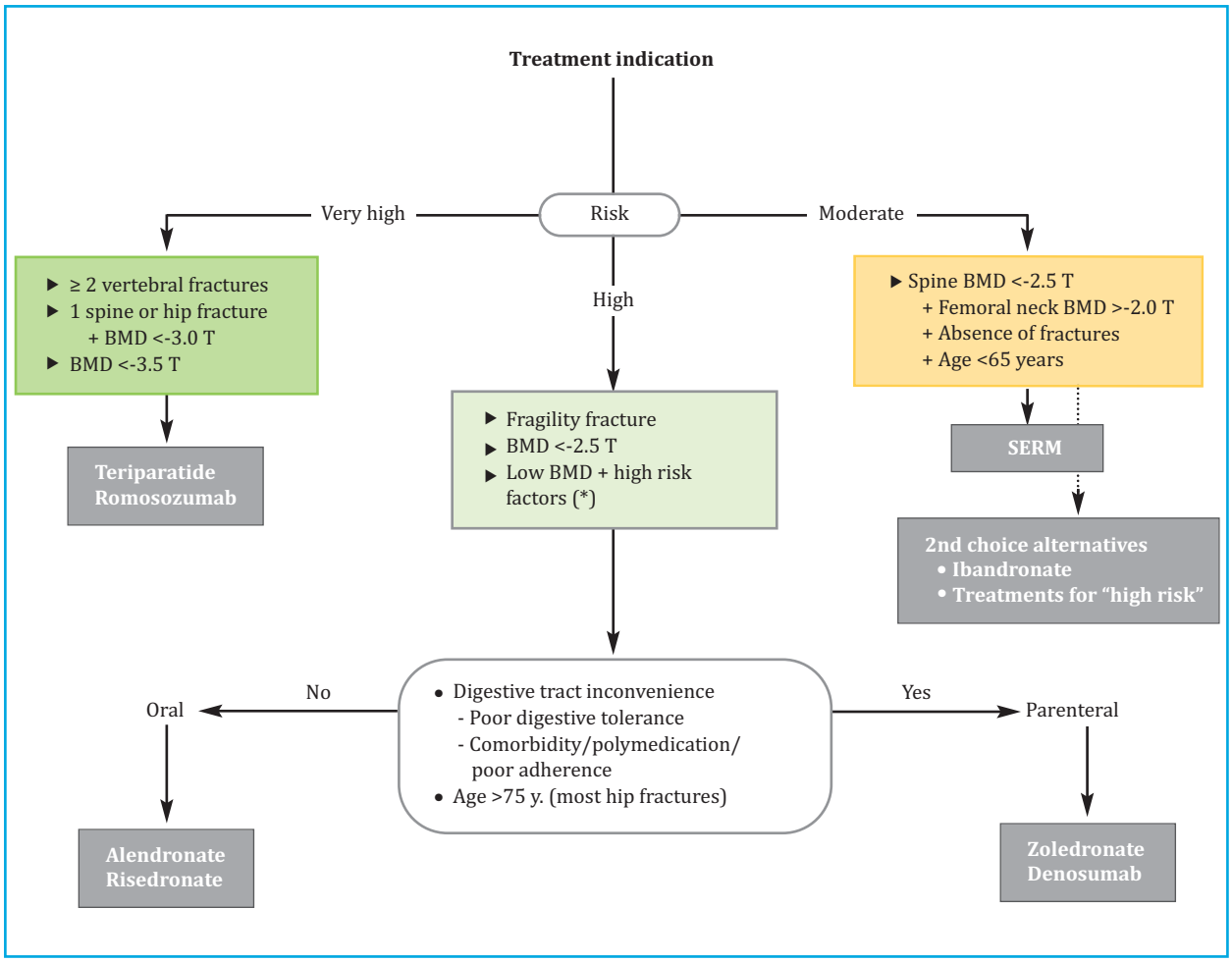
Some situations require an individualised assessment of the clinical characteristics. In young women with only slightly low BMD and no fractures or other risk factors, delaying treatment can be considered because the absolute risk of fracture is low. By contrast, the coincidence of several important risk factors may lead to earlier treatment consideration. Scales that help estimate fracture risk (e.g., FRAX) may be helpful, although their validity in the Spanish population is limited.

2. Control of the therapeutic response

If necessary, adherence to treatments can be monitored using BTMs, whose changes predict therapeutic response.

The beneficial effect of the treatment is confirmed by the evolution of BMD and the absence of new fractures. A change of treatment may be considered due to a possible inadequate response if two new fractures appear during treatment or two of the following events occur: a new fracture, a significant decrease in BMD (e.g., 4-5%), or a decrease of the BTM less than the minimum significant change (approximately 25%).

Figure 1. Algorithm for selection of initial treatment in postmenopausal osteoporosis



(*): especially if T ≤-2 and factors strongly associated with fracture risks, such as hypogonadism, early menopause, or treatment with glucocorticoids or sex hormone antagonists. These general criteria may need to be adapted based on other clinical determinants of fracture risk, the characteristics of individual patients, and their preferences.
BMD: Bone mineral density, fx: fracture, SERM: selective oestrogen receptor modulator.

3. Duration of treatment

Several aspects must be considered. Although the treat-to-target strategy is theoretically attractive, the aims to be achieved in treating osteoporosis are not well defined, limiting its practical application. For some experts, the absence of new fractures and an increase in BMD would be the most appropriate. Various experts have recommended a T score greater than -2.0 or -2.5 as a target, especially in the hip.

Several studies demonstrated the persistence of the effect by maintaining zoledronate for 6 years or alendronate or denosumab for 10 years. However, side effects (particularly ONJ and AFF) may increase with the duration of treatment. Therefore, it is recommended to reassess patients treated with BP at 3 (zoledronate) or 5 years (oral BP) and those treated with denosumab at 5-10 years.

Treatment should be continued (with the same drug or with another) if any of the following circumstances occur:

a. BMD at the femoral neck <-2.5 T.

b. The appearance of fragility fractures in the 3–5 years before evaluation.

c. Some experts also recommend continuing treatment if the patient has a history of hip or vertebral fracture at some point in life.

If none of these circumstances occurs, treatment with BP can be withdrawn, at least temporarily ("therapeutic holidays"): For risedonate, 1 year; for alendronate, 2 years; and for zoledronate, 3 years. In the case of denosumab, temporary interruptions should not be considered.

4. Sequential and combined treatment

4.1. Bisphosphonates after denosumab

After discontinuation of denosumab, bone turnover increases beyond baseline values ("rebound effect"). This is associated with a rapid decrease in bone mass gained and vertebral fractures in some cases. To avoid this occurrence, a powerful BP should be administered[13]. The first dose of zoledronate should be prescribed when denosumab is discontinued (i.e., 6 months after the last dose) and repeated when elevated BTMs are detected, generally at 6 or 12 months.

If the BTMs cannot be measured, the administration of zoledronate should be repeated 6 and 12 months after the previous administration, and the need for new doses should be individually considered. In patients who have received denosumab for fewer than 2.5 years, alendronate can be used instead of zoledronate.

4.2. Antiresorptive agents after anabolics
After finishing treatment with anabolic drugs such as teriparatide or romosozumab, the administration of a BP or denosumab is recommended.

4.3. Anabolic drugs after antiresorptives drugs
The previous use of BP slightly reduces the BMD gain obtained with teriparatide. Therefore, the preferred sequence is first an anabolic drug and then an antiresorptive. However, previous treatment with BP does not contraindicate the administration of anabolics. Of course, teriparatide should not be started as the only treatment in the months after stopping denosumab, given the risk of the accelerated loss of bone mass.

4.4. Combined treatment
There are not enough trials to recommend it routinely. The combination of teriparatide with denosumab or zoledronate may be considered in particularly severe cases with a high risk of hip fracture.

5. Therapeutic decision algorithms
5.1. Initial treatment (choice of drug, figure 1)
The main criterion for the choice of the initial drug is the level of fracture risk:

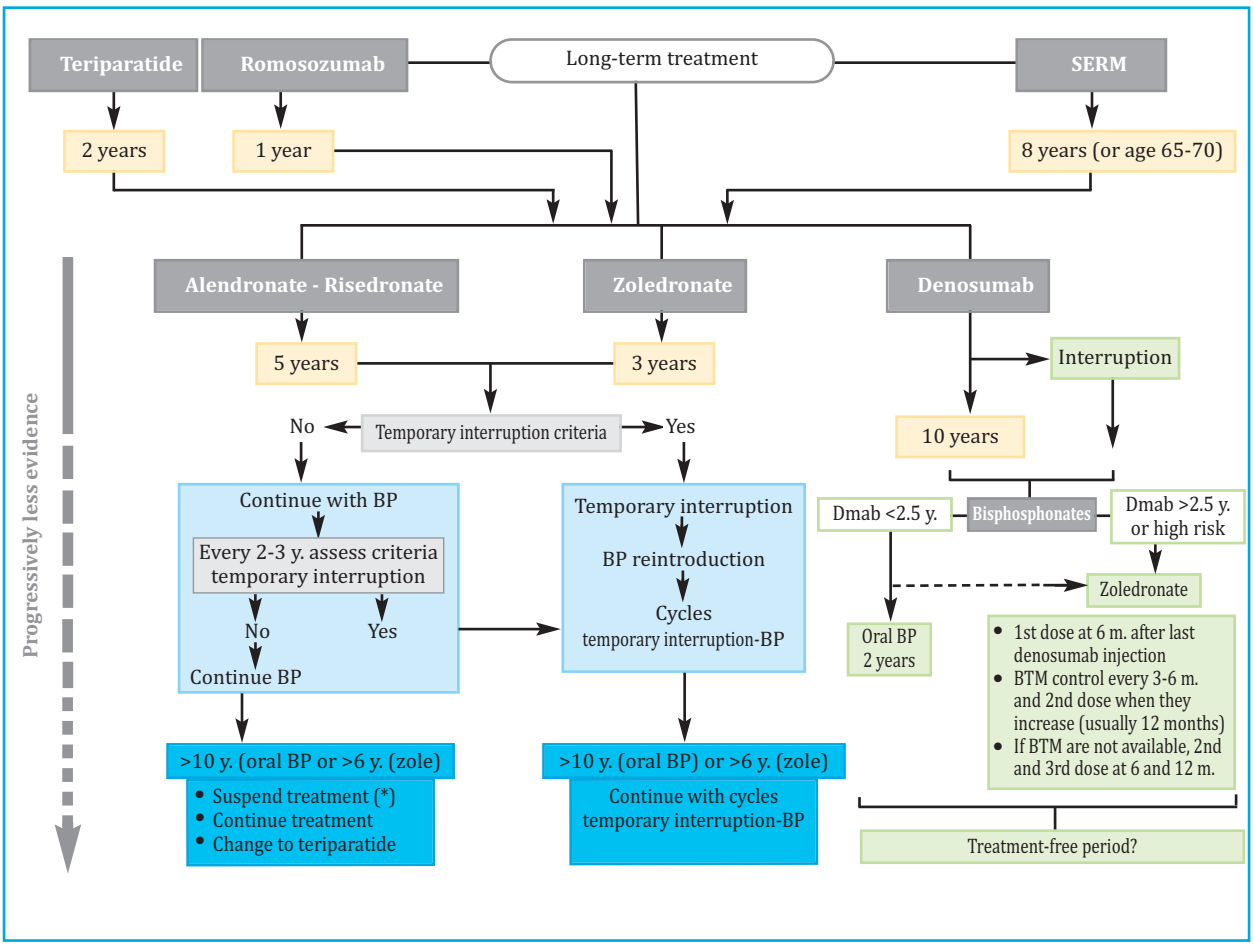
1) **Moderate risk**. This level corresponds to the risk profile of a woman under 65 years of age, with no history of fracture, moderately low BMD in the spine (T score between -2.5 and -3.0) and preserved in the hip (T >-2). In this situation, it is advisable to use a SERM and thus delay the use of drugs with possible long-term adverse effects. Ibandronate and other antiresorptives are alternative options.

2) **High risk.** This level corresponds to most of the cases. Alendronate, risedronate, zoledronate, and denosumab are indicated. Oral BPs are preferred in patients without inconveniences for oral administration (digestive problems, polypharmacy, adherence) and preferably under 75 years of age.

3) **Very high risk.** This level corresponds to women with a) 2 or more vertebral fractures, or equivalent situation (e.g., vertebral and hip fracture); or b) very low BMD (T <-3.5; or c) vertebral or hip fracture together with T <-3.0. There may be other situations (difficult to systematise) in which clinical factors determine very high fracture risk and require individualised consideration. For this level of risk, bone-forming drugs are preferable.

5.2. Long-term treatment (figure 2)
Romosozumab should only be given for 1 year and teriparatide for 2 years. SERMs can be continued for 8 years or until the patient reaches 65-70 years. Then it will be necessary to administer another antiresorptive, BP or denosumab.

Figure 2. Long-term treatment continuation algorithm



(*): there are not enough data to establish a recommendation after that treatment time, so the possible options are listed before a decision that must be individualized.
BP: bisphosphonates; SERM: selective estrogen receptor modulators; BTM: bone turnover markers.

The continued use of denosumab is recommended for 5–10 years. There is no information available regarding more prolonged use, so at that time, continuing treatment or discontinuing it should be carefully considered. In any case, a BP should be administered subsequently.

After the initial treatment cycle with BP, an interruption can be considered if the requirements to start a "therapeutic holiday" are met (see the end of section 3). No quality studies are available to guide decision making after 10 years.

MALE OSTEOPOROSIS

Most of the drugs have shown gains in BMD like those observed in women, suggesting that their efficacy for fractures is also similar. Alendronate, risedronate, and zoledronate have been shown to reduce vertebral fractures in men. Denosumab has been shown to increase BMD in men and reduce fracture risk in those undergoing androgen deprivation. Teriparatide has also shown beneficial effects in men[14]. For this reason, a strategy for choosing a drug like that for women should be proposed for men: a) risedronate or alendronate (although the latter is not approved in Spain for treating male osteoporosis) as the treatment of choice for most patients; b) zoledronate or denosumab in the elderly or when the oral route is not advisable; and c) teriparatide in very high-risk patients.

GLUCOCORTICOID-INDUCED OSTEOPOROSIS

The drugs of choice are BPs. If there are vertebral fractures, preferential treatment with teriparatide is justified due to its greater anti-fracture effect[15]. Calcium and vitamin D should also be given.

Postmenopausal women and men older than 50 years who are to receive doses of ≥5 mg/d of prednisone for >3 months should be treated. In premenopausal women and men <50 years of age, treatment is indicated only if there are previous fractures, BMD is low, or the dose of glucocorticoids is very high (>30 mg/d). Denosumab is an alternative when other antiresorptive agents cannot be used.

The authors' conflicts of interest are detailed in annex II of the full version of the Guide.

Additional material

The full text is available in the Guide.

Additional material. Annex

Members of the SEIOMM Expert Group for the revision of the Osteoporosis Guidelines.

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Clinical practice guidelines for postmenopausal, glucocorticoid-induced and male osteoporosis: 2022 update

Spanish Society for Bone and Mineral Metabolism Research (SEIOMM)

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Summary
This update incorporates the most relevant information that has emerged during the seven years since the publication of the previous version, with a particular focus on diagnostic procedures and therapeutic options. Among the diagnostic procedures, we highlight the use of the Trabecular Bone Score (TBS) and densitometry for identifying the risk of vertebral fractures. Novel therapeutic modalities include the use of anabolic drugs with comparative studies focused on their efficacy for the treatment of severe osteoporosis. Guidelines for actions to be taken after discontinuation of antiresorptive agents, sequential therapy and current recommended treatment schemes are included

Key words: osteoporosis, fractures, densitometry, anabolic, antiresorptive.

Introduction

Seven years have passed since the publication of the previous version of the Osteoporosis Guidelines of the Spanish Society for Bone Research and Mineral Metabolism (SEIOMM) that was created in accordance with the standard methodology of evidence-based medicine[1]. This update incorporates the most essential information that has appeared since the publication of the previous version, with particular reference to new diagnostic procedures and therapeutic options. Novel diagnostic modalities discussed in these guidelines include the Trabecular Bone Score (TBS) and the detection of vertebral fractures by densitometry. Among the therapeutic options, we discuss the use of novel anabolic drugs (abaloparatide and romosozumab). Studies that compare the efficacy of various drug regimens for the treatment of severe osteoporosis are also considered. Likewise, the guidelines for action after the withdrawal of antiresorptive drugs and other sequential and combined treatment schemes are assessed.

To prepare this update, a group of experts (see author listing) reviewed each of the sections and incorporated new findings from reports published in recent years. The initial draft of the manuscript was then critically examined by a group of experts. Once their comments were considered, the new text was distributed to other interested parties, including SEIOMM partners, patient associations, the Spanish Agency for Medicines and Health Products, and pharmaceutical industries so that each might provide additional comments and contributions to the document. The document was then re-analyzed again by the group of experts tasked with drafting the guidelines. The recommendations were graded according to the level of evidence as indicated in Tables S1 and S2.

The topics reviewed in this document include (1) diagnostic and therapeutic aspects of primary osteoporosis in postmenopausal women, (2) specific findings associated with osteoporosis in males, and (3) new information on the diagnosis and treatment of glucocorticoid-induced osteoporosis.

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ASSESSMENT OF PATIENTS AT RISK FOR OSTEOPOROSIS

1. Fracture risk factors

The main factors associated with the risk of bone fractures in patients presenting with osteoporosis include gender, age, bone mineral density (BMD), history of fragility fracture, history of hip fracture in a first-degree relative, and low body weight (i.e., body mass index [BMI] <20 kg/m^2). Paradoxically, obesity can also be a risk factor for some peripheral fractures, including those of the humerus and distal third of the radius. Recognised risk factors also include various diseases including hypogonadism, early menopause, prolonged amenorrhea, anorexia nervosa, malabsorption, rheumatoid arthritis, diabetes (particularly type 1), immobilization, as well as their treatments, e.g., glucocorticoids, inhibitors of aromatase or gonadotropin-releasing hormone agonists[2,3]. Other disorders and medications that may be associated with the development of osteoporosis, (although probably less strongly) are hyperparathyroidism, hyperthyroidism, selective serotonin reuptake inhibitors, proton pump inhibitors, and anticonvulsants, as well as smoking and excessive alcohol consumption. Calcium deficiency and vitamin D deficiency have traditionally been considered risk factors for osteoporosis, although their precise role continues to be a subject of debate (Table 1).

Factors associated with an increased risk of falls, including postural instability, inability to get up from a chair, visual impairment, and some neurological problems are also associated with an increased risk of fractures.

After a first fracture, the greatest risk of sustaining a new fracture occurs within the first two years, particularly if the first fracture was vertebral[4-6]. This has led to the concept of an "imminent risk" of fracture. The main factors that have been associated with imminent risk are older age, female gender, white race, recent fracture, falls, and some comorbidities and treatments (e.g., very low bone mass, cardiovascular disease, obstructive pulmonary disease, chronic and depression, and anxiety, as well as the use of sedatives, hypnotics, glucocorticoids, and muscle relaxants).

In conclusion, recent evidence suggests that an assessment of clinical risk factors combined with the measurement of BMD is an effective method for assessing fracture risk (Recommendation A).

2. Bone densitometry and related techniques

Dual-energy X-ray absorptiometry (DXA) can be used to quantify BMD and is thus the procedure most commonly used to estimate fracture risk[7]. The results are expressed in terms of T-score, which is the number of standard deviations (SDs) by which the BMD value obtained differs from that of the normal young adult population (i.e., 20–29 years of age). The World Health Organization (WHO) guidelines state that osteoporosis can be diagnosed when the BMD is less than -2.5 T[8]. The organization has since clarified that this value must correspond to a measurement made on the neck of the femur using data from the National Health and Nutrition Examination Survey (NHANES III) study as a reference[9]. By contrast, the International Society for Clinical Densitometry (ISCD)[10] states that this diagnosis can be established based on a -2.5 T value detected in the lumbar spine or total hip as well as the femoral neck. The WHO also defined normal bone density, osteopenia (i.e., low bone mass), as well as established or severe osteoporosis (Table 2).

BMD measured at the mid-third of the radius may also be used to diagnose osteoporosis when the hip and lumbar spine cannot be used or interpreted[11].

In, The ISCD recommends that instead of T-scores, Z-scores adjusted for ethnicity or race be used when diagnosing osteoporosis in premenopausal women, men younger than 50 years of age, and children. Z-scores ≤-2.0 are identified as "low bone mineral density for age chronological" or "below expected range for age". Z-scores >-2.0 are identified as"within expected range for age".

Evaluation of therapeutic efficacy is an indication for densitometry. This examination might be repeated after two to three years of treatment.

Other measurement techniques, including quantitative ultrasonometry and quantitative computed tomography, among others, also provide values that are related to fracture risk. However, they are not recommended as diagnostic procedures at this time.

Lateral projections of DXA studies can be used to identify vertebral fractures (i.e., VFA, or “vertebral fracture assessment”). However, the accuracy of this procedure is lower than that of conventional radiography, most notably for the diagnosis of fractures of the upper thoracic vertebrae.

The Trabecular Bone Score (TBS) is a parameter that describes bone texture based on data obtained from a DXA image of the lumbar spine. TBSs are typically reduced in patients who have sustained fragility fractures, and it is a useful value for assessing fracture risk in women and men over 50 years of age, independent of BMD findings. The combination of BMD and TBS is superior to BMD alone for the prediction of fracture risk. A TBS may be particularly useful in assessing fracture risk in patients diagnosed with diabetes or primary hyperparathyroidism as well as those treated with glucocorticoids. The TBS is also expressed in absolute terms and as a T- score.

A TBS value <1.230 (T <-3) is indicative of a degraded trabecular microstructure and a high risk of fracture. The TBS has been included in the Fracture Risk Assessment Tool (FRAX) which can be used to calculate the absolute risk of fracture in a given patient.

Despite the proven usefulness of DXA for assessing patients with an elevated risk of sustaining a fracture, the sensitivity and specificity of this modality remain limited. DXA does not identify all subjects at risk of fracture; more than 50% of peripheral fractures occur in patients with a T-score >-2.5[12,13]. Current trends suggest that BMD measurements might be considered together with the clinical risk factors when calculating an absolute fracture risk[14,15].

There are no universally accepted criteria regarding when to perform densitometry. The general recommendation is that this procedure might be performed when risk factors that are strongly associated with osteoporosis or fractures emerge (Table 1), including:

a) Disorders frequently associated with osteoporosis, such as rheumatoid arthritis, early menopause, hyperparathyroidism, hyperthyroidism, malabsorption, and anorexia nervosa, among others.

b) Treatments with negative effects on the bone, such as glucocorticoids, antiestrogens, and antiandrogens, among others.

c) Other factors (especially if two of them are observed in a single patient): age over 65 years (according to some authors), low weight (BMI <20 kg/m^2), family history of osteoporosis, alcoholism, and smoking, among others.

In conclusion, DXA can be used to measure BMD in the proximal femur and lumbar spine to assess the risk of fracture (Recommendation A). A TBS can provide additional information on the risk of fracture in an individual patient (Recommendation B).

3. Markers of bone turnover

Bone turnover markers (BTMs) provide information on the dynamics of bone turnover. Among the markers of bone formation, significant research has focused on levels of osteocalcin, bone alkaline phosphatase, and the carboxy –and amino-terminal propeptides of type I procollagen (PICP and P1NP). Markers of bone resorption include the carboxy– and amino-terminal telopeptides of collagen I (CTX in blood, s-CTX and NTX in urine) and tartrate-resistant acid phosphatase 5b (FATR 5b). Various international organizations (for example, the International Federation of Clinical Chemistry) have recommended the use of P1NP and s-CTX as markers of bone formation and resorption, respectively, for ongoing and future clinical studies. It is important to control the variability of these measurements by obtaining biological samples consistently between 08:00 and 10:00 hrs after an overnight fast.

While BTMs are not useful for diagnosing osteoporosis, this information may be combined with other risk factors to identify, patients with a higher risk of sustaining a fracture. These values are particularly useful for the early assessment of responses to both antiresorptive and anabolic therapy (Evidence 2a)[16,17]. For example, measurements of s-CTX and PINP are recommended as an effective means to monitor bone turnover after discontinuation of denosumab[18].

In conclusion, BTMs can be useful for evaluating therapeutic responses (Recommendation B), but they must be measured under standardised conditions. They are not used routinely to diagnose osteoporosis.

4. Identification of vertebral fractures

Conventional radiography is not sufficiently sensitive or specific when used to assess changes in bone mass[8]. However, the use of this modality is essential when attempting to identify fractures.

A diagnosis of a vertebral fracture requires a decrease of at least 20–25% in height[19]. This is because slight wedging can be confused with deformities of another origin (e.g., sequelae of Scheuermann's disease, small wedging of a degenerative type)[20]. Thus, VFA by DXA may be useful as a first step. Spinal radiography (or DXA) is recommended for patients over the age of 70 years with suspected osteoporosis who present with back pain, glucocorticoid treatment, or a significant decrease in height (>4 cm based on historical data or >2 cm in confirmed height)[21].

In conclusion, reliable identification of vertebral fractures is important in decision-making because these lesions represent a risk for future fractures. Evaluation can be done by radiography or by VFA. However, radiography should not be used as a method of assessing bone mass to establish a diagnosis of osteoporosis (Recommendation A).

Table 1. Diseases and treatments that constitute risk factors for osteoporosis

1. Factors clearly associated with osteoporosis
   - Hypogonadism
   - Early menopause, amenorrhea
   - Anorexia nervosa
   - Malabsorption
   - Rheumatoid arthritis
   - Diabetes (particularly type 1)
   - Immobilization
   - Cushing's disease
   - Drugs
     - Glucocorticoids
     - Aromatase inhibitors
     - Gonadotropin-releasing hormone agonists

2. Other factors associated with less consistency
   - Hyperparathyroidism. Hyperthyroidism
   - Calcium deficiency
   - Vitamin D deficiency
   - Drugs and toxic
     - Selective serotonin reuptake inhibitors
     - Proton-pump inhibitor
     - Anticonvulsants
     - Antiretrovirals
     - Alcohol, tobacco

Table 2. WHO diagnostic criteria for osteoporosis

- Normal: BMD T ≥−1
- Osteopenia or low bone mineral density: BMD T <−1 and >−2.49
- Osteoporosis: BMD T ≤−2.5
- Severe osteoporosis: BMD T ≤−2.5 + fracture

BMD: bone mineral density; T (T-score or T index): comparison with the BMD value reached in a young reference population.

5. Study protocol

In addition to anamnesis and a physical examination, an evaluation of a patient with suspected osteoporosis should include a complete blood count and determination of basic biochemical parameters (kidney and liver function and serum levels of calcium, albumin, phosphorus, alkaline phosphatase, thyrotropin (TSH), and 25-hydroxyvitamin D, as well as a serum protein electrophoresis study). It is useful to quantify calciuria. These tests should be performed before starting treatment and then repeated if clinically indicated. The usefulness of parathyroid hormone (PTH) levels and BTMs remains controversial (see the previous section). Bone densitometry and an assessment of potential vertebral fractures by VFA or radiology will almost always be required. Pertinent studies should be performed to rule out secondary causes of osteoporosis (e.g., hypercortisolism, celiac disease, and systemic mastocytosis, among others) in younger patients (Recommendation C).

6. Risk prediction tools

Various scoring scales have been developed to assess either the risk of developing osteoporosis (i.e., low DXA), or sustaining osteoporotic fractures. Current scoring scales used to assess the risk of densitometric osteoporosis do not include BMD but are useful in deciding when densitometry evaluations should be performed.

The simplest method, known as the Osteoporosis Self-assessment Tool [OST])[22,23] includes only patient age and weight which are variables included in all assessment strategies.

To assess the risk of fractures, the addition of findings from DXA to the clinical data results in their improved predictive value. Several instruments have been developed for this purpose, including FRAX[24], the Garvan Medical Research Institute scale[25], and the QFracture Index[26]. All three have similar discriminatory capacities albeit with only moderate performance[27,28]. FRAX is the most widely used of these instruments on a worldwide basis. Unfortunately, its adaptation in Spain has been inadequate[29] and it underestimates the risk of fracture, most notably major osteoporotic fractures. Other tools, such as EPIC, which has been adjusted to the Spanish population, are currently undergoing validation.

In conclusion, although fracture risk prediction tools may be helpful in decision-making in some cases, their predictive value for our population is limited. Adaptations of FRAX may be used with caution pending the development and validation of newer and more precise instruments (Recommendation C).

AVAILABLE TREATMENTS FOR POSTMENOPAUSAL OSTEOPOROSIS

1. Non-pharmacological interventions

A balanced diet should be maintained by all patients diagnosed with postmenopausal osteoporosis. This would include a protein intake of 1–1.5 g/kg/day. While sun exposure will promote essential vitamin D synthesis, additional supplementation may be needed (see below)[30]. Furthermore, recent evidence suggests that physical exercise that loads the skeleton has a positive effect with respect to preventing falls and reducing the risk of fracture[31]. Routine exercise is recommended, for example, walking every day for at least 30 minutes.

Smoking and excessive alcohol consumption should be avoided, as both are factors associated with decreased bone mass and an increased risk of fractures[32,33].

Although the efficacy of fall prevention programs (beyond basic physical exercise) remains controversial, recent evidence suggests that they are useful in institutionalised elderly patients who undergo repeated falls[34,35].

Hip protectors are slightly effective at reducing the risk of hip fracture. However, poor tolerance by some patients, poor adherence, and a slight increase in the risk of pelvic fractures limit its application[36].

2. Calcium and vitamin D

Patients treated with antiresorptive or anabolic drugs for osteoporosis should be certain to maintain an adequate intake of calcium and vitamin D[37,38]. Serum levels of 25-hydroxyvitamin D (25(OH)D) should be maintained above 20–25 ng/ml, preferably above 30 ng/ml[39]. The recommended daily dose of vitamin D is generally between 800–1200 IU/day, although some patients may need higher doses to maintain adequate serum levels of 25(OH)D. While bi-weekly or monthly equivalents can be considered, administration of large amounts of vitamin D in a single dose (e. g., 500,000 IU/year)[40] is not recommended. The standard dose of calcifediol (25(OH)D3) is 0.266 micrograms every 15–30 days. This form of vitamin D may be preferable in patients with advanced liver disease or problems with intestinal absorption. Occasionally, these patients may require parenteral administration.

Daily intake of calcium should be maintained at 1000-1200 mg/day[30]. While it is preferable to obtain this amount from dietary sources, supplements can be added as necessary. The general population, particularly the elderly, should be advised to maintain adequate nutrient intake, including appropriate levels of calcium and vitamin D. However, the isolated effects of calcium and vitamin D on the progression of osteoporosis are not well-understood; if they exist at all, their impact seems to be limited[41-43].

In conclusion, patients at risk for developing osteoporosis and those undergoing treatment with antiresorptive or anabolic drugs should receive and be certain that they are taking in an adequate supply of calcium and vitamin D. However, these nutrients alone are insufficient treatments in patients who have developed osteoporosis (Recommendation A).

3. Calcitonin

Although treatment with calcitonin was associated with a slight reduction in the risk of vertebral fractures, it has no impact on the risk of peripheral fractures. Furthermore, long-term calcitonin use has been associated with an increased risk of tumors. Thus, calcitonin is not approved for the treatment of osteoporosis[44,45].

4. Thiazides

Although numerous observational studies suggested that treatment with thiazides resulted in increased bone mass and a concomitant reduction in the risk of fracture[46], we have no data that can be construed as recommending its use as a treatment for osteoporosis. Thiazide treatment (e. g., 12–50 mg/day of hydrochlorothiazide or chlorthalidone) can be considered for patients presenting with hypercalciuria[47] (Recommendation D).

5. Estrogen therapy

The results of several clinical trials have revealed the efficacy of estrogens for the prevention of fractures. A recent network meta-analysis revealed that estrogen therapy (with or without progesterone) reduced the risk of vertebral fracture by 34% (hazard ratio [HR], 0.66; 95% confidence interval [CI], 0.49–0.89); hip fracture by 29% (HR, 0.71; 95% CI, 0.52–0.98); and non-vertebral fractures by 21% (HR, 0.79; 95% CI, 0.70-0.90)[48]. However, the side effects of estrogen therapy revealed by the Women's Health Initiative (WHI) study and other trials include an increase in cardiovascular events and breast cancer. Thus, estrogen is not recommended as a treatment for osteoporosis ex cept in women with early menopause or at a high risk of fracture in which there is no other therapeutic option available[49]. Estrogens may be an effective treatment for osteoporosis in women already receiving these drugs as therapy for the climacteric syndrome.

In conclusion, although estrogen therapy is effective in preventing osteoporotic fractures, it is not recommended for routine use given the possibility of serious side effects (Recommendation A). Estrogens can be considered in patients exhibiting early menopause who have no other contraindications and/or in cases in which no other therapeutic options are available (Recommendation D).

6. Selective Estrogen Receptor Modulators (SERMs)

Results from several recent studies document that these drugs can increase BMD in the spine over follow-up pe-

riods as long as eight years[50,51]. A recent meta-analysis revealed that raloxifene and bazedoxifene reduce the risk of vertebral fracture by 40%, although neither drug has any impact on non-vertebral fractures[48]. The main complication associated with this class of drugs is an increased risk of venous thromboembolic disease.

In conclusion, SERMs may be indicated for the treatment of osteoporosis because they reduce vertebral fractures, but they do not reduce the risk of non-vertebral fractures (Recommendation A).

7. Tibolone

Although the use of this drug will reduce the risk of both vertebral and non-vertebral fractures in women under 60 years of age (or <10 years of menopause)[52,53], its cardiovascular side effects limit its use. At this time, tibolone may be prescribed for patients who are not at high risk for cardiovascular disease or breast cancer who cannot be treated with other drugs (Recommendation B). This drug has not been approved for the treatment of osteoporosis in Spain.

8. Phytoestrogens and isoflavones

Isoflavones may have a favorable effect on BMD[54]. However, they are not currently recommended for the treatment of osteoporosis due to the lack of data focused on their efficacy in preventing fractures.

9. Bisphosphonates (BPs)

9.1. Etidronate

While etidronate reduces the incidence of vertebral fractures by about 40%[55], it has no impact on non-vertebral fractures (Evidence 1a; Recommendation A). This drug has fallen into disuse as more effective BPs have become available.

9.2. Alendronate

Alendronate increases BMD at the lumbar spine and the hip in both treatment and prevention studies performed in osteoporotic women (Evidence 1a). Both daily and weekly administration of this drug result in similar efficacy (Evidence 1a). At a dose of 70 mg/week, alendronate reduces the incidence of vertebral, non-vertebral, and hip fractures by ~45%, 25–30%, and 45–55%, respectively[56,57] (Evidence 1a). Most clinical trials focused on this drug included a treatment period of three to five years. However, administration over longer periods may sometimes be recommended. One extension study revealed that patients who discontinued treatment after five years had a higher risk of suffering clinical vertebral fractures than those who continued on this drug[58]. Older patients with low BMDs at the femoral neck at the time of treatment withdrawal exhibit a greater risk of fracture, including non-vertebral fractures[59,60]. Several meta-analyses and studies with data from real-world practice documented efficacy findings that were similar to those reported previously[48,61]. Alendronate is generally well tolerated, although it can result in some side effects (described below). Long-term use of this drug has been associated with an increase in atypical fractures. Recently, there has been speculation as to its possible beneficial cardiovascular ef fects[62].

In conclusion, alendronate has a definitive role in the treatment of osteoporosis as it reduces the risk of vertebral, non-vertebral, and hip fractures in susceptible individuals (Recommendation A).

9.3. Risedronate

A recent systemic review and network meta-analysis documented the efficacy of risedronate in preventing vertebral, non-vertebral, and hip fractures in postmenopausal women with osteoporosis or osteopenia. The reduction in risk of fracture compared to placebo was 39% for vertebral fracture, 27% for hip fracture, and 22% for non-vertebral fractures[48,63] (Evidence 1a). Risedronate can be administered in single doses of 35 mg per week or 75 mg on two consecutive days per month[64,65]. A new gastro-resistant formulation has been developed that does not require fasting before its administration[64]. Risedronate is well tolerated with side effects similar to those of other BPs as described below.

In conclusion, administration of risedronate results in reductions in the incidence of vertebral, non-vertebral, and hip fractures. Thus, this drug has a definite role in the treatment of osteoporosis (Recommendation A).

9.4 Ibandronate

Ibandronate can be administered orally at 150 mg/dose once a month or intravenously at 3 mg every 3 months intravenously (NB: the intravenous formulation is not marketed in Spain). Ibandronate reduces the risk of vertebral fractures by ~60% but has no impact on non-vertebral fractures (Evidence 1b). In a meta-analysis that included 107 trials focused on drugs that can be used to treat osteoporosis, ibandronate was identified as somewhat less efficacious at reducing the incidence of fractures than other BPs[48].

In conclusion, ibandronate reduces the risk of vertebral fractures (Recommendation A), although had no apparent effect on non-vertebral fractures.

9.5. Zoledronate

Zoledronate administered intravenously at a dose of 5 mg/year reduces the incidence of vertebral, non-vertebral, and hip fractures by 70%, 25%, and 40%, respectively[66] (Evidence 1b). Patients who, continue treatment with zoledronate for an additional three years after completion of an initial three years of treatment benefit from an additional 50% reduction in the risk of vertebral fracture compared to those who are not maintained on this regimen[67]. In a clinical trial that included women with what was called “osteopenia” who were older than 65 years of age, administration of this drug every 18 months also reduced the incidence of vertebral and non-vertebral fractures[68]. The side effects of this drug are described in the section to follow. While one network meta-analysis identified no differences between zoledronate and any of the other BPs studied in terms of fracture prevention[69], two other studies revealed that zoledronate was shown to be more effective than the other formulations[70,71].

In conclusion, zoledronate also reduces the incidence of vertebral, non-vertebral, and hip fractures, and thus plays an important role in osteoporosis treatment (Recommendation A).

9.6. Adverse effects of bisphosphonates[72,73]

BPs are generally safe and well-tolerated drugs. However, given their central role in the treatment of osteoporosis, possible adverse effects are discussed in detail below. It should be noted that other beneficial effects of these drugs have been described, including a decrease in mortality, especially that associated with cardiovas-

cular events, and a reduction in the incidence of some cancers. However, the actual extent of these effects remains controversial[74-76].

a) Adverse effects on the upper digestive tract have been described in patients taking oral BPs (i.e., esophagitis and esophageal ulcers). These responses can be largely avoided if the drug is ingested with a glass of water with an upright position maintained for the following 30–60 minutes. Contrary to what was suggested in some of the initial studies, these drugs do not increase the incidence of cancer of the esophagus or stomach[77,78]. However, BPs should not be prescribed for patients with disorders of the upper digestive tract, notably those with difficulty swallowing or Barrett's esophagus.

b) Acute-phase response or flu-like symptoms have been described mainly in response to intravenous BPs. This reaction typically appears within 24–36 hours of drug administration, can be relieved with acetaminophen, and usually disappears within three days[79]. This response had been reported in 25–35% of patients receiving intravenous zoledronate for the first time. The intensity typically diminishes in response to subsequent injections.

c) Studies regarding the association of BP treatment (especially intravenous) with atrial fibrillation have led to discordant results[80]. This has not been identified as a potential limitation for treatment in cases in which these drugs are indicated. Of note, several studies documented a reduced incidence of cardiovascular events in patients treated with BPs[81,82].

d) BPs are not recommended in patients with renal failure with glomerular filtration rates (GFRs) ≤30 ml/min. However, even in patients with normal GFRs, BPs can promote the development of renal failure if administered via the intravenous route without due caution. Overly rapid administration (i.e., over a period of <15 minutes for zoledronate), simultaneous use of potentially nephrotoxic agents (NSAIDs, diuretics), and drug administration to dehydrated patients must be avoided[83,84].

e) Intravenous BPs can result in clinically significant hypocalcaemia, especially when administered to patients with decreased GFRs, vitamin D deficiency, insufficient calcium intake, or very high bone turnover.

f) The risk of developing osteonecrosis of the jaw (ONJ) among patients treated with BPs for osteoporosis is very low (1/1,500 –1/100,000 patient-years, depending on the specific study)[85,86]. The incidence of this complication is related to the patient's state of oral health (i.e., periodontitis) and a history of dental trauma; a decrease in bone turnover is most likely involved. However, BTM measurements are not useful for identifying people at risk. Temporary suspension of drug treatment does reduce the frequency of this complication.

g) The incidence of atypical fractures of the femur (AFF) is very low[87,88]. In a recent study from the United States, 1.7 patients with AFF were identified for every 10,000 treated with BPs. The relative risk (RR), compared with those not treated, increased with the time of exposure to BPs (RR = 2.5 with treatments < three years; RR = 8.9 with treatment for three to five years; RR = 19.9 with five to eight years of treatment, and RR = 43.5 for treatments lasting longer than eight years). Despite the observed increase in RR, the absolute risk is very low compared to the risks associated with osteoporotic fractures. Current estimates suggest that for each atypical fracture appearing during the first three years of treatment, ~270 clinically-relevant fragility fractures are prevented, including 70 hip fractures[89]. Risk factors for AFF include Asian race, low weight, and femoral curvature. The incidence of AFF appears to decline rapidly after drug withdrawal. The usefulness of the synthetic parathyroid hormone, teriparatide, for the treatment of AFF remains controversial.

h) Various types of inflammatory reactions of the eye have been described in association with the use of BP (e.g., episcleritis, keratitis, and uveitis). These adverse effects are very infrequent but would require discontinuation of treatment[90].

i) Diffuse osteoarticular and muscular pain can develop in patients undergoing BP drug treatment. The discomfort typically disappears after the drug has been withdrawn[91].

10. Denosumab

Denosumab is a monoclonal antibody with a powerful antiresorptive effect that translates into a reduction in the risk of fracture. In general, it has shown greater antiresorptive potency and results in a greater increase in BMD than achieved with BPs.

Denosumab therapy results in reductions in the risk of vertebral, non-vertebral, and hip fractures of ~70%, 20%, and 40% respectively[92] (Evidence 1b). A post hoc analysis of these data suggests that its efficacy in reducing hip fracture may be greater in subjects older than 75 years of age[93] (Evidence 2b). Its beneficial impact on fracture risk appears to be maintained during treatment and persists for at least 10 years[94].

In the months following drug withdrawal, an increase in BTMs and a loss of the bone mass gained with subsequent stabilization at baseline values are observed. In some patients, these responses have been associated with multiple vertebral fractures[95]. Therefore, any interruption of denosumab therapy should be followed by the administration of a BP for six months following the final dose. However, the ideal regimen has not yet been established (see below)[18].

Denosumab is generally well tolerated. It is not associated with an increased risk of neoplasms, cardiovascular events, or infections and is safe to use in patients with diabetes[96]. As with BPs, the risk of AFF and ONJ is very low. In a study performed with patients treated for a prolonged period (up to 10 years), the risk of AFF was determined to be ~1/10,000 patient-years. The risk of developing ONJ was 1/2,000 patient-years[94]. Furthermore, denosumab can be used safely in patients with GFRs <30 ml/min and even in those on dialysis with no need for dose adjustment. However, hypocalcaemia may develop, especially in patients with advanced renal failure. Close follow-up will be necessary for these patients, together with an adequate supply of calcium and vitamin D.

In conclusion, denosumab therapy can reduce the incidence of vertebral, non-vertebral, and hip fractures. Thus, this agent has a definitive role in the treatment of osteoporosis (Recommendation A).

11. Strontium ranelate

Strontium ranelate reduces the incidence of vertebral and non-vertebral fractures by ~40% and 16%, respectively[97]. However, the administration of this agent results in an increased incidence of cardiovascular events. It is not currently available for use in Spain or any other European country.

12. Parathyroid hormone (PTH) 1-34 (teriparatide)
Teriparatide is the amino-terminal (1-34) peptide fragment of human parathyroid hormone (PTH) that promotes bone formation. Administration of teriparatide reduces the risk of vertebral fracture by 65% and non-vertebral fracture by 50%[98] (Evidence 1a). While teriparatide has not yet been evaluated in trials designed to assess its specific impact on hip fractures, a review of observational studies suggested reductions of ~56%[99]. A more recent meta-analysis found that teriparatide therapy resulted in no significant reductions in hip fractures[48], although another three reviews concluded that it reduced hip fractures between 56% and 65%[61,100,101]. One study directly compared the effects of the BP, risedronate, and teriparatide in postmenopausal women with severe osteoporosis and vertebral fractures; the teriparatide-treated group experienced fewer vertebral and clinical fractures than the BP-treated group (5.4% versus 12.0% and 4.8% versus 9.8%, respectively)[102]. Teriparatide is administered as a daily subcutaneous injection for two years. The benefits with respect to BMD that are achieved with this drug decrease progressively after its withdrawal; thus, sequential treatment with an antiresorptive drug is recommended. Teriparatide is generally well tolerated. Several biological and biosimilar drugs have been approved for clinical use because they have met the standard bioequivalence requirements established for these drugs.

In conclusion, teriparatide reduces both vertebral and non-vertebral fractures and, although it is not approved for this indication, it probably also reduces the incidence of hip fractures (Recommendation A).

13. PTH (1-84)
This formulation is not currently licensed for osteoporosis treatment.

14. Abaloparatide
Abaloparatide is an analog of the 1-34 region of PTH and is a PTHrP (PTH-related peptide). The results of a clinical trial found that administration of abaloparatide reduced the risk of vertebral and non-vertebral fractures by 86% and 43%, respectively, compared to placebo[103]. A recent meta-analysis revealed that the use of

this drug resulted in 87%, 50%, and 61% reductions in vertebral, non-vertebral, and wrist fractures respectively[104]. Abaloparatide is approved for use in the US but not in Europe. It is not available in Spain.

15. Romosozumab
Romosozumab is a sclerostin-neutralizing monoclonal antibody. Sclerostin is a small protein pathway which is essential for osteoblastic activity. Various experimental and clinical studies have shown that romosozumab has a dual effect. Administration of romosozumab increases bone formation and also decreases the rate of bone resorption. The latter effect has been associated with the impact of this drug on levels of the osteoclast NKL. Consistent with its dual effect, romosozumab increases the levels of bone formation markers, such as PINP, and decreases the levels of resorption markers, such as CTX. Romosozumab induces notable increases in BMD in both the spine and the hip. The anabolic effects of this drug disappear after 6–12 months of treatment. Therefore, it is typically administered for periods of one year, after which an antiresorptive agent must be used to maintain or increase BMD.

The results of three pivotal trials and several meta-analyses[48,105-108] reveal that treatment with romosozumab for 12 months reduces the incidence of vertebral fractures in postmenopausal women and men with osteoporosis (relative risk reduction [RRR], 66%–73%)[109]. Likewise, the combined analysis revealed that romosozumab therapy decreases the risk of non-vertebral (RRR 33%) and hip fractures (RRR 56%). In postmenopausal women with severe osteoporosis and a history of previous fragility fractures, treatment with romosozumab for one year followed by alendronate significantly reduced the risk of new vertebral, hip, and clinical fractures, compared with treatment over the entire period with alendronate alone[107].

Romosozumab is generally well-tolerated, although the results of several studies suggested that it may increase the incidence of cardiovascular events[110]. While this difference was small in absolute terms (1.3% of events versus 0.9% in the control group), romosozumab is not indicated in patients with a history of myocardial infarction or stroke and should be carefully considered in patients presenting with multiple cardiovascular risk factors.

In conclusion, romosozumab has a defined role in the treatment of osteoporosis as it reduces the risk of both vertebral and peripheral fractures (Recommendation A). Potential cardiovascular risks and specific contraindications should be assessed in each patient.

16. Vertebroplasty and kyphoplasty
Although many uncontrolled studies have shown that these procedures are associated with a marked analgesic effect, randomised clinical trials have offered contradictory results[111-114] and controversy regarding a potential increased risk of fracture in the adjacent vertebrae remains. Therefore, these procedures are not routinely recommended[87] for patients with asymptomatic vertebral fractures, mild pain, or those with symptoms that have persisted for more than one year. These procedures can be considered in patients who present with fractures that are less than six weeks old and severe pain despite appropriate medical treatment and in patients with fractures that have evolved over six weeks to one year ago with persistent pain that responds poorly to analgesics and evidence of edema on magnetic resonance imaging studies[115]. These procedures may also be useful in patients who present with contraindications or poor tolerance to analgesics. Vertebroplasty and kyphoplasty are similar in terms of effectiveness and safety[116]. There is insufficient evidence on the relative usefulness of procedures that include the insertion of expanding implants specifically when compared to vertebroplasty and balloon kyphoplasty (Recommendation B).

In conclusion, vertebroplasty, kyphoplasty, and related techniques are not routinely recommended for the treatment of vertebral fractures, although these procedures may help to control symptoms in carefully selected patients (Recommendation C). In any case, its use must be accompanied by medical treatment of osteoporosis to prevent new fractures.

INITIATION AND FOLLOW-UP OF TREATMENT
1. Decision to commence treatment
There is no internationally agreed or apstandard on when to initiate treatment for osteoporosis. SEIOMM suggests that, in general, patients that present with the following attributes should be treated:

1. Patients who present with one or more fragility fractures, especially those of the vertebrae, hip, humerus, and pelvis, regardless of whether their T-scores indicate "osteoporosis".

2. Patients with a BMD <-2.5T at the lumbar spine, femoral neck, or total hip.

3. Women with osteopenia (particularly with T < -2.0) who also present with factors that are strongly- associated with an increased risk of fracture (e. g., hypogonadism or early menopause, treatment with glucocorticoids or antiestrogens, among others).

However, we recognise that some situations may require exceptions to these recommendations. All patients must undergo a careful, individualised assessment that considers the risk factors for fracture as well as other clinical characteristics. For example, it may be possible to delay the start of treatment in young women who present with only slightly low BMD, without fractures or other risk factors. By contrast, a patient who presents with several important risk factors may require early treatment. Scales that help estimate fracture risk (e. g., FRAX) may be helpful, although these instruments have not yet been fully validated for use in the Spanish population, as mentioned above.

2. Control of the therapeutic response

Adherence and therapeutic responses to treatment regimens can be assessed by changes in BTMs.

The beneficial effect of a given treatment regimen can be confirmed by increases in BMD and the absence of new fractures. However, it is critical to recognise that a single fracture while on a treatment regimen is not necessarily indicative of therapeutic failure. Elderly patients and those with dementia, poor quality of life, and/or multiple fractures are at greater risk for therapeutic failure. In cases where oral BPs have failed, parenteral drugs (zoledronate, denosumab, and [de- pending on patient characteristics] teriparatide or romosozumab) may represent good therapeutic alternatives.

Changes to treatment regimens due to a potential inadequate response may be considered in the following circumstances[117]:

a) development of two successive fractures; or

b) coincidence of two of the following three factors, including the de velopment of a new fracture; decrease in BMD greater than the minimum significant change (nb: this varies based on the densitometer and the skeletal region studied but is usually between 4–5%); or decrease in BTMs below the minimum significant change, usually ~25% (Recommendation D).

Before proceeding with a therapeutic change, the following factors should be considered as possible causes of an inadequate response: a) vitamin D deficiency; b) secondary forms of osteoporosis; c) in- adequate compliance; d) tendency to fall; e) defective techniques used to measure BMD and/or BTMs; f) serious bone deterioration, leading to the likelihood of new fractures despite active drug treatment.

If the reasons for the changes observed include an apparent lack of an appropriate response, the following options are recommended[117,118] (Recommendation D):

- Select the drug with the highest anti-fracture effect.
- Select a drug that is anabolic rather than antiresorptive.
- Select an injectable drug rather than one that is taken orally.

3. Duration of treatment

Interruption of treatment is justified when the risk/benefit ratio becomes unfavourable. These situations can include: a) therapeutic objectives have been achieved; b) loss of effectiveness; or c) increased risk of developing secondary effects.

a) Attainment of objectives

Although the "treat to target" strategy is theoretically an attractive approach, the objectives to be achieved in the treatment of osteoporosis are not well defined, which limits its practical application. For some experts, the absence of new fractures and the increase in BMD would be the most appropriate objectives to consider. Other experts have recommended objectives that include reaching a T-score greater than -2.0 or -2.5, especially in studies focused on the hip[119-121].

b) Loss of effectiveness

The increase in BMD induced by antiresorptive drugs is more marked during the first years of treatment. However, that does not mean that these drugs subsequently lose effectiveness. Although there is no general agreement, the results of several studies have revealed that fracture risk reduction persists with treatment with zoledronate for six years and with alendronate or denosumab for 10 years, especially in patients who maintain a high baseline risk.

c) Increased risk of developing undesirable long-term side effects

ONJ and AFF induced by BPs and denosumab are particularly relevant to this concern. The absolute risk of ONJ in patients treated with antiresorptive agents for osteoporosis is very low and similar to that reported for the general population. Likewise, there is currently no evidence that short-term discontinuation of treatment reduces the risk of ONJ or disease progression in patients who need dental procedures. The absolute risk of AFF is also very low, although the relative risk increases with the duration of exposure to BPs (see the previous section).

Based on these facts, the following recommendations are proposed. These recommendations represent expert consensus, albeit without published studies to provide definitive support[122-126] (Recommendation D):

1. Patients treated with BPs should be evaluated after three (zoledronate) or five years (oral BP) of treatment. Patients treated with denosumab should be evaluated after 5–10 years of treatment.

2. After this evaluation, treatment should be continued (with the same or another drug) if any of the following circumstances occur:

a. BMD at the femoral neck at <-2.5 T.

b. The appearance of fragility fractures in the 3–5 years prior to evaluation.

c. Some experts also recommend continuing treatment if the patient has a history of hip or vertebral fracture at any time.

If none of these circumstances arise, BP treatment can be withdrawn, at least temporarily.

If treatment is maintained, the possibility of its withdrawal should be periodically reassessed at various intervals thereafter. There is currently no guidance as to how often each patient should be reassessed, nor if there is a defined maximum duration of treatment. A limit of 10 years is often set, as there are no studies that have evaluated the impact of these drugs over the longer term. However, if the patient remains at risk, anti-osteoporotic treatment should not be withdrawn. If anti-resorptive treatment is withdrawn, and the patient remains at risk for fracture, a drug from another class should be administered, for example, an anabolic.

When BP treatment is withdrawn, the suspension must be temporary (i.e., a "drug holiday"). It is not known how long the treatment regimen can be safely suspended or fully discontinued. Typically, the drug can be suspended for a period of 1 to 3 years, depending on the BP used (e. g., perhaps one year for risedronate, two years for alendronate, and three years for zoledronate). Some experts have suggested that BTMs and BMD measurements can help with this decision, although we are not in a position to confirm this. In theory, if the BMD remains above a "target" value (e. g., T >-2 or -2.5), drug withdrawal can be considered.

"Drug holidays" should not be scheduled for patients treated with denosumab, because after its withdrawal, not only is there no residual effect, but bone turnover increases to levels above baseline values (i. e., a "rebound effect"). This increased bone turnover has been associated with a rapid loss of bone mass and an increased risk of developing multiple vertebral fractures. Therefore, continuing denosumab therapy in- definitely is recommended. In cases in which denosumab must be discontinued, it should be replaced with a potent BP (see below)[18].

There are some data available that address the efficacy and safety of SERMs (raloxifene and bazedoxifene) for up to eight years. In these cases, the treatment regimen can be maintained through this time or until the risk of hip fracture or complications, such as thromboembolic disease, increases. It is not usually recommended in patients older than 65-70 years of age.

Treatment with teriparatide or romosozumab should be maintained for 24 and 12 months, respectively, followed in both cases by an antiresorptive drug.

4. Sequential and combined treatment

4.1. Bisphosphonates (BPs) after denosumab

As stated above, a BP must be administered after discontinuation of denosumab to limit the rebound effect (Recommendation A). Pending the results of ongoing trials focused on the optimal BP regimen, patients with a low risk of fracture and who have been treated with denosumab for a relatively short period (up to 2.5 years), can be treated for another two years with an oral BP, such as alendronate. IV zoledronate is another alternative. Zoledronate is preferable in cases of prior intolerance to oral BPs, foreseeable poor adherence, or polypharmacy. Patients who have been treated with denosumab for a longer period (i. e., more than 2.5 years) or who remain at high risk of fracture should be treated with zoledronate for 1–2 years. The first dose of zoledronate should be administered once denosumab has been discontinued (i. e., six months after the last dose) and repeated when elevations in BTMs are detected, generally at 6 or 12 months later. If BTM measurements are not available, zoledronate administration can be repeated 6 and 12 months after the first dose[18,127]. The need for additional doses should be considered on an individual basis (Recommendation D).

There are no trials that established the best therapeutic options for patients who have sustained a vertebral fracture after discontinuation of denosumab. However, the following options have been recommended in this situation:

a) restart denosumab;
b) administer zoledronate;
c) administer teriparatide together with denosumab (Recommendation D)[18]. In the months following the discontinuation of denosumab, treatment with teriparatide alone should be avoided, because it causes a transient loss of bone mass[128].

4.2. Antiresorptive agents after anabolics

Progressive loss of BMD will follow after discontinuing treatment with teriparatide[129]. Several studies have shown that this loss of bone mass could be prevented by the sequential administration of an antiresorptive agent; additional increases in BMD might also result from this new drug regimen[130], although there are no data available on fracture prevention. Likewise, after completion of treatment with romosozumab, current recommendations include that the patient shouldontinue with an antiresorptive agent[131,132].

In conclusion, after completion of treatment with anabolic drugs, such as teriparatide or romosozumab, further treatment with powerful antiresorptive drugs, such as a BP or denosumab, is recommended (Recommendation A).

4.3. Anabolic after antiresorptive drugs

The anabolic effects of PTH depend on the type of antiresorptive drug used in the previous treatment regimen. Several studies have confirmed that the previous use of a BP result in an overall decrease and slightly reduces the rate of increase in BMD that resulted from teriparatide treatment[133,134]. However, the reduction in fracture risk associated with the use of teriparatide is not affected by prior treatment with a BP[135].

One study focused on the impact of switching to romosozumab or teriparatide among women previously treated with a BP (particularly alendronate). Both groups exhibited increases in spine BMD, but those who switched to romosozumab exhibited these increases 12 months or more after those achieved in patients who switched to teriparatide; this was especially notable in the hip[136].

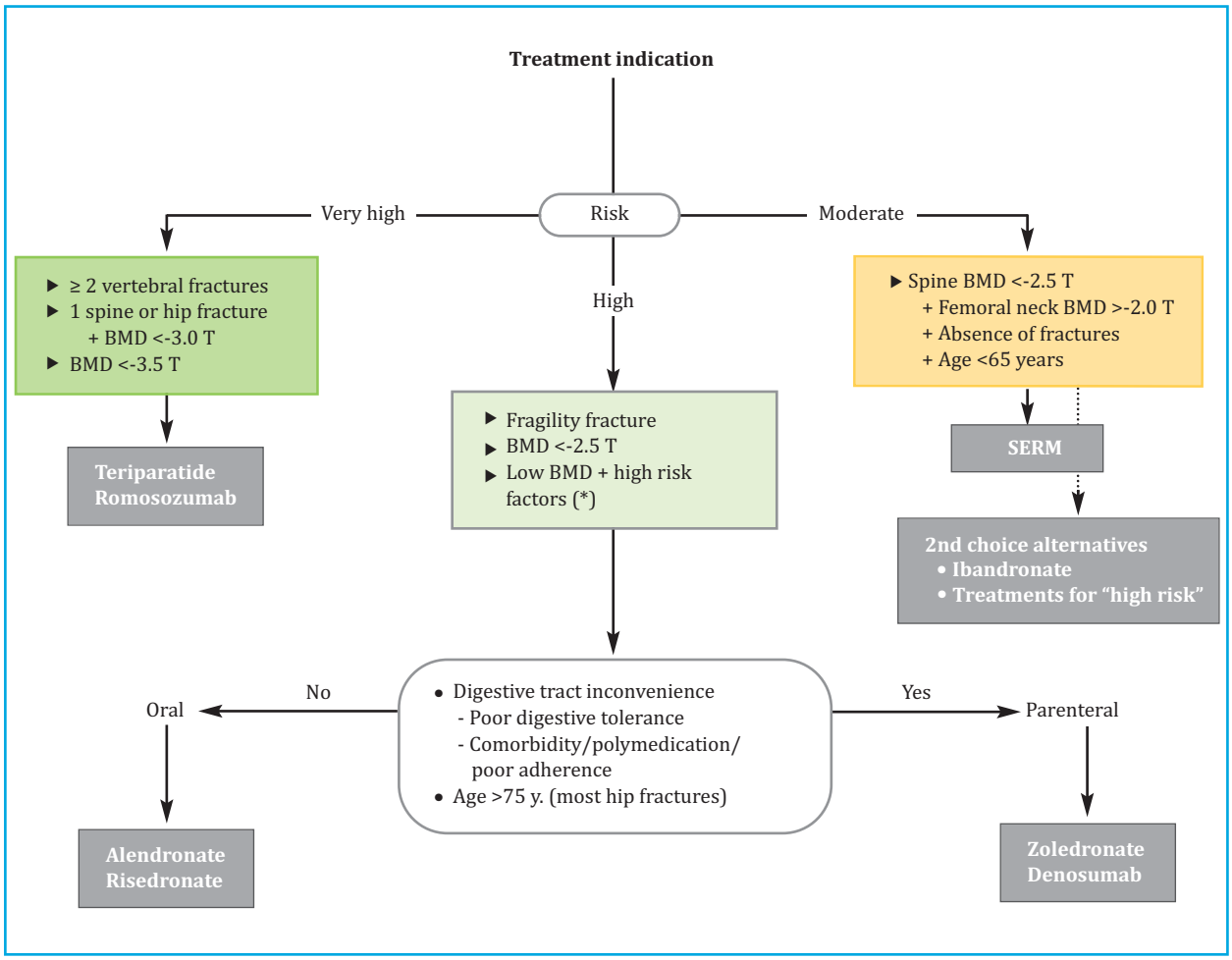
By contrast, initiation of teriparatide in postmenopausal women who had completed a course of treatment with denosumab resulted in a transient decrease in BMD[128]. Therefore, teriparatide should not be administered after discontinuation of denosumab.

In conclusion, although the preferred sequence is an anabolic followed by an antiresorptive drug, prior treatment with a BP is not a contraindication for subsequent administration of teriparatide or romosozumab and is considered adequate to reduce the risk of fracture. (Recommendation A). Teriparatide in the months following denosumab suspension should be avoided, given the risk of accelerated bone loss (Recommendation A).

4.4. Combination treatments

- The combination of two antiresorptive drugs (e.g., estrogens and a BP) can enhance the gain in bone mass achieved individually[137], but there are doubts regarding the risk-benefit ratio of this association compared to results achieved with each drug alone. This combination is not recommended.
- Studies focused on the combination of a BP and teriparatide have not shown clear benefits over individual administration of each drug. Thus, this combination is not recommended. However, in one study, the combination of zoledronate and teriparatide resulted in a higher value for hip BMD than what was achieved in response to teriparatide alone[138].

Figure 1. Algorithm for selecting the initial treatment in postmenopausal osteoporosis



SERM: selective estrogen receptor modulator; (*): especially if T ≤-2 and factors strongly associated with fracture risk, such as hypogonadism, early menopause, or treatment with glucocorticoids or sex hormone antagonists. These general criteria may need to be adapted based on other clinical determinants of fracture risk, the characteristics of individual patients, and their preferences.

• In one trial, the use of denosumab combined with teriparatide resulted in greater increases in BMD at the hip and spine than those achieved with each drug alone[139].

In conclusion, given the lack of data on fracture prevention and the higher costs and side effects associated with these types of regimens, combination therapy is not generally recommended at this time. However, combinations of denosumab or zoledronate with teriparatide can be considered on an individual basis in particularly severe cases associated with a very high risk of hip fracture. In these cases, it may be preferable to delay the start of antiresorptive for one to two months after initiating teriparatide to take advantage of the anabolic effect (Recommendation grade D).

5. Therapeutic decision algorithms

The proposed algorithm is based on data from published trials and considerations that are summarised below.

5.1. Initial treatment (Choice of a drug; Figure 1)

The main criterion for choosing the initial drug is the risk of fracture. We distinguish three levels of risk, including "moderate", "high", and "very high".

1) Moderate risk. This category corresponds to the risk profile of a woman under 65 years of age, with no history of fracture, a spinal T-score between -2.5 and -3.0, and a relatively preserved hip BMD (T-score >-2). In this situation, a SERM is recommended because one can then delay the use of prolonged treatment strategies that can elicit AFF or ONJ. However, ibandronate and antiresorptive agents that are typically recommended for high-risk situations are the second choice in this situation. These drugs represent acceptable alternatives if for some reason SERMs are to be avoided.

2) High risk. Most of the cases seen in the clinic will present this level of risk (see section above "Decision to start treatment"). These patients do not meet the criteria that define either moderate or very high-risk cohorts as described further below. Alendronate, risedronate, zoledronate, or denosumab are indicated for the treatment of patients in the high-risk cohort. Oral BPs are considered preferable for patients <75 years of age when there are no inconveniences with respect to oral administration (digestive problems, polypharmacy, adherence). Injectable antiresorptive drugs are considered preferable in all other cases. As most individuals who have sustained hip fractures are over 75 years of age and belong to the second group, injectable antiresorptive agents are generally preferred for this group. Given the rebound effect after discontinuation of denosumab, zoledronate may be the preferred agent if there are doubts regarding compliance.

3) Very high risk. We consider women to be at very high risk in any of the following situations: a) two or more vertebral fractures, or an equivalent risk (i.e., T-score <-3.5); or b) vertebral or hip fracture with a T-score <-3.0. There may be other clinical situations that suggest that a patient is at a very high risk of fracture; these will require individualised consideration. For this level of risk, bone-forming drugs such as teriparatide or romosozumab should be used. Romosozumab may have a better cost-benefit ratio (although its marketing price was not known at the time that these guidelines were written), albeit a less favorable risk-benefit ratio due to the potential increase in cardiovascular events. Romosozumab should be avoided in all patients with or at high risk of developing cardiovascular disease. However, these guidelines and recommendations should be understood as provisional at this time, pending marketing in Spain and further experience with this drug in our population.

Although some authors have suggested that all patients with a recent fracture, especially a vertebral fracture, might benefit from treatment with a bone-forming drug. However, there is currently no consensus on this point among our panel of experts. Regardless of the treatment that is ultimately selected, therapy should be initiated as soon as possible given that these patients are at very high risk for new fractures.

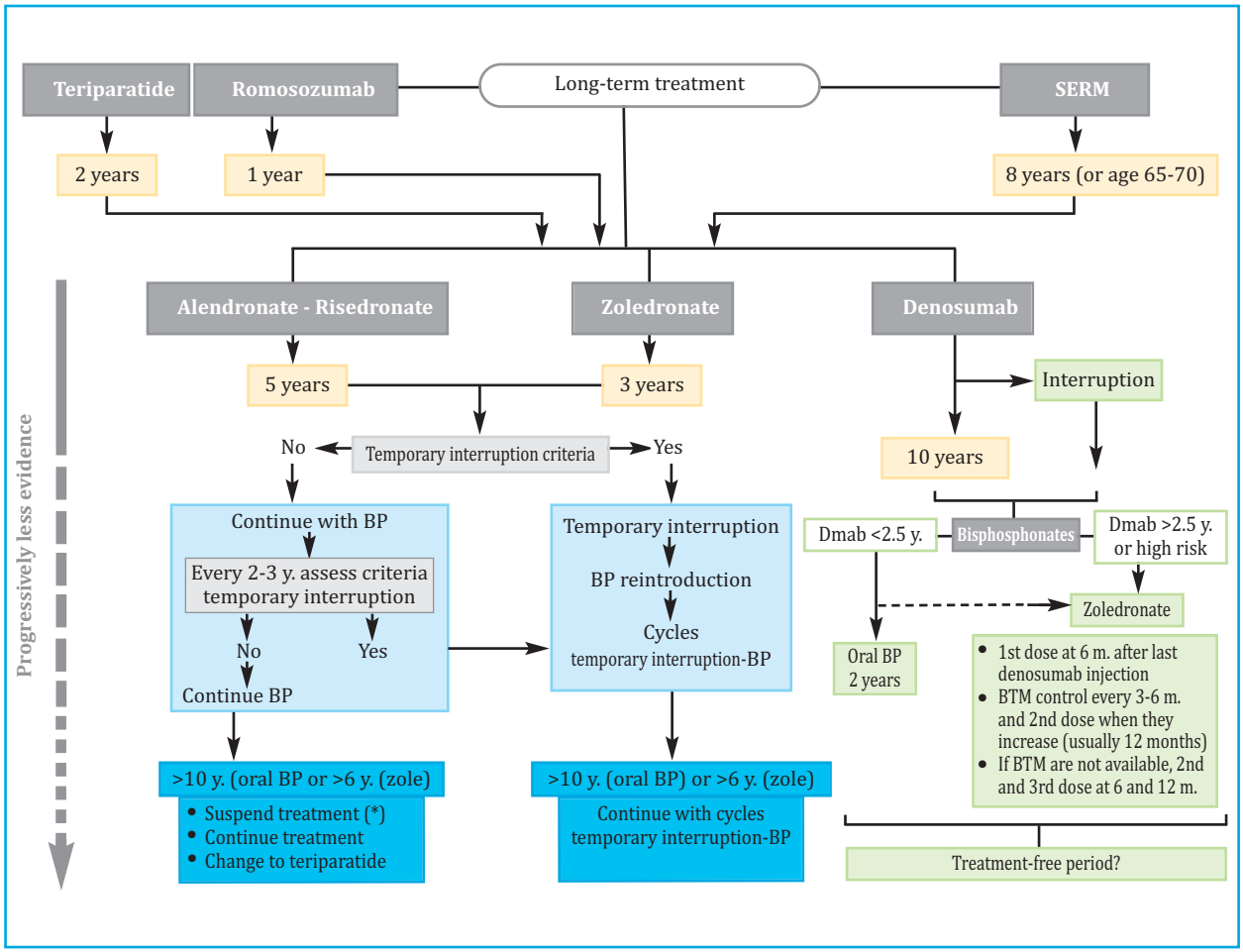
5.2. Long-term treatment (Figure 2)

Romosozumab should only be administered for one year; teriparatide therapy is limited to two years. Likewise, given that efficacy and safety data are available for up to eight years of treatment only, withdrawal of SERMs should be considered after that period, when the patient reaches 65-70 years of age or if the risk of fracture increases. After one or more of these milestones are reached, it will likely be necessary to administer another antiresorptive. The discussion on long-term treatment is thus restricted to a consideration of BPs and denosumab. One key differentiating factor at this time is the potential impact of a temporary interruption or "therapeutic vacation" or "drug holiday". While this is discouraged for individuals undergoing treatment with denosumab, it is currently accepted for BP regimens.

1) Denosumab. This agent can be administered continuously for 5–10 years. No information is currently available regarding longer periods of use. Thus, the decision to continue or discontinue drug treatment should be made carefully. Once administration of denosumab has been interrupted, the patient should be treated with a BP, for example, alendronate or zoledronate. Zoledronate is preferred if denosumab treatment was prolonged for more than 2–3 years. (See section 4.1).

2) Bisphosphonates (BPs). Three periods of use have been described:

Figure 2. Long-term treatment continuation algorithm



BP: bisphosphonates; SERM: selective estrogen receptor modulators; BTM: bone turnover markers; (*): there are not enough data to establish a recommendation after that treatment time, so the possible options are listed before a decision that must be individualized.

• First period: Current recommendations suggest that these drugs should be administered without interruption for five years (for oral BPs) or three years for zoledronate.

• Second period: After the first period (above), treatment can be temporarily interrupted if the requirements for a "drug holiday" are met (see above). The need to reinstate treatment should be periodically assessed. Once reinstated, the possibility of a second temporary suspension can be reassessed at frequent intervals.

• Third period (after 10 years of continuous or intermittent treatment with an oral BP, or six years of treatment with zoledronate): No high-quality studies are available that can be used to guide decision-making. By extrapolation of what was proposed for the second period, it is reasonable to assume that a patient that meets the appropriate requirements can be converted to a "drug holiday" regimen. Otherwise, one of the following three options should be chosen depending on context and clinical judgement:

a) Maintain treatment: This increases the risk of complications but may keep the risk of osteoporotic fractures comparatively low;

b) Withdraw treatment: This strategy reduces the risk of complications but could increase the risk of developing osteoporotic fractures;

c) Change the regimen: Teriparatide can be prescribed. This drug can reduce the risk of complications as well as the risk of developing osteoporotic fractures.

MALE OSTEOPOROSIS

There is very little evidence available to guide the treatment of male osteoporosis. Of the information that does exist, most of the studies focus on increasing BMD as a primary objective. The results are largely similar to those obtained from studies in women and suggest that drug efficacy in men is similar with respect to the prevention of fractures. Interestingly, administration of BPs such as alendronate, risedronate, and zoledronate resulted in a decrease in vertebral fractures in male patients[140-144]. Denosumab reportedly increases BMD in men and reduces the risk of fracture specifically in those undergoing androgen deprivation therapy[145,146]. Teriparatide also has beneficial effects in men[147,148]. For this reason, a drug selection strategy similar to that designed initially for women might be proposed for men:

a) Risedronate or alendronate (nb: the latter drug is not approved in Spain for male osteoporosis) for patients who have no restrictive criteria for oral administration, as described for women with postmenopausal osteoporosis;

b) Zoledronate or denosumab in patients with these restrictive criteria or who are older and therefore are at a higher risk of hip fracture;

c) Teriparatide in patients with established osteoporosis and with a high risk of fracture. Although, as in women, romosozumab also induces gains in BMD in men[109], its use to treat osteoporosis in men is not currently approved.

Proper calcium intake is also recommended, preferably through diet and vitamin D supplements in cases of insufficiency. Androgens are only justified if there is associated hypogonadism and no contraindications for their use. Even in cases of hypogonadism, some of the aforementioned drugs might have significant anti-fracture efficacy. Lastly, when hypercalciuria is detected, administration of thiazides may be considered (Recommendation D).

GLUCOCORTICOID-INDUCED OSTEOPOROSIS

BPs are the drugs of choice for glucocorticoid-induced osteoporosis[149-151]. However, if a patient presents with vertebral fractures, treatment with teriparatide is justified due to its greater anti-fracture effect[152,153] (Recommendation A). Calcium and vitamin D should also be administered. The active metabolites of vitamin D by themselves have some preventive effect on bone loss, but we do not have convincing evidence regarding their role in fracture prevention at this time[154].

Postmenopausal women and men over the age of 50 years who receive or are about to receive doses of prednisone equal to or greater than 5 mg/day (or the equivalent dose of other corticosteroids) for more than three months should receive treatment for this condition. In premenopausal women and men under 50 years of age, treatment is indicated only in cases of previous fractures, low BMD, or very high glucocorticoids dose (e. g., >30 mg/day of prednisone for more than 3 months).

Drug treatment should be maintained while the patient remains on corticosteroids. Once they are withdrawn, the risk of fracture must be evaluated in each patient. If the risk is not overly high, it may be possible to stop osteoporosis therapy entirely.

Denosumab results in a greater increase in BMD than that achieved by BPs in patients receiving corticosteroids. However, the reduction in fracture risk is similar with both drugs, as are the adverse effects[155-157]. Given, on the one hand, the rebound effect observed in some patients when denosumab is discontinued[158] and, likewise, the possibility of withdrawing antiresorptive treatment when discontinuing corticosteroids, denosumab should be indicated when it is not possible to use other antiresorptive agents and the risk of fracture is high.

In patients receiving corticosteroids, densitometric evaluation performed at shorter intervals may be justified (Recommendation D).

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Annex I
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Annex II
Conflicts of interests

Author	Shares, employee	Conference fees	Travel costs	Research grants	Advisory councils
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Carbonell Abella, Cristina		Amgen, UCB, Stada, Theramex, Angelini Pharma, Gebro	Amgen, Rubio		
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Ciria Recasens, Manuel		Grünenthal, Angelini Pharma, Gedeon Richter, Theramex, Rubio, Gebro Pharma	Amgen, Lilly, Rubio		
Corral-Gudino, Luis					
Del Pino Montes, Javier		Gedeon Ritcher, Grünenthal, UCB	Amgen		
Del Río Barquero, Luis Miguel		Amgen, Gedeon Richter			
Díaz Curiel, Manuel			Rubio		
Díez Pérez, Adolfo	Active Life Sci	Amgen, Lilly, Theramex			
García Vadillo, Alberto		Lilly, Amgen, Gebro Pharma, Theramex	UCB, Lilly, Amgen		
Gómez Alonso, Carlos	Faes	Stada, Grünenthal, Amgen, UCB	Amgen	Stada, Kyowa Kirin, Faes	Amgen, Kyowa Kirin
Gómez de Tejada Romero, María Jesús					
González Macías, Jesús		Amgen-UCB, Gedeon Richter, Menarini, Theramex	Lilly	Faes	
Guañabens, Nuria		Eli Lilly, Amgen, UCB	Eli Lilly, Amgen, UCB		Amgen, UCB
Hawkins Carranza, Federico					
Jodar Gimeno, Esteban	SICAM SL, Cajal PME, H&B	Amgen, Asofarma, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Faes, Janssen, Lilly, MSD, Novartis, Novo Nordisk, Viatrix	Amgen, Lilly, Novo Nordisk, UCB	Amgen, AstraZeneca, Boehringer Ingelheim, Faes, Janssen, Lilly, MSD, Novo Nordisk Pfizer & Sanofi	Amgen, AstraZeneca, Faes, Fresenius, Italfármaco, Janssen, Lilly, MSD, Mundipharma, Novo Nordisk, Shire & UCB

Annex II (cont.)
Conflicts of interests

Author	Shares, employee	Conference fees	Travel costs	Research grants	Advisory councils
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Monegal Brancos, Ana			Amgen, Lilly		
Muñoz Torres, Manuel		Amgen, UCB, Grünenthal Pharma, Stada, Meiji, Gedeon Richter, Ferrer			Amgen, UCB, Meiji
Naves Díaz, Manuel		Grünenthal, Gedeon Richter	Amgen, UCB		
Nogues, Xavier		UCB, Amgen, Lilly, Faes, Italfarmaco	Amgen		UCB, Amgen
Nolla, Joan M		Amgen, Lilly	Amgen, Lilly		
Pérez-Castrillón, José Luis		MSD, Lilly, Amgen, UCB, Gedeon-Ritcher, Grünenthal	Gedeon-Ritcher, MSD, Amgen, Italfarmaco	Pfizer	Faes
Peris Bernal, Pilar		Amgen, UCB, Lilly, Kyowa Kirin			
Quesada Gómez, José Manuel		Amgen, Faes, Ferrer, Gebro Pahrma, Grünenthal, Procare Health Iberia, S.L, Theramex	Amgen, Faes	Faes	Amgen, Shire
Riancho, José A.		Amgen, UCB, Lilly, Merck	Amgen, UCB, Lilly, Merck, Takeda	Alexion, Kyowa Kirin	
Rodríguez García, Minerva		Amgen, Kiowa Kyrin	Rubió, Amgen, Vifor Pharma		
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Annex III
Supplementary tables

Table S1. Levels of evidence according to the Oxford Center for Evidence-Based Medicine for studies evaluating therapy, prevention or harm

Level	
1a	Systematic reviews of RCTs with homogeneity between individual studies or several RCTs with similar results
1b	Single RCT with narrow confidence interval
2a	Systematic review of cohort studies with homogeneity between individual studies
2b	Individual cohort study or a low-quality RCT
2c	'Results' research; ecological studies
3a	Systematic review of case-control studies with homogeneity between individual studies
3b	Individual case-control study
4	Case series and low-quality cohort and case-control studies
5	Expert opinions without explicit critical appraisal, or based on physiology, basic research or "first principles"

RCT: randomized clinical trial.

Table S2. Grades of recommendation from the Oxford Center for Evidence-Based Medicine according to levels of evidence

Recommendation	Type of studies
A	Consistent level 1 studies (randomized clinical trials). By consistency we mean homogeneity (concordance) in the results of the different individual studies
B	Consistent level 2 (cohort studies) or 3 (case-control studies) studies or extrapolations from level 1 studies
C	Level 4 studies (case series and low-quality cohort or case-control studies) or extrapolations from level 2 or 3 studies
D	Level 5 evidence (inconclusive expert opinions or studies or problematic inconsistency between them, whatever their level)

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Differential inflammatory environment in patients with osteoporosis and type 2 diabetes mellitus

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Summary

Objetive: Type 2 diabetes mellitus (DM2) and osteoporosis are diseases associated with a pro-inflammatory environment, the prevention of which through new therapeutic strategies could prevent their development. However, there are few studies that evaluate the inflammatory profile of osteoporosis in patients with DM2.

This study focuses on evaluating the inflammatory immune response through serum concentrations of nine cytokines, two of them anti-inflammatory (IL-10, IL-5) and six pro-inflammatory (IL-2, IL-6, IL-12 (p70), IL-17A, TNFα and IFNγ) in 163 individuals with DM2 and 47 controls. A subpopulation, made up of 43 DM2 patients without osteoporosis, and 33 with osteoporosis, was analyzed in greater depth at the level of bone parameters. Furthermore, we have assessed the calciotropic hormones, bone remodeling markers, bone mineral density and vertebral fractures in the population, and we have analyzed the relationship of the cytokines tested with DM2, osteoporosis and prevalent vertebral fractures.

Patients with DM2 had significantly higher serum concentrations of IL-10 compared to the control group (0.5±1 vs. 0.14±0.3 pg/ml; p=0.016) and the levels of IL12 p70 were shown lower in patients with DM2 compared to controls (2.9±1.6 vs. 3.9±3.1 pg/ml; p=0.027).

In the group of patients with DM2 and osteoporosis, the levels of the cytokine IL-6 were elevated compared to the group with DM2 without osteoporosis (10.9±14.6 vs. 4.5±7.0; p=0.017). An association of IL-5 was also observed, with its lowest levels in the DM2 group with osteoporosis (1.7±0.2 vs. 3.8±0.6; p=0.032). Furthermore, IL-5 showed a direct correlation with the levels of the bone formation biomarker alkaline bone phosphatase (r=0.277, p=0.004) in the subpopulation of patients with DM2. The rest of cytokines did not show significant differences.

In conclusion, our findings indicate that in our study population, patients with DM2 compared to healthy subjects present an inflammatory profile opposite to what is expected in a hyperglycemic situation, probably as a compensatory response to the inflammation caused. The cytokine profile is modified in the subpopulation of diabetic patients, depending on the presence of osteoporosis. In this case, the inflammatory profile in the presence of osteoporosis is consistent with the expected response.

Key words: type 2 diabetes mellitus, osteoporosis, inflammation, cytokines.

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Introduction

Diabetes mellitus (DM2) and osteoporosis are increasing prevalence diseases due to the aging of the population, and gender, genetic and environmental factors, such as an unbalanced diet, obesity and a sedentary life. DM2 increased alarmingly in 2014, affecting more than 420 million people worldwide[1]. Patients with DM2 present a higher risk of falls and increased prevalence and incidence of fragility fractures have been observed in these patients[2-5], causing significant mortality, morbidity and increased healthcare costs.

DM2 affects bone homeostasis[6,7], and is associated with a higher risk of fractures[8], despite the fact that patients exhibit higher bone mineral density (BMD)[4,9-12]. Furthermore, reduced circulating levels of bone turnover markers have been observed in DM2[13], which should influence the high fracture risk in patients with DM2.

On the other hand, inflammation is gaining prominence in the development of the disease and its complications. Multiple studies show an increase in inflammatory cytokines in DM2, which confer a chronic state of low-grade inflammation.

In DM2, it is common for patients to have an inadequate lifestyle, with excessive caloric intake and lack of physical exercise, which promotes central adiposity and obesity, so that there is a greater infiltration of macrophages in the adipose tissue, potentially altering the secretion of cytokines[14]. The release of these inflammation-mediating proteins is thus the result of the activation of immune cells accumulated in metabolic tissues and that by altering the secretion of cytokines, promote systemic insulin resistance (IR) and damage to β cells. producing insulin. Thus, an inflammatory environment is associated with altered levels of circulating cytokines, which could alter insulin sensitivity, leading to a greater risk of suffering from DM2[15]. On the other hand, patients with DM2 have accelerated aging, a process that leads to an increased risk of developing bone fragility prematurely, especially in patients with poorly controlled blood glucose[16]. Inflammatory cytokines also increase their production during aging, being crucial for skeletal homeostasis. Inflammatory cytokines have been observed to alter RANKL: OPG ratios and may result in increased osteoclastogenesis[17]. Thus, the immune system is strongly linked to maintaining healthy bones.

In order to prevent the progression of osteoporosis and related fractures in patients with DM2, bone health should be evaluated and interventions for the prevention of fractures should be implemented in this population, and if DM2 and osteoporosis are established, pharmacological interventions should be found and effective lifestyles. In this sense, the most innovative treatments for DM2 include blocking the pathological overproduction of pro-inflammatory cytokines by antagonists of the receptor of the cytokine of interest, or by neutralizing antibodies to it. Currently, vaccine treatments are being developed, consisting of repeated injection of the cytokine to produce an overexpression of neutralizing antibodies against the injected cytokine. Specifically, drugs that block the effect of the cytokine IL-1β have emerged as first-line therapy. Monoclonal antibodies directed against IL-1 β[18,19] and vaccines[20] are being tested, which turn out to be beneficial in terms of glycemic and inflammatory parameters in patients with DM2.

Due to the increasing prevalence of DM2 and its comorbidities, such as osteoporosis, there is a growing demand for personalized therapies, the efficiency of which is periodically monitored by evaluating biomarkers of disease progression.

This study aims to expand the knowledge of the mechanisms involved in bone homeostasis, by evaluating inflammatory cytokines associated with osteoporosis in patients with DM2. We have focused on 9 circulating cytokines, which could be involved in the systemic inflammation of osteoporosis in patients with DM2. In this way, we intend to contribute to the knowledge of the cytokines involved in the pathogenesis of both diseases, facilitating and simplifying the design of anti-inflammatory therapies to prevent the progression of osteoporosis in patients with DM2.

Population and methods

Design and study population

This cross-sectional study encompasses a total of 210 participants, which include 47 control individuals and 163 patients with DM2 diagnosed with diabetes, according to the criteria of the American Diabetes Association. Diabetic patients were on therapy for their disease, including metformin, sulfonylureas, insulin, or a combination of these. Patients treated with thiazolidinediones were excluded because they affected bone metabolism and cytokine release.

The specific study in presence vs. absence of osteoporosis in the DM2 population was performed on 43 patients without osteoporosis and 33 patients without osteoporosis. We use the World Health Organization Criteria for osteoporosis[21]. Due to the special characteristics of the pathophysiology of type 2 diabetes condition the appearance of fractures without densitometric alterations, patients with osteoporosis will also be those with prevalent vertebral fractures, even without meeting the criteria of BMD ≤−2.5 standard deviations (SD) of the T-score in the lumbar spine, total hip or femoral neck.

All participants were Caucasian, 35 to 65 years old.

Exclusion criteria for the population of patients with DM2 include a previous history of systemic inflammation due to other diseases or chronic diseases different from DM2, anti-inflammatory treatments or high alcohol consumption. None of the subjects were treated with medication known to modify bone mass.

The population was recruited at the San Cecilio University Hospital in Granada, Spain, and the samples were managed by the Biobank of the Andalusian Public Health System. The study was approved by the Andalusian Biomedical Research Ethics Committee.

Anthropometric, clinical and biochemical measurements

Anthropometric data were collected, including body mass index (BMI) (weight in kilograms divided by the square of height in meters).

For the measurement of various biochemical parameters in serum, venous blood samples were taken in the morning after an overnight fast. Sera were stored at -80°C until examination.

The biochemical parameters of fasting glucose, glycated hemoglobin (HbA1c), calcium, phosphorus, and creatinine were measured using standard automated laboratory techniques. Calciotropic hormones measured were iPTH (immunoassay; Roche Diagnostics SL) and 25-hydroxyvitamin D (RIA; DiaSorin). The biomarkers of bone turnover measured were osteocalcin (RIA, DiaSorin Stillwater, MN); Bone alkaline phosphatase - (immunoassay, Hybritech Europe), CTX (immunoassay,

Elecsys CrossLaps; Roche Diagnóstica) and tartrate-resistant acid phosphatase 5b -TRAP5b- (immunoassay, IDS Ltd.).

For the measurement of bone density and vertebral fractures, bone mineral density (BMD) was evaluated in the lumbar spine (LS) L2-L4, in the femoral neck (CF) and in the total hip (TH) by means of dual absorptiometry of X-ray of (DEXA) with a Hologic QDR 4500 densitometer (Waltham, MA; coefficient of variation 1%). We use the World Health Organization Criteria for osteoporosis[21]. The presence of prevalent vertebral bills was evaluated in conventional lateral view radiographs of the spine, at the thorax level and at the lumbar level (T4-L5). Traumatic vertebral fractures were excluded. Vertebral fractures were identified according to the method of Genant et al.[22] Only moderate and severe fractures were considered in our study.

Cytokine measurement

The concentration of nine cytokines (IL-10, IL-4, IL-5, IL-2, IL-6, IL-12 (p70), IL-17, TNFα and IFNγ) was measured by multiplex assays with luminex technology, using the Millipore Human Th17 Magnetic Bead Panel kit (Cat. # HTH17MAG-14K), according to manufacturer's instructions. The reading was carried out on the Bio-Plex® 200 system (Bio-Rad). Data are expressed in pg x mL^{-1}. The intra-assay coefficient of variation was less than 10% and the inter-assay coefficient of variation was less than 15% for all the analytes studied. The assayed kit incorporates internal cytokine controls designed for use in quality control during accuracy and precision monitoring of cytokine analyzes carried out.

Statistic analysis

Data were analyzed using SPSS-23 software (SPSS, Inc.). Continuous variables were expressed by means and standard deviation, and categorical variables by percentages. The normal distribution was evaluated using the Kolmogorov-Smirnov test. The variables with normal distribution were studied using the Student's t-test, and the variables that did not meet normality were analyzed using the Mann-Whitney U test. The x^2 tests were used to compare categorical variables. Values of $p<0.05$ were accepted as statistically significant values.

Results

Clinical characteristics of the population of patients with DM2 and controls

The baseline characteristics of the entire study population, both the group of patients with DM2 and controls, are described in table 1. Due to the inclusion criteria, individuals with DM2 have significantly higher levels of glucose and HbA1c than the control group ($p<0.001$). Calciotropic hormones iPTH, osteocalcin and biomarkers CTX and TRAP5b were higher in controls.

Cytokine profile in patients with DM2 and controls

As presented in table 1, the cytokines that show differences in the comparison of their serum concentrations correspond to IL-10 and IL-12 p70. Serum IL-10 concentrations are higher in the group of patients with DM2 compared to the control group (0.5±1 vs. 0.14±0.3 pg/ml; $p<0.05$).

In the case of IL-12 p70, lower serum values are shown in patients with DM2 compared to healthy controls (2.9±1.6 vs. 3.9±3.1 pg/ml; $p<0.05$) . On the other hand, the values of the cytokines IL-5, IL-6, IL17A, TNFα and IFNγ do not show differences between the study groups, although IL-5 and IFNγ approach significance in the comparison of groups. In addition, the levels of the cytokine IL-4, IL-2 and IL-17A were not detectable in most cases. Therefore the data have not been presented in this study.

In figure 1, the comparison of the serum levels in the DM2 groups and the cytokine controls is graphically shown, being visualized in A) IL-10 and in B) IL-12 (p70).

Clinical characteristics of the group of patients with DM2 and its relationship with bone metabolism

The characteristics of the patient population with type 2 diabetes mellitus, based on the presence or absence of osteoporosis, are presented in table 2.

Regarding the calcium hormones 25 (OH) vitamin D and parathyroid hormone, no differences were observed for the first one. However, parathyroid hormone levels are elevated in the DM2 group in the presence of osteoporosis compared to the DM2 group without osteoporosis (45.9±4.0 vs. 31.1±1.4; $p=0.01$).

The bone remodeling markers CTX and TRAP5b and bone alkaline phosphatase show no differences between groups.

Regarding the DEXA measurement parameters, it can be verified both in the T-scores and in BMD, that these values correspond to the selection criteria of this sample of patients with DM2, according to their bone status. The group with osteoporosis presented all the parameters of BMD and T-score with a lower value compared to the group without osteoporosis.

Cytokine profile in patients according to the presence of osteoporosis in the DM2 population

Among the cytokines studied, IL-6 is shown with a higher serum concentration in the DM2 group with osteoporosis, compared to the DM2 group without osteoporosis (10.9±14.6 vs. 4.5±7.0; $p=0.01$). On the contrary, the cytokine IL-5 presented lower values in the same group of diabetics with osteoporosis (1.7±0.2 vs. 3.8±0.6; $p=0.032$). The cytokines studied IL-10, IL-12 (p70), TNFα and IFNγ did not show differences in the comparison between both groups.

In figure 2C, the levels of IL-6 are graphically shown in both DM2 groups, with and without osteoporosis, and in figure 2D the levels of IL-5 in the same groups are shown.

On the other hand, we found a lack of association in the analysis between the presence of fractures and the cytokines studied in the group of osteoporotic diabetics.

Relationship between cytokines and markers of bone formation and resorption

A correlation study has been carried out between the cytokines tested and the biomarkers of formation (bone alkaline phosphatase and osteocalcin) and bone resorption (TRAP5b and CTX), in the total population, in the type 2 diabetic population and in the osteoporotic diabetic population with prevalent vertebral fractures. The results indicate a significant direct correlation in the case of alkaline phosphatase and interleukin 5, both for the total population ($r=0.162$, $p=0.049$), and for the type 2 diabetic population ($r=0.276$, $p=0.004$) . This last correlation is shown in figure 2. In the case of the population of osteoporotic diabetics with prevalent vertebral fractures, the correlation is lost.

Table 1. Anthropometric and biochemical parameters and cytokine concentrations in the study population of patients with type 2 diabetes mellitus (DM2) and the control group

	DM2 group (n=163)	Control group (n=47)	P value
Age (years)	63±9	54±8	≤0.001*
Male/female (n)	91/72	29/18	0.028*
BMI (kg/m^2)	31.6±5.8	31.4±7.7	0.056
Glucose (mg/dL)	159±59	90±11	≤0.001*
HbA1c (%)	8.2±1.9	4.9±0.4	≤0,001*
Creatinine (mg/dL)	1.8±8.0	0.8±0.2	0.106
Calcium (mg/dL)	10.8±9.9	9.3±0.4	≤0.001*
Phosphorus (mg/dL)	3.8±3.3	3.4±0.48	0.779
25 (OH) D (ng/mL)	18.2±9.9	21.3±10.8	0.069
iPTH (pg/mL)	46.3±43.9	51.7±18.7	0.003*
Osteocalcin (ng/mL)	1.4±1.2	4.3±4.9	0.002*
Bone alkaline phosphatase (µg/L)	15.9±9.8	13.7±7.3	0.06
CTX (ng/ml)	0.23±0.13	0.35±0.15	≤0.001*
TRAP5b (UI/L)	1.4±0.92	1,8±0,87	0.019*
Vertebral fracture (%)	27.7	0	≤0.001*
Osteoporosis (%)	43.42	0	≤0.001*
Cardiovascular disease (%)	49	0	≤0.001*
Cytokines:			
IL-5 (pg/mL)	3.2±4.2	4.1±4.2	0.07
IL-10 (pg/mL)	0.5±1	0.14±0.3	0.016*
IL-2 (pg/mL)	1.3±2.8	0.3±0.6	0.57
IL-6 (pg/mL)	6.7±11.1	9.8±18	0.66
IL-12 (p70) (pg/mL)	2.9±1.6	3.9±3.1	0.027*
IL-17A (pg/mL)	2.7±2.2	2.1±1.7	0.41
TNF-a (pg/mL)	1.8±4.5	1.0±1.9	0.65
IFN-g (pg/mL)	1.3±1.4	0.8±1.2	0.07

Data are shown as mean ± standard deviation, percentages or total number (n) *: p-value <0.05 between groups.
BMI: body mass index; HbA1c: hemoglobin A1c; 25 (OH) D: 25 hydroxy-vitamin D; IL: interleukin; TNFα: tumor necrosis factor alpha; IFNγ: interferon gamma.

Discussion

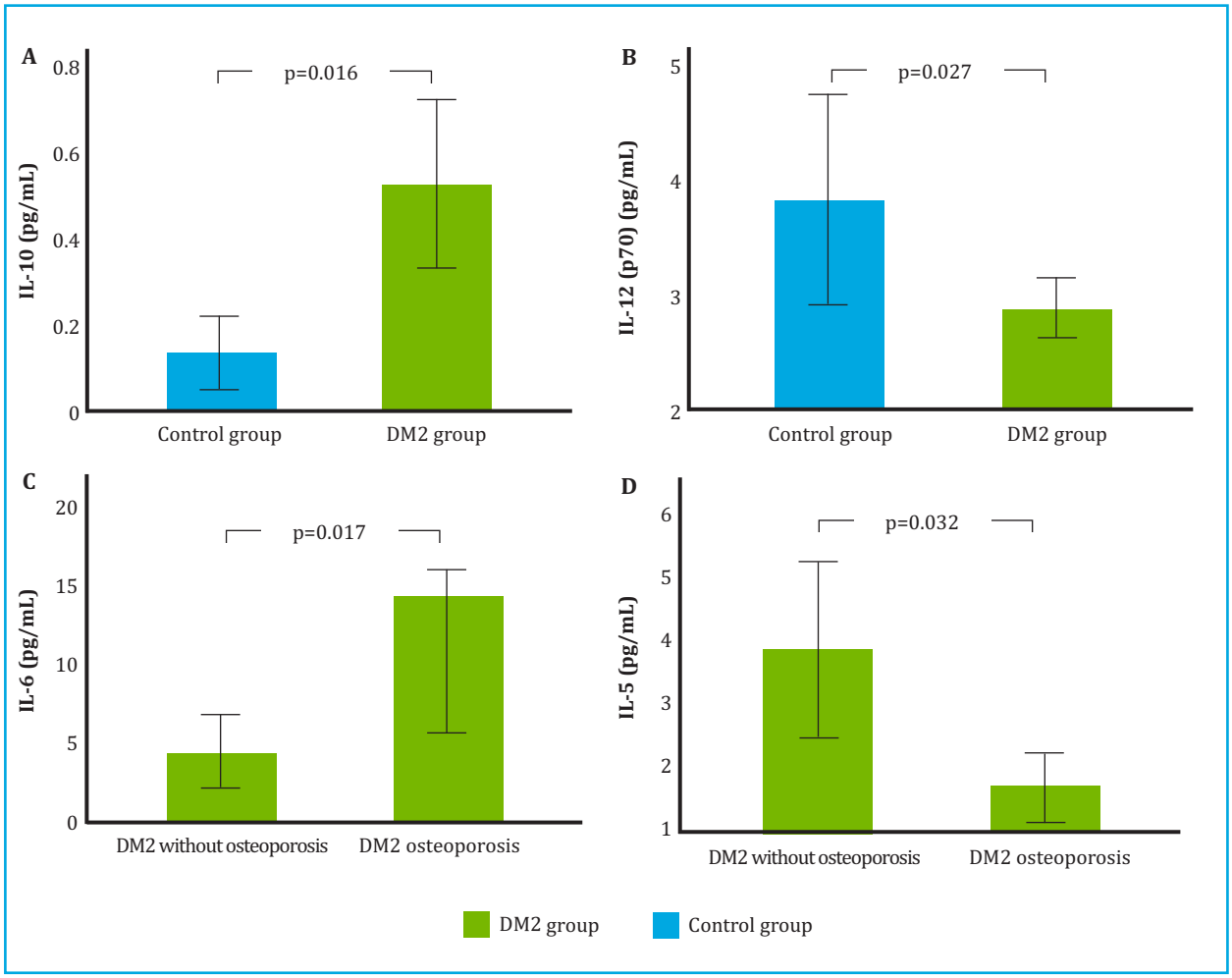
DM2 is an extremely complex and multifactorial chronic and systemic inflammatory disease. Clinical evidence shows that the risk of other complications such as osteoporosis is greatly increased in these patients. Insulin resistance can affect abnormal cytokine secretion and, in turn, produce alterations in bone metabolism, resulting in bone deterioration and osteoporosis[23]. However, the specific factors and molecular mechanisms that cause osteoporosis in patients with DM2 have not yet been elucidated.

In this study we have explored the relationship of the inflammatory environment with the presence of DM2 and osteoporosis. First, we have evaluated the association of the levels of various pro-inflammatory and anti-inflammatory serum cytokines (IL-2, IL-4, IL-17, IL-5, IL-6, IL-10, IL12 p70, TNFα and IFNɣ) in 210 individuals, of which 163 corresponded to patients with DM2 and 47 healthy individuals. Second, in the DM2 population, we have analyzed the association of these cytokines with osteoporosis, characterizing the population from the point of view of bone metabolism.

The results show higher serum IL-10 concentrations in DM2 compared to the control group, lower levels of IL-12 (p70) in patients with DM2, as well as higher circulating concentrations of IL-6 and lower IL-5 in the DM2 population with osteoporosis compared to DM2 patients without osteoporosis (see figure 1).

An outstanding finding of the present study involves increased levels of the anti-inflammatory cytokine IL-10, which has been shown to be elevated in patients with DM2 compared to the control group. Previously, it has been suggested that this anti-inflammatory cytokine is part of a complex interaction between pro-inflammatory

Figure 1. Association in the entire population, between control groups and patients with type 2 diabetes mellitus, with serum concentrations of: A) IL-10 and B) IL-12 (p70). C) association in the group of patients with type 2 diabetes mellitus, in relation to the presence and absence of osteoporosis, with the circulating concentration of IL-6 and D) of IL-5



and anti-inflammatory molecules, where the high levels of the latter would compensate and limit the damage caused by the inflammatory environment. This hypothesis was formulated in the context of both DM2, in a recent study with a low number of patients (n=25)[24], and in a study of osteoarthritis, where it is observed that inflammatory cytokines such as IL-6 and TNFα were expressed in parallel with the anti-inflammatory cytokine IL-10 as a compensatory mechanism for inflammation[25]. In fact, the physiological role of IL-10 is to limit the immune inflammatory response, inhibiting the activity of various cell types, especially the activation of macrophages and also preventing the production of other pro-inflammatory mediators such as IL-6 or TNFα[26]. On the other hand, it has been possible to verify that macrophages exposed to high levels of glucose show a resistance or low response to the effect of IL-10, preventing its anti-inflammatory action[27], so that the high levels of IL-10 could also be due to an attempt In addition, our findings are in line with those found by Wang et al.[28], who observed a progressive increase in IL-10 among patients without DM2, prediabetics and with type 2 diabetes. There are studies in contrast to our results, such as the one carried out with 15 patients with DM2 with respect to the same number of controls, in which a low expression of IL-10 is observed in DM2, and its levels were correlated with the levels of Glycosylated hemoglobin, for which it was proposed as a predictor of glycemia[29].

The pro-inflammatory cytokine IL-12 is a heterodimeric glycoprotein formed by the p40 and p35 subunits, its bioactive form being IL12 p70[30]. In our study we found a low serum concentration of the pro-inflammatory cytokine IL-12 p70 in patients with DM2 compared to controls. There are several studies with conflicting results. Thus, it has been shown that IL-12 increases in DM2 and is involved in the pathogenesis of atherosclerosis, macrovascular complications, diabetic retinopathy and endothelial dysfunction, especially in those patients with greater insulin resistance[31-33]. Several studies establish that the interruption in the expression of IL-12 triggers angiogenesis, protecting the endothelial tissues in type 2 diabetes. In addition, studies have been shown in murine models of DM2 in which IL-12[33] deficiency promotes overload. -expression of anti-inflammatory cytokines and reduces the expression of pro-inflammatory ones.

In line with our results, an increase in IL-10 and a decrease in IL-12 p70[34] have been observed in patients with DM2. In this study, it was suggested that interleukin IL-10 suppresses the activation of Th1 cells, which require IL-12 for their differentiation. In this way, the reduced level of IL-12 and the high concentration of IL-10 found in our study in patients with DM2, would contribute to

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Table 2. Anthropometric, physical and biochemical parameters of bone metabolism and serum cytokine concentrations in a subpopulation of the group of patients with type 2 diabetes mellitus (DM2), in relation to the presence and absence of osteoporosis

	Group DM2 and OP (n=33)	Group DM2 without OP (n=43)	P value
Age (years)	59.5±5.2	56.33±6.8	0.02*
Male/female (n)	19/14	22/21	0.37
BMI (kg/m^2)	33.1±6.5	29.8±4.4	0.02*
HbA1c (%)	7.6±1.8	8.1±1.8	0.4
Glucose (mg/dL)	163.3±65	180.5±58.5	0.08
Creatinine (mg/dL)	0.88±0.17	0.9±0.21	0.27
Calcium (mg/dL)	9.5±0.5	9.6±0.5	0.24
Phosphorus (mg/dL)	3.6±0.4	3.7±0.6	0.84
25 (OH) D (ng/mL)	19.2±11.8	16.5±10.6	0.38
Osteocalcin (ng/mL)	1.7±1.4	1.3±1.0	0.43
iPTH (pg/mL)	45.9±4.0	31.1±1.4	0.013*
Bone alkaline phosphatase (µg/L)	15.1±7.5	14.7±5.6	0.76
CTX (ng/ml)	0.24±0.1	0.18±0.09	0.14
TRAP5b (UI/L)	1.3±0.9	1.4±1.01	0.58
Fracture (%)	60.6	0	≤0.001*
DEXA parameters			
BMD CL (g/cm^2)	0.9±0.1	1.0±0.1	0.001*
BMD CF (g/cm^2)	0.7±0.1	0.8±0.1	0.005*
BMD CT (g/cm^2)	0.8±0.1	0.9±0.1	0.007
T-score CL	-2.0±1.4	-0.9±1.1	0.001*
T-score CF	-1.1±1.0	-0.27±0.7	0.001*
T-score CT	-1.1 ±1.0	-0.3±0.7	0.002*
Cytokines			
IL-5 (pg/mL)	1.7±0.2	3.8±0.6	0.032*
IL-10 (pg/mL)	0.7±1.2	0.4±0.7	0.97
IL-6 (pg/mL)	10.9±14.6	4.5±7.0	0.017*
IL-12 (p70) (pg/mL)	2.7±0.2	2.8±0.2	0.328
TNF-a (pg/mL)	1.0±2.1	1.2±1.7	0.41
IFN-g (pg/mL)	1.3±1.7	1.4±1.3	0.38

Data are shown as mean ± standard deviation, percentages, or total number (n). *: P value <0.05 between groups.
25 (OH) D: 25 hydroxy-vitamin D; iPTH: intact parathormone; CTX: carboxy-terminal telopeptide; TRAP5b: tartrate-resistant acid phosphatase 5b; FAO: bone alkaline phosphatase; BMD: bone mineral density; CL: lumbar spine; CF: Femoral neck; CT: total hip; IL: interleukin; TNFα: tumor necrosis factor alpha; IFNɣ: interferon gamma.

stopping the activation of the subpopulation of Th1 cells, the main producers of the pro-inflammatory cytokine IFNɣ, resulting in homeostasis of relevant tissues.

Thus, in our study we observed that the differences found in cytokine levels between patients with DM2 and controls seem to be the opposite of what would be expected in hyperglycemia, an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines. This could indicate a response to the increase in inflammation derived from hyperglycemia, rather than to the factors intrinsic to DM2.

The cytokine IL-6 has been described in multiple epidemiological studies as a powerful predictor of diabetes, suggesting that it interferes with the insulin signal and alters the function of beta cells[35,36]. We have investigated the potential of IL-6 as a factor involved in osteoporosis in patients with DM2, but did not find extensive references in the literature on this subject. The cytokine IL-6 performs two parallel functions that aggravate the osteoporotic condition: it stimulates the osteoclasts and inhibits the activity of the osteoblasts, resulting in a loss of bone density[37]. This effect of loss of bone density has been shown

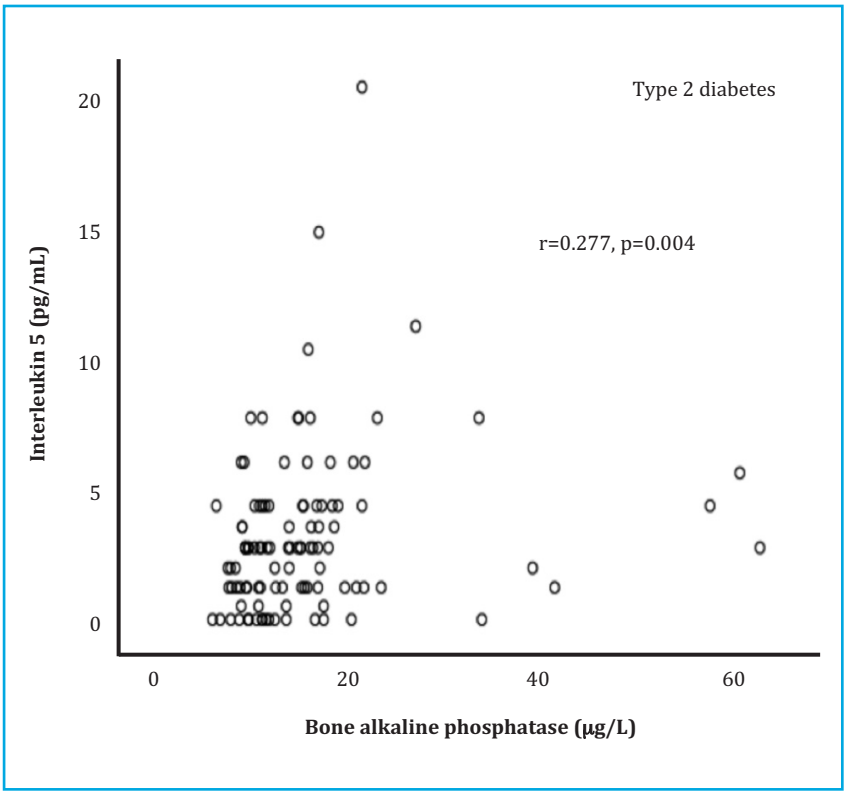
mainly in menopausal women[38]. Here it is shown that IL-6 is also increased in DM2 patients with osteoporosis. The anti-inflammatory interleukin IL-5 has been shown to lower levels in patients with DM2 and osteoporosis. Furthermore, we have observed a direct relationship of this IL-5 with the osteoblastic activity marker bone alkaline phosphatase.

These results should be expanded with larger studies that clarify the role of the association of inflammatory markers, DM2 and osteoporosis, as well as its possible extension to therapeutic intervention.

Conclusions

DM2 is a disease with a low degree of chronic inflammation, being extremely complex and multifactorial. In this study we have shown that patients with DM2 have altered levels of some pro-inflammatory and anti-inflammatory cytokines, which could be factors involved in the evolution of the disease. The inflammatory profile varies depending on the progression of the disease, the presence or absence of osteoporosis in patients with DM2. Taking into account the existence of differentiated profiles, it would be necessary to develop more precise options for the treatment of patients and include them in clinical practice guidelines.

Figure 2. Correlation graph showing the relationship between IL-5 and the bone formation biomarker alkaline bone phosphatase in the subpopulation of patients with type 2 diabetes mellitus



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25-OH-vitamin D and reversal of metabolic comorbidities associated with obesity after bariatric surgery

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Summary

Introduction: Obesity is a public health concern in which defects in the endocrine system occur, which may lead to metabolic diseases. Bariatric surgery (BS) has proved to be more effective in weight loss and reversal of comorbidities (especially inflammatory and metabolic). The underlying mechanisms related to the reversal of comorbidities are still poorly understood. Patients undergoing BS routinely receive vitamin D supplements, so its role in the reversal of comorbidities may be relevant.

Objectives: To determine the relationship between 25-OH-vitamin D levels, the prevalence of metabolic comorbidities before BS and 6 months post-op.

Results: 328 patients were evaluated, who showed significant loss of weight and lean mass 6 months after BS. Serum levels of 25-OH-vitamin D increased in parallel with an increase in supplementation. However, no correlations were observed with the presence of baseline metabolic comorbidities or at 6 months of BS. Serum levels of 25-OH-vitamin D were correlated with some parameters of body composition independently of the reversal of comorbidities.

Conclusions: Bariatric surgery was associated with a significant improvement in metabolic comorbidities in the patients studied independently of 25-OH-vitamin D serum levels.

Key words: vitamin D, obesity, metabolic comorbidities, reversal.

Introduction

Obesity is a chronic metabolic disease with an increasing incidence that is associated with the development of multiple metabolic and mechanical comorbidities, it has also been associated with a higher incidence of tumors, a worse evolution of autoimmune diseases (SEEDO/WHO)[1,2] and a increase in all-cause mortality[3,4]. According to Spain's Ministry of Health data, the prevalence of obesity in the adult population (25-65 years) is 14.5% (17.5% in women; 13.2% in men), with a parallel increase with people's age (21.6% and 33.6% in women and men over 65 years of age, respectively). This situation is a public health challenge, not only because of its prevalence, but also due to the increase in morbidity and mortality, accelerated aging, the economic costs and associated social implications[5,6].

To date, intensive medical treatment and lifestyle modifications in obese patients have not shown a significant decrease in the development of complications during follow-up (10-20 years)[7-9]. In contrast, bariatric surgery (BS) is an intervention that entails significant weight loss (25-58% at 10 years), associated with a significant improvement in comorbidities directly and indirectly related to the disease[7,10]. Additionally, BS reduces the risk of mortality by 51%[10]. Different meta-analyzes suggest reductions in cardiovascular mortality (OR, 0.58, 95% CI, 0.46-0.73), all-cause mortality (OR: 0.70, 95% CI, 0.59-0 ,84) and increased life expectancy of up to 7 years in patients with underlying cardiovascular disease[11].

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On the other hand, 25-OH-vitamin D levels have been closely related to the development of cardiovascular disease, specifically, its deficit has been associated with higher cardiovascular and all-cause mortality[12]. After BS, there is a significant reversal of metabolic comorbidities, as well as reduced cardiovascular risk and mortality from all causes in this population[10,13,14]. Likewise, the use of vitamin D supplements is carried out systematically during the follow-up of patients undergoing BS[15,16]. In this sense, it is not clear whether this supplementation can modulate the post-BS inflammatory response and that it could be related to the improvement of comorbidities in this patient group.

In this context, we analyze the levels of 25-OH-vitamin D in obese patients undergoing BS and its relationship with the reversal of metabolic comorbidities 6 months post op.

Patients and methods

Patients

The clinical variables of 328 patients undergoing BS at the Reina Sofía Hospital in Cordoba, Spain were analyzed. Our ethics committee, whose protocol was designed in accordance with the Declaration of Helsinki and with national and international guidelines for biomedical research approved the study. Each individual signed a written informed consent before taking part. Inclusion was carried out consecutively, all patients who underwent surgery and voluntarily decided to participate in the study. Bariatric surgery in our center is performed in men and women between 18 and 65 years old with BMI >40 Kg/m^2 or BMI >35 Kg/m^2 and at least one metabolic, mechanical or psychological comorbidity that indicates it, as established clinical practice guidelines[17-20]. A 6-month follow-up was carried out following the protocol of our hospital, which is based on international clinical practice guidelines. For this control, 260 patients of those initially evaluated were included. The general clinical characteristics of the included patients are summarized in the table 1.

The patients were treated according to the available clinical guidelines[15,17,19,21], an anthropometric assessment was also performed, with bioimpedance measurement (TANITA MC-780MA multifrequency impedance meter) and analytical. The determination of 25-OH-vitamin D was carried out by chemiluminescence with acridinium ester, with capture of streptavidin-biotin. The presence and disappearance of metabolic comorbidities was determined as part of the clinical history in the evaluation of the patient, and it was confirmed by analysis or determination of blood pressure in the consultation.

Statistic analysis

U-Mann Whitney tests were used to assess clinical associations. The xi-square test was used to compare categorical data, as well as Kruskal-wallis tests and ANOVA for multiple comparisons. Statistical analyzes were performed using the statistical software SPSS version 20 and Graph Pad Prism version 7. The graphs and tables present the data expressed as mean ± standard deviation or median ± interquartile range. The proportions were expressed as a percentage. In all analyses, p values <0.05 were considered statistically significant.

Table 1. Characteristics of the baseline population and 6 months after surgery

Characteristic	Baseline (n=328)	Post-BS (6 meses)	*p*
Age	47.54 ± 9.78		
Sex			
Female (%)	65.7		
Male (%)	34.3		
Metabolic comorbidities (%)	62.7	53.3	0.004
BMI (Kg/m^2)	47.5 ± 6.63	34.14 ± 5.55	<0.001
Fat mass (Kg)	59.09 ± 16.49	31.67 ± 11.86	<0.001
Lean mass (Kg)	58.29 ± 12.46	58.29 ± 2.45	0.639
Abdominal perimeter (cm)	131.18 ± 15.26	110.7 ± 14.80	<0.001
25-OH-vitamin D (ng/dl)	16.43 ± 9.95	30.08 ± 12.86	<0.001
Calcifediol supplementation (%)	26.2	93.8 (16/260)	<0.001
Supplementation dosage			
Calcifediol 0.266 mg every 30 days	5.2 (17/328)	31.2 (81/260)	
Calcifediol 0.266 mg every 21 days	4.9 (16/328)	33.8 (88/260)	
Calcifediol 0.266 mg every 15 days	12.8 (42/328)	23.8 (62/260)	
Calcifediol 0.266 mg every 10 days	2.4 (8/328)	0.8 (2/260)	
Calcifediol 0.266 mg every 7 days	1.2 (4/328)	3.1 (8/260)	

Results

The 328 operated patients presented a significant decrease in the presence of metabolic comorbidities at the sixth month after surgery, in parallel with the decrease in BMI and fat mass, but not in lean mass (figure 1A; table 1). Only 26.2% of the patients received calcifediol supplementation before surgery. This percentage increased to 93.8% 6 months after surgery (table 1). The presence of arterial hypertension (HT), dyslipidemia (DLP) and type 2 diabetes mellitus (DM2) were evaluated as metabolic comorbidities, which decreased from 62.7% to 53.3% (table 1). Supplementation at discharge from BC and at 6 months was significantly higher, as was the percentage and absolute number of patients with an increase in the dose interval (figure 1B).

Baseline 25-OH-vitamin D levels did not show differences between patients with or without metabolic comorbidities (figure 2A), both groups increased their serum levels in parallel (figure 2B) and the values did not affect the presence or absence of metabolic comorbidities. at the sixth month of BS (figure 2C). When analyzing metabolic comorbidities separately, no differences were observed between their presence and baseline 25-OH-vitamin D levels (figures 2 D-F); patients with HT had significantly higher levels of 25-OH-vitamin D at 6 months (figure 2H) while in patients with DM2 the increase was not significant (figure 2G); patients with DLP showed a trend that did not reach statistical significance (figure 2I).

From the anthropometric point of view, baseline BMI together with fat mass were negatively correlated with serum levels of 25-OH-vitamin D, while these values were positively correlated with baseline lean mass and at 6 months of BC. For its part, the determination of 25-OH-vitamin D at 6 months was only negatively correlated with pre-surgery abdominal girth and weight after BS (figure 3).

Discussion

This study is presented in which possible associations between 25-OH-vitamin D levels and the reversal of metabolic comorbidities after bariatric surgery are analyzed in a large cohort of patients undergoing this procedure. The aim is to determine the relationship between 25-OH-vitamin D levels, the prevalence of metabolic comorbidities before BS and 6 months post operative.

Vitamin D has been associated with an increased risk of developing DM2, HT, myocardial infarction, peripheral arterial disease, some types of cancer, autoimmune and inflammatory diseases, and even with increased mortality[22]. It also has an essential role in homeostasis and insulin secretion mechanisms[23]. After BC, there are numerous effects on mineral and bone metabolism, including calcium and/or vitamin D deficiency, secondary hyperparathyroidism, and loss of bone mass[24-27]. The changes in its metabolism seem to be influenced first by abnormalities in bone metabolism prior to surgery (related to morbid obesity), and later by changes in calciotropic hormones after BS and nutrient malabsorption. It is also unknown whether in the long term, these changes persist or stabilize after the body adapts to the new weight, hormonal secretion and environmental habits[28].

Likewise, calcidiol levels have been related to modulation of the inflammatory response in different diseases, conditioning their evolution and prognosis[29]. In this context, the mechanisms underlying the improvement in comorbidities are dependent and independent of the percentage of weight lost[30,31], and to a large extent are related to the improvement in insulin resistance and the improvement in β-cell function-pancreatic[32]. Vitamin D has been reported to improve insulin sensitivity and decrease the risk of developing diabetes, which is why it could have an additive (or essential) effect in the reversal of comorbidities[33]. However, in our cohort, no significant changes were observed in the evolution of metabolic comorbidities in the patients evaluated. This can be explained by the follow-up time of the patients, considering that, if there is no re-gain in weight, a greater reversion of comorbidities would be expected in these patients.

On the other hand, vitamin D deficiency is more common in obese patients, in this sense different mechanisms have been postulated, including a lower dietary intake, lower skin synthesis, decreased intestinal absorption and alteration in its metabolism[34]. There are

Figure 1. Clinical changes after BS. A) evolution of anthropometric changes 6 months after BS; B) interval of prescription of calcifediol supplementation after 6 months from the BS

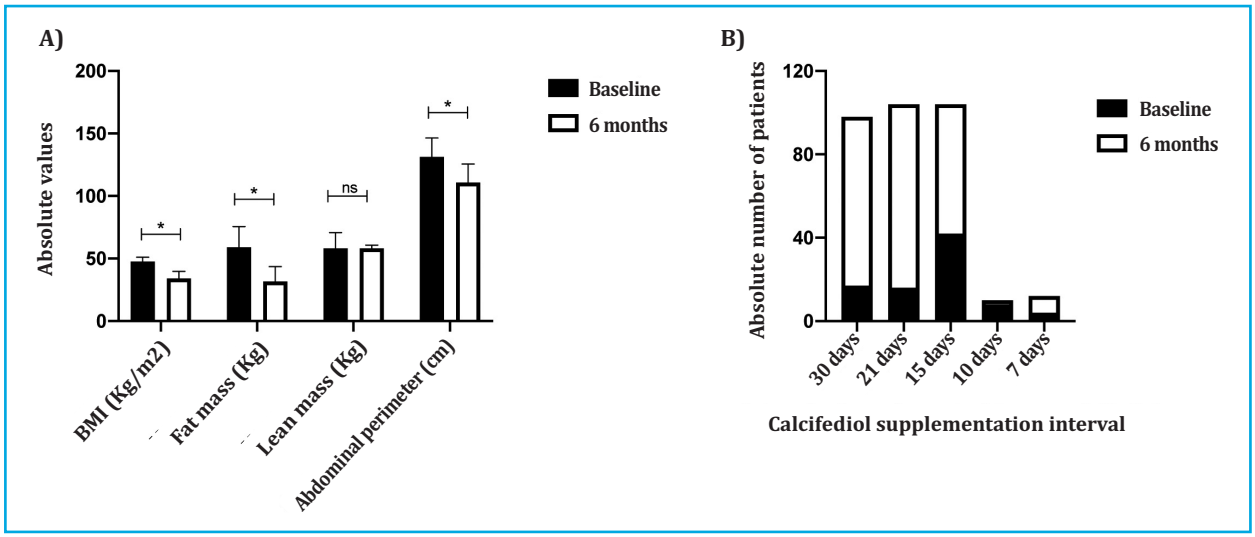

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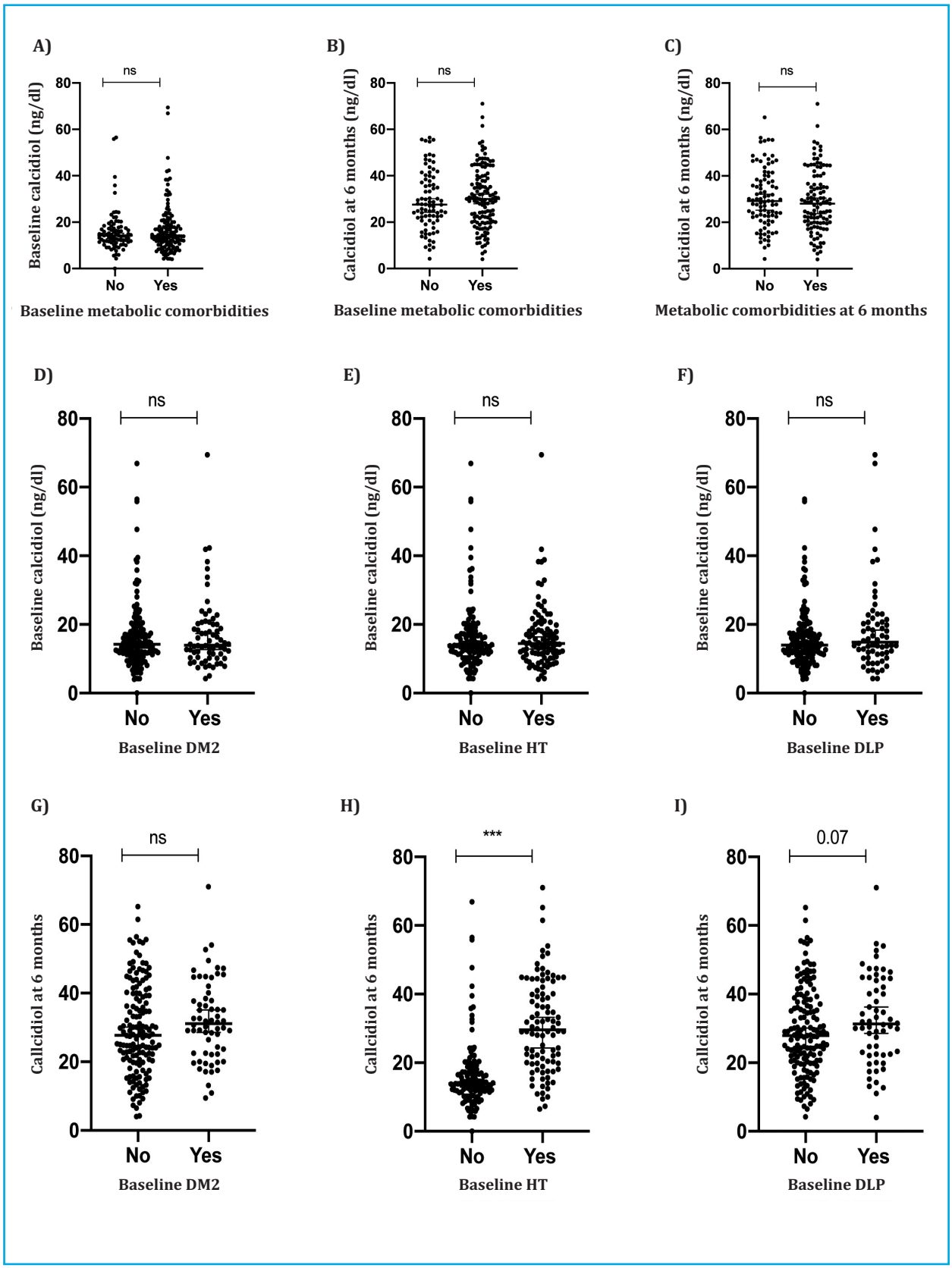
Figure 2. Association between metabolic comorbidities and serum levels of 25-OH vitamin D (calcidiol). A) metabolic comorbidities and basal calcidiol; B) metabolic and calcidiol comorbidities at 6 months; C) metabolic and calcidiol comorbidities 6 months after BS; D) presence of baseline DM2 and basal calcidiol levels; E) presence of baseline HTN and basal calcidiol levels; F) presence of baseline DLP and basal calcidiol levels; G) presence of baseline DM2 and calcidiol levels at 6 months; H) presence of baseline HTN and calcidiol levels at 6 months; I) presence of baseline DLP and calcidiol levels at 6 months

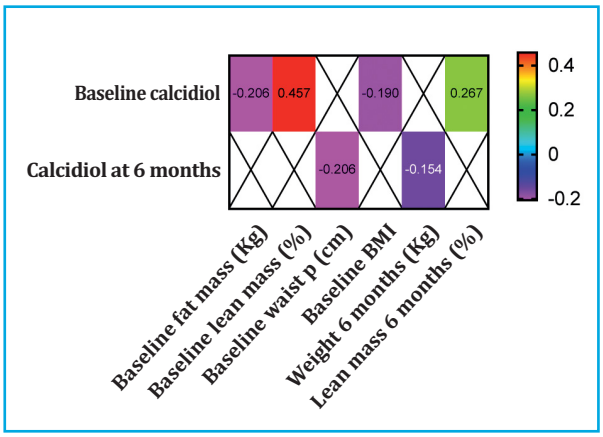


also hypotheses about the "sequestration" of 25-OH-vitamin D by adipose tissue, accompanied by less hepatic activation due to a decrease in 25-hydroxylase activity[35,36]. The specific mechanisms, however, are not known and are still under study. In our cohort, serum 25-OH-vitamin D levels were negatively correlated only at baseline with fat mass, and in contrast, they were positively correlated with lean mass both before and 6 months after surgery.

Among the strengths of this study is the number of patients included, as well as the availability of bioimpedance measurement in all of them, the technique for determining 25-OH-vitamin D and the fact that it is a prospective study at 6 months. However, as limitations we should point out the evolution time, which is limited to 6 months and that, at the time of the analysis, only 260 of the initially included patients had been evaluated. If the follow-up of these patients is continued, a longer-term evolution of the comorbidities and the behavior/adherence of the supplementation may provide additional information and with greater specificity. Finally, in this study, associations are observed that do not demonstrate a direct causal relationship.

To sum up, in our cohort, no relationship was observed between serum levels of 25-OH-vitamin D, the presence or evolution of metabolic comorbidities, but with the body composition of the individuals evaluated.

Figure 3. Correlations between anthropometric parameters and serum levels of 25-OH vitamin D (calcidiol) at baseline and at 6 months after BS. Only statistically significant correlations are represented (p<0.05)



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Effect of a calcium-rich diet on mineral and bone metabolism in rats

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Summary

Objetive: A diet rich in calcium has generally been recommended to maintain adequate bone health. However, recent studies have sparked controversy over its benefits. In this sense, most of the existing studies in animal models are carried out with diets deficient in vitamin D. In this study, the effect of a diet rich in calcium on mineral metabolism and bone histomorphometry in rats is evaluated. In addition, in UMR-106 cells, the direct effect of calcium supplementation on the expression of osteogenic genes is assessed.

Material and methods: A group of male wistar rats of approximately 3 months of age was fed a normal calcium content diet (0.6%) while another group received a high calcium content diet (1.2%). After 20 days urine samples were collected 24h, blood for biochemical analysis and the femur for bone histomorphometry study. *In vitro*, the gene expression of *Runx2, Osterix* and *Osteocalcin* was studied in UMR-106 cells cultured under conditions of high calcium content.

Results: The ingestion of a diet rich in calcium reduced the concentration of PTH and calcitriol in plasma, increased calciuria and decreased phosphaturia. At the bone level, a drastic decrease in osteoblastic activity was observed, consistent with the decrease in PTH. However, the trabecular volume remained similar in both groups. *In vitro*, calcium supplementation did not decrease the expression of osteoblastic markers in UMR-106, indicating that the *in vivo* effects are mostly indirect and due to the decrease in PTH.

Conclusions: A high-calcium diet reduces the concentration of PTH and calcitriol in plasma, which results in a decrease in osteoblastic activity.

Key words: Calcium, PTH, calcitriol, bone histomorphometry.

Introduction

The body's main reservoir of calcium is bone, where about 99% of total calcium is stored in the form of hydroxyapatite. Thus, the calcium content present in extracellular fluids only represents a small fraction of total calcium.

In healthy individuals, the concentration of calcium in the blood varies between 8.6 and 10.4 mg/dl, with around 40% being bound to proteins and 6% to phosphate, citrate or bicarbonate salts. The metabolic activity of calcium is attributed to ionic calcium, which represents 54% of total calcium in the blood and is very precisely regulated so that plasma values remain in a range between 4.4 and 5.4 mg/dl (1.1-1.35 mM)[1].

With regard to bone health, the benefits of a diet rich in calcium on bone homeostasis are under debate[2]. Thus, for example, the calcium supplement has been commonly recommended for the maintenance of bone health and for preventing osteoporosis. Nevertheless, meta-analysis studies have shown that this calcium

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supplement does not always have a positive effect. In a general adult population, it has been observed that neither the supplement with vitamin D, calcium nor the combination of both are associated with a decrease in the risk of fracture[3]. Thus the controversy concerning the effectiveness of these supplements has increased. In the same sense, in a prospective longitudinal study carried out in Sweden in which the incidence of fractures and osteoporosis in adult women was studied over 19 years, calcium intake was estimated by means of a questionnaire, it concluded that a higher intake was not associated with a reduced risk of fracture or osteoporosis[4].

In animal models, most of the studies on the bone effects associated with calcium are developed in models in which vitamin D levels are reduced, either through diets deficient in vitamin D or in knock out animal models for the vitamin receptor D (VDR). In male wistar rats fed a diet deficient in vitamin D from prenatal stages, a vitamin D-deficient diet has been shown to decrease bone mineral density, femur length and cause histological changes such as osteoid accumulation, increased osteoblastic activity or decreased osteoclastic activity. When these rats with a diet deficient in vitamin D were fed with a calcium supplement in the diet, the bone mineral density was partially recovered, as well as the length of the tibia, the volume of osteoid decreased and the osteoblastic activity, while the number of osteoclasts, which produced a decrease in trabecular bone volume[5]. In another similar study, animals fed a diet deficient in vitamin D decreased the calcium content in the bone, which was reestablished with the infusion of calcium and phosphorus, indicating that the effects of vitamin D on the bone must be mainly indirect and derived from its function on the regulation of mineral metabolism[6].

On the other hand, in knock out mice for the vitamin D receptor and for 25-hydroxyvitamin D 1-α-hydroxylase, which are hypocalcemic, an increase in bone formation, bone volume and the number of osteoblasts was observed, associated with the consequent increase in parathyroid hormone (PTH) levels even though the animals were fed a lactose-free diet with a high calcium content. However, the number of osteoclasts was not associated with PTH levels in these animals and remained similar to that of mice with wild-type phenotype and normal PTH levels. When the animals were fed a rescue diet (2% calcium, 1.25% phosphorus, 20% lactose, and 2.2 units/g of vitamin D) it was possible to prevent hypocalcemia, hyperparathyroidism and, consequently, the number of osteoblasts, the mineral apposition rate and bone volume were reduced[7].

Based on these premises, the objective of this study was to investigate the effect of a diet with a high calcium content on bone histomorphometry in rats, as well as on the osteogenesis of UMR-106 cells.

Materials and methods

All experimental procedures carried out in this study were approved by the Research Ethics and Animal Welfare Committee of IMIBIC/University of Cordoba in accordance with the provisions of Directive 2010/63/EU of the European Parliament and the Council of Europe of the September 22, 2010, the institutional guidelines for the care and use of laboratory animals and the Declaration of Helsinki, protocol authorization number 03/14/2018/026.

Experimental design

Male wistar rats with approximately 3 months of age were used to avoid interactions related to sex since in rats there is a sexual dimorphism in the bone phenotype that appears to be multifactorial[8]. The animals were fed with diets of normal content (0.6% Ca; n=6) or high content of calcium (1.2% Ca; n=9) and both diets had a phosphorus content of 0.2%. After 20 days, the rats were placed in metabolic cages to collect the 24-hour urine. The following day the animals were sacrificed by puncture of the abdominal aorta and exsanguinated under general anesthesia with sevoflurane. The blood was processed to separate the plasma and the right femur was placed in 70% ethanol for subsequent inclusion in methylmethacrylate.

Biochemistry in blood and urine

The blood samples were collected in heparinized tubes (BD Vacutainer, Franklin Lakes, NJ, USA) and centrifuged at 2000 x g, for 10 minutes at 4ºC to separate the plasma that was stored at -80ºC until the biochemical determinations were carried out. The 24h urine samples were centrifuged at 2000 x g, for 10 minutes at 4ºC to discard the sediment and the aliquots were stored at -20ºC until analysis. Colorimetric kits (BioSystems SA, Barcelona, Spain) were used to determine the content of phosphorus, total calcium and creatinine. The fraction of phosphorus excretion, expressed as a percentage, was calculated according to the formula: (urine phosphorus x plasma creatinine x 100)/(plasma phosphorus x urine creatinine). Ionic calcium quantification was performed in plasma just after sacrifice and before freezing in an ion analyzer (Spotlyte Ca^{2+}/pH (Menarini Diagnostics, Barcelona, Spain). Circulating bioactive PTH contents were determined by ELISA (Immutopics, San Clemente, CA, USA) and intact FGF23 (Kainos Laboratories, Tokyo, Japan). Calcitriol concentration was measured by radioimmunoassay (Immunodiagnosticsystems, Boldon, UK). All kits were used following manufacturer's instructions.

Inclusion in methylmethacrylate and bone histomorphometry analysis

After the sacrifice, the right femur was removed from each animal and embedded in 70% ethanol. Subsequently, the femurs were dehydrated in alcohol, rinsed with xylene, and embedded in 75% methyl methacrylate, 25% dibutyl phthalate and 2.5% w/v benzoyl peroxide. Histomorphometry analysis was performed on 5 µm sections without decalcification stained with Villanueva's modified Goldner trichrome method[9]. Briefly, bone sections were fixed with 50% ethanol under pressure for 24h at 37ºC, then rehydrated and stained with 1:1 hematoxylin-ferric chloride, subsequently rinsed with 1% hydrochloric acid and blued with lithium carbonate saturated. After washing with water, sections were stained with Goldner trichrome stain for 20 minutes and then rinsed with 1% acetic acid. Subsequently, the samples were stained with a 1% w/v alcoholic saffron solution for 5 minutes, dehydrated with ethanol and mounted. Calcified tissue was stained green and areas stained red were considered osteoid. Bone histomorphometric parameters were evaluated at 200x in a Leica DM4000B optical microscope (Leica Microsystems Wetzlar, Germany) with an Olympus DP72 camera (Olympus, Tokyo, Japan) using OsteoMeasure Software (OsteoMetrics, Decatur, IL, USA). The distal part of the bone within the secondary cancellous was analyzed (1 mm away from

the growth plate and at a distance of approximately 0.25 mm from the endocortical bone). The bone histomorphometry parameters were calculated according to the American Society for Bone and Mineral Research (ASBMR) recommendations[10].

In vitro experiments

The effect of high calcium concentrations on the osteogenesis of UMR-106 cells was also evaluated. The cells were cultured with DMEM (Sigma-Aldrich) supplemented with 10% FBS (Lonza), 2 mM ultraglutamine (Lonza), 1 mM sodium pyruvate (Lonza), 20 mM HEPES (Sigma-Aldrich), 100U/ml penicillin and 100 mg/ml streptomycin. Once the cells reached approximately 90% confluence, the culture medium was changed to calcium-free DMEM (Gibco, Grand Island, NY) supplemented as indicated above, and a 0.1 M calcium chloride solution (Sigma-Aldrich) to increase the calcium concentration in the culture medium to 1.25 mM (normal concentration in blood) and 1.8 mM (equivalent to hypercalcemia). After 48h, cells were lysed and processed for total RNA isolation. 3 independent experiments were carried out with 4 replications for each group.

RNA isolation and RT-PCR

Total RNA was extracted with Trizol (Sigma-Aldrich) and the final concentration was quantified by spectrophotometry (ND-1000, Nanodrop Technologies). RNA samples were post-treated with DNAse (Sigma-Aldrich) and real-time PCR was performed with 50 ng of DNAse-treated RNA with the SensiFAST SYBR No-ROX One-Step Kit (Bioline). The primers used were: *Runx2* (Sense 5'CGG-GAA-TGA-TGA-GAA-CTA-CTC3 'Antisense 5'CGG-TCA-GAG-AAC-AAA-CTA-GGT3'), *Osterix* (Sense 5'GTA-CGG-CAA-GGC -TTC-GCA-TCT-GA3 'Antisense 5'TCA-AGT-GGT-CGC-TTC-GGG-TAA-AG3'), *Osteocalcin* (Sense 5'TCT-GAG-TCT-GAC-AAA-GCC-TTC-ATG3 'Antisense 5'TGG-GTA-GGG-GGC-T GG-GGC-TCC3') and *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* (Sense 5'AGG-GCT-GCC-TTC-TCT-TGT-GAC3 'Antisense 5'TGG-GTA-GAA-TCA-TAC-TGG- AAC-ATG-TAG3'). The RT-PCR amplification was carried out in a Lighcycler 480 (Roche Molecular Biochemicals). The expression of the target genes was normalized by method $2^{(-\Delta\Delta Ct)}$ using *GAPDH* as constitutive.

Statistic analysis

Values are shown as mean ± standard error. The differences between the two groups were studied using the non-parametric Mann-Whitney U test. The groups were considered significantly different for a $p<0.05$. Statistical analyzes and graph editing were performed with the GraphPad program (GraphPad Software, La Jolla, CA, USA).

Results

Biochemistry in blood and urine

The group of animals fed a diet rich in calcium showed plasma levels of calcium and phosphorus similar to those of the group fed normal calcium (figure 1a and b respectively). However, the high calcium diet produced both a decrease in plasma concentrations of intact PTH (figure 1 c) and calcitriol (figure 1d). The intact FGF23 levels remained similar in both groups (figure 1e). As expected, the 24-hour urinalysis showed an increase in calciuria and a decrease in phosphaturia in rats fed a calcium-rich diet (figure 1f and g respectively).

Bone histomorphometry

The volume of trabecular bone and the volume of osteoid in the rats fed the calcium-rich diet remained similar to that of the rats fed a normal calcium diet (figure 2a and b), while a significant reduction in the osteoid surface area of the calcium was observed (figure 2c) in the group of animals fed a diet rich in calcium, which was consistent with a decrease in the bone surface covered by osteoblasts (figure 2 d). Both the resorption surface and the bone surface covered by osteoclasts were similar in both groups (figure 2e and f respectively). At the level of trabecular micro-architecture, no differences were observed with respect to trabecular thickness, trabecular separation and number of trabeculae (figure 2 g, h, i).

Effect of calcium on the osteogenesis of UMR-106

To study *in vitro* the direct effect of calcium on osteoblasts we used the rat osteoblast cell line UMR-106. The culture medium of these cells was supplemented with calcium until reaching a concentration of 1.8 mM and it was compared with cells cultured with culture media with normal calcium content (1.25 mM). After 48h of treatment, it was observed that the high levels of calcium did not modify the expression of osteogenic genes such as *Runx2, Osterix* or *Osteocalcin* (figure 4 a, b and c respectively).

Discussion

In this study, a Ca-rich diet for 21 days did not promote significant differences in plasma levels of Ca or P at the expense of increasing calciuria and decreasing phosphaturia which had a direct impact on plasma PTH and calcitriol levels. This effect could be due to a transient hypercalcemia at the beginning of the experiment, resulting in a subsequent decrease in PTH production, hypercalciuria and calcitriol synthesis, since it seems consistent with an activation of the calcium receptor (CaSR) in the parathyroid glands. and in the kidney, which has been widely described to result in a decrease in PTH and an increase in urinary calcium excretion[11,12]. The levels of calcium in plasma, which remained similar in both groups, could also be due to this excessive calciuria or to an adaptation of the body to the prolonged high intake of calcium in the diet. With respect to bone, animals fed a diet high in calcium showed a reduction in osteoblastic activity associated with the decrease in PTH and a tendency to decrease trabecular bone volume. It is interesting to note that in animals fed a diet deficient in vitamin D, osteosclerosis occurs at the trabecular level and the infusion of calcium and phosphorus results in a decrease in osteoblastic activity[13]. These observations support our results suggesting that increased calcium loading reduces osteoblasts on the bone surface. Furthermore, in our study, osteoclastic activity did not show significant differences compared to the group fed a diet with normal calcium content despite the decrease in PTH, suggesting that other mechanisms must be involved.

A limitation of this *in vivo* study is that no calcein-type marking with specific fluorochromes was performed in these animals. Therefore, formation and mineralization kinetic parameters could not be determined.

In this study the intake of a diet rich in calcium during 3 weeks did not produce significant changes in various parameters bone histomorphometry (volume trabecular

bone, volume osteoid separation trabecular and number of trabeculae) despite a decline significant of the osteoblastic activity and a similar osteoclastic activity.

In vitro, UMR-106 cells that were treated with high levels of calcium did not show changes in the gene expression of *Runx2, Osterix* and *Osteocalcin*. This suggests that the decrease in osteoblastic activity observed in bone is not directly influenced by a high calcium concentration, but could be more closely related to the decrease in PTH concentration. The rat osteosarcoma cell line UMR-106 is a widely used model with an osteoblastic phenotype in which the response to extracellular calcium and PTH has been well characterized[14,15]. Previously, our group has published that the activation of CaSR by a calcimimetic increases osteogenesis and bone remodeling, therefore, presumably, its natural activators such as ionic calcium and others should have a similar effect[16]. In this previous study, the effect of calcimimetic on UMR-106 with a very low concentration of calcium (0.5 mM) was examined, so that treatment with the drug produced a more significant response. Therefore, in this *in vitro* experiment with UMR-106 with a normal calcium concentration (1.25 mM), in which the CaSR would be in a high degree of activation based on that described in parathyroid glands[17], a high calcium (1.8 mM) should not produce a significant additional activation and therefore a significant increase in the expression of osteogenic genes would not be observed. Probably 1.8 mM calcium causes only a slight increase in CaSR activation with respect to 1.25 mM calcium, which only produces tendencies to increase *Osterix* and *Osteocalcin* as observed in this study.

In a study with young and healthy volunteers, the effects of the acute administration of 400 mg of oral calcium were evaluated and after 10 hours it was observed that the serum

Figure 1. Biochemistry in plasma and urine. Bars represent mean ± standard error. U of Mann-Whitney test. * p<0.05 vs 0.6% Ca. ** p<0.01 vs 0.6% Ca

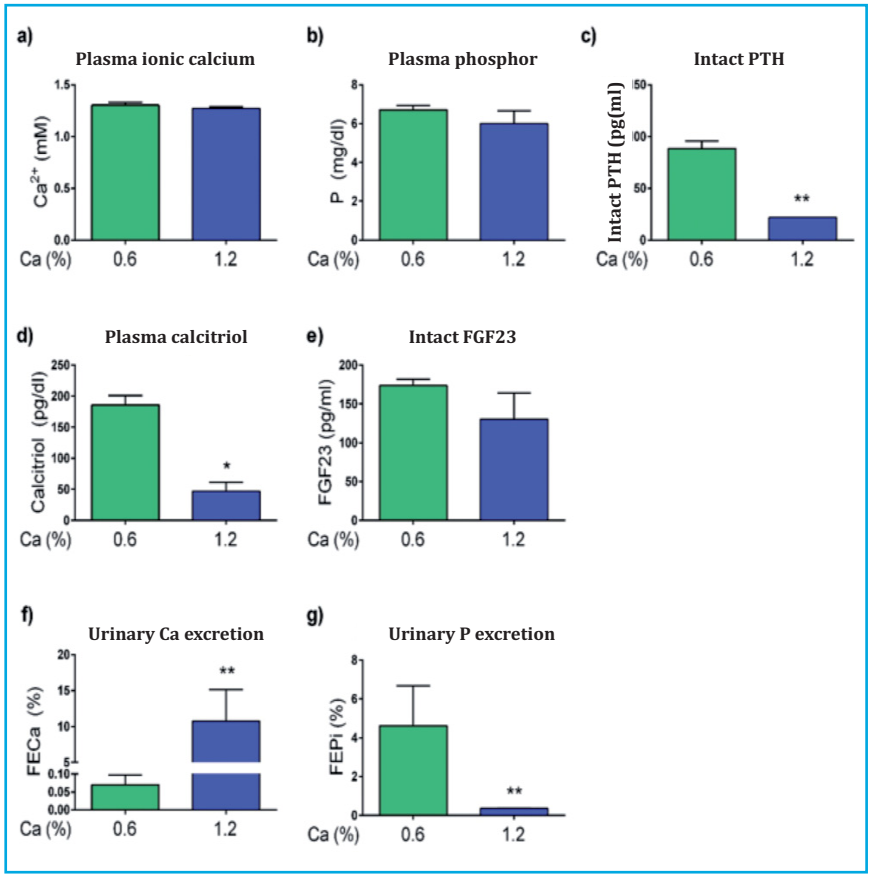


Figure 2. Bone histomorphometry analysis. Bars represent mean ± standard error. U of Mann-Whitney test. * p<0.01 vs 0.6% Ca

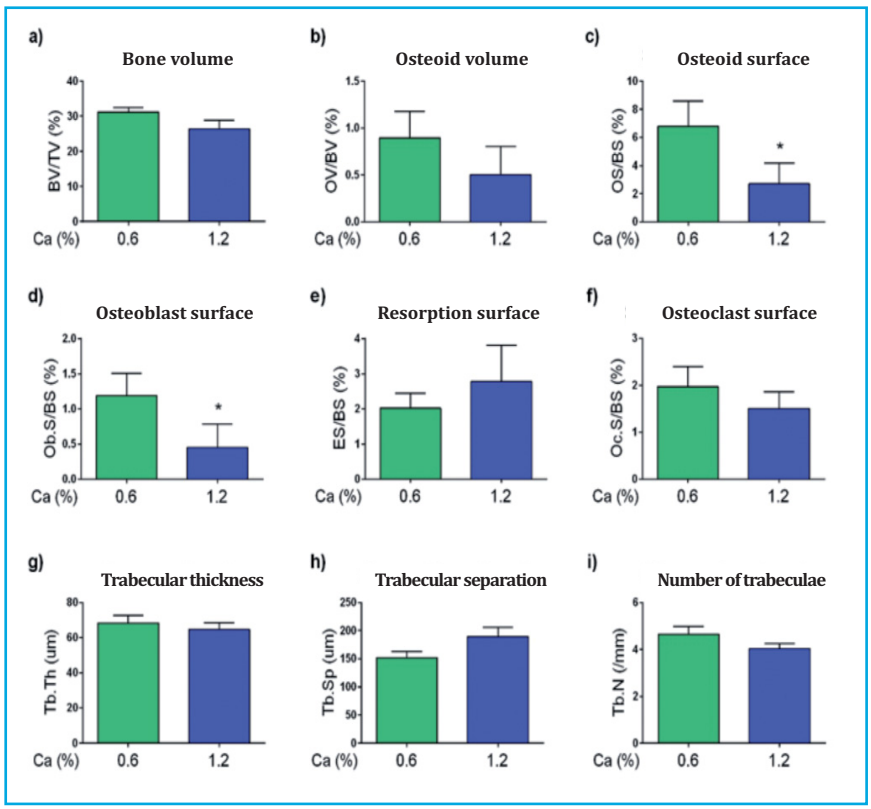
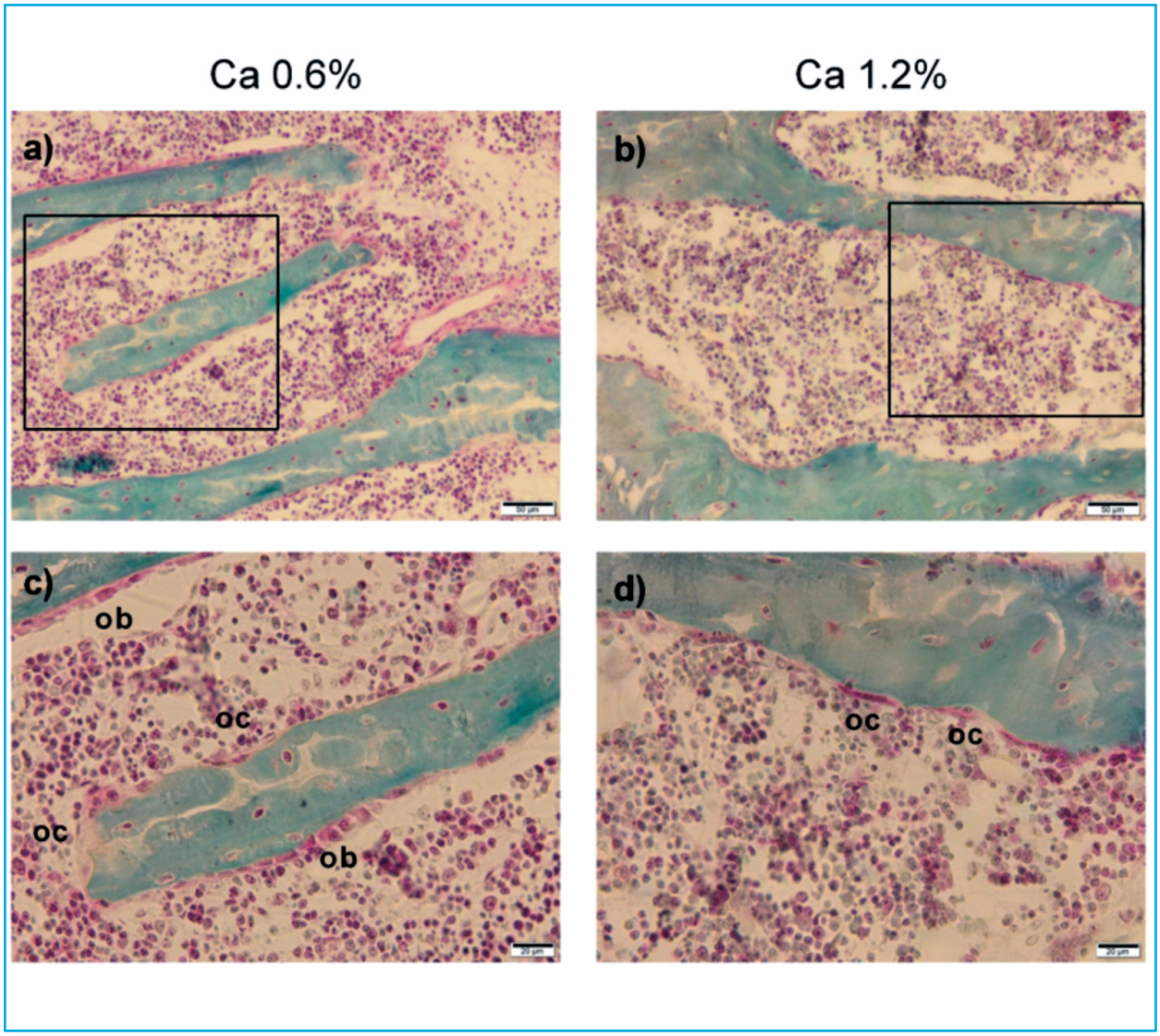


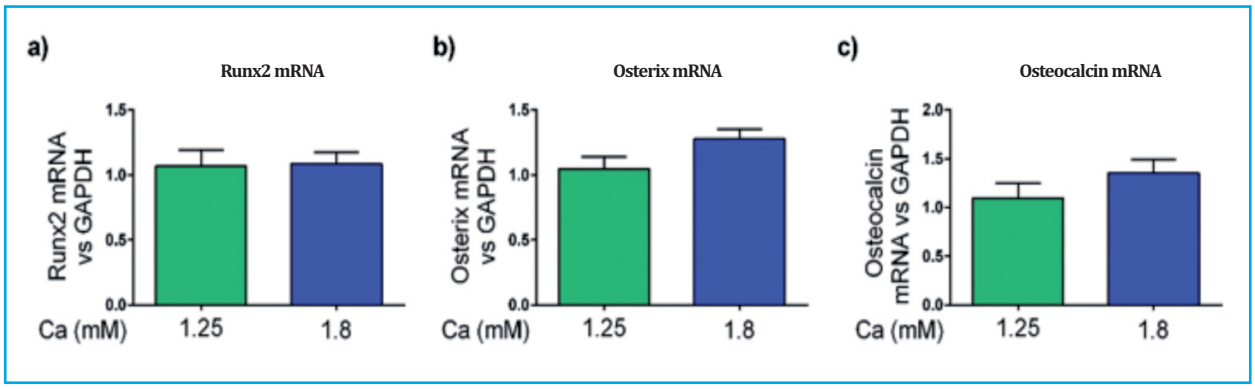
Figure 3. Photomicrographs of the bone samples stained with Goldner's trichrome. oc: osteoclasts. ob: osteoblasts. (a and b) magnification: 100x. Scale bar: 50 µm. The inset indicates the area magnified in the following photomicrographs. (c and d) magnification: 200x. Scale bar 20 µm



PTH concentration decreased, accompanied by a decrease in the serum levels of collagen telopeptides type I did not, however, show data related to bone formation[18]. This study supports our observations that dietary calcium supplementation reduces PTH levels, resulting in changes at the bone level. In this study acutely, the decrease in PTH produced a decrease in osteoclastic activity that we did not observe in our study with rats and that is probably due to prolonged treatment with a diet rich in calcium. It is important to note that the expression of 25 (OH) D-1α-hydroxylase is directly regulated by PTH[19], and that therefore an increase in calcium intake would result in a decrease in calcitriol synthesis, consistent with the results obtained in our study.

In conclusion, a diet rich in calcium could lead to a reduction in osteoblastic activity due to a decrease in PTH production that would also result in a decrease in active vitamin D.

Figure 4. Expression of osteogenic genes in UMR-106 cells treated with high levels of calcium. Calcium chloride (0.1 M) was added until reaching levels corresponding to a situation of hypercalcemia (1.8 mM) and they were compared with normal levels (1.25 mM). Exposure to different levels of extracellular calcium was 48h. Bars represent mean ± standard error



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Conflict of interests: The authors declare no conflict of interest.

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Knowledge and clinical decisions of Colombian dentists about the risk of osteonecrosis of the jaws in patients receiving treatment for osteoporosis

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Summary
Introduction: Osteonecrosis of the jaws is a rare, severe adverse reaction associated with the administration of drugs used to treat osteoporosis and cancer, such as bisphosphonates and denosumab. However, many professionals suspend these medications or defer the procedures until they have the referring physician's authorization. This study evaluates the knowledge and attitudes of a group of Colombian dentists regarding the risk of developing maxillary osteonecrosis with the use of bisphosphonates and denosumab.
Methods: A survey was designed from a focus group that was endorsed by experts. A tool of 30 questions was obtained, which was sent to a group of dentists, maxillofacial surgeons, periodontists and oral rehabilitators affiliated with dental societies through the Survey Monkey software.
Results: The responses of 187 dentists (42.6% with postgraduate studies) were analyzed. 50.3% of dentists mistakenly considered the use of bisphosphonates an absolute contraindication for major dental procedures and 51.3% believed the same regarding denosumab use. 74.6% of professionals would unnecessarily request approval from the referring physician to schedule procedures in patients receiving bisphosphonates and 43.8% for patients receiving denosumab. Our findings were similar regardless of years of experience or level of education.
Conclusion: Our results suggest that the respondents had little knowledge as to the risk of developing maxillary osteonecrosis with the use of medications for the management of osteoporosis.

Key words: osteonecrosis of the jaws, bisphosphonates, denosumab, osteoporosis, dentists.

Introduction
Maxillary osteonecrosis (ONJ) is a rare severe adverse reaction to drugs used to treat osteoporosis and cancer, such as bisphosphonates and denosumab. This complication consists of the progressive destruction of the mandibular and/or maxillary bone, with exposure of the necrotic bone in the oral cavity, which occurs more frequently with the use of antiresorptive agents in cancer and multiple myeloma[1,2].

The risk of ONJ with bisphosphonates and denosumab in osteoporosis therapy is very low, close to 0.01%, as it is a low-dose and short-exposure therapy, unlike when they are used in cancer patients, with a risk of around 1.3%[3,4]. The prevalence of ONJ in patients receiving long-term oral bisphosphonate therapy was reported to be 0.1% (10 cases per 10,000), which increased to 0.21% (21 cases per 10,000) in patients older than 4 years. bisphosphonate exposure[5].

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Although the risk of ONJ is very low with the use of bisphosphonates and denosumab in osteoporosis, dental professionals still perceive a high risk of presenting this complication. They frequently request authorization for dental procedures to the prescribing physician, leading to dental complications due to delays in carrying out the procedures or associated with the suspension of treatment for osteoporosis[6,7].

This study aims to ascertain the degree of knowledge and the clinical decisions that Colombian dentists make regarding ONJ risk associated with the use of bisphosphonates and denosumab in osteoporosis.

Methods

A survey was designed to assess two areas. The first related to the level of knowledge of dentists regarding the risk of developing ONJ with bisphosphonates and denosumab evaluated with general questions about the topic. The second involved the clinical decisions made by professionals, which was assessed with hypothetical clinical cases.

The survey development process initially included a focus group, in which a dental professional, a clinical psychologist and epidemiologist expert in qualitative research, a rheumatologist and two internal medicine residents took part. Some initial questions were proposed that were subsequently submitted to a group of experts for approval and correction. The resulting tool was applied to a group of 30 students in their final year of dentistry studies at the Pontificia Universidad Javeriana (Bogotá) as a pilot test, seeking to evaluate the ease of response and understanding. Their comments were taken into account to make the final adjustments to the survey prior to its application.

The survey was hosted in the SurveyMonkey program (Supplement 1) and sent to dentists, maxillofacial surgeons, periodontists and oral rehabilitation specialists, affiliated with the Colombian Odontological Federation, during the period from October 2019 to August 2020. They were invited to participate by sending registered email by each professional, up to a maximum of 3 times. Professionals who reported no clinical practice for one year and those with exclusive pediatric practice were excluded.

The demographic characteristics of the participants are presented in absolute numbers, proportions or as measures of central tendency and dispersion, depending on the type of variable. The comparison analysis between subgroups was performed using a Chi square test. Statistical analysis was carried out using Stata software (Stata: version 15, TX Stata Corp LLC).

The study was approved by the Ethics and Research Committee of the University Hospital San Ignacio and the Pontificia Universidad Javeriana.

Results

1,000 Colombian dentists were invited to participate. 340 (34%) responded to the survey and of these 19 (5.5%) were excluded because they had no clinical practice in the last 12 months, 57 (16%) due to their exclusive practice with pediatric patients and 77 (22%) because they did not complete the survey. In total, the responses of 187 dentists were analyzed (algorithm 1). The median age was 42 years (interquartile range 39-45). The majority were women (70.2%), with a greater presence of dentists from Bogotá (56.2%). Table 1 shows the demographic characteristics of the group of dentists who took part in the study.

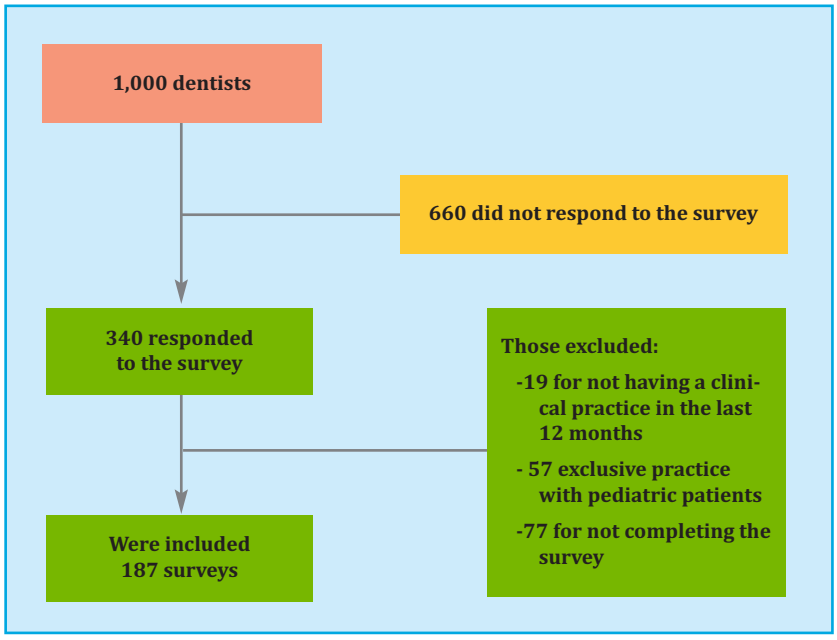
Knowledge assessment

When evaluating the risk of developing ONJ, 50.2% of the respondents considered that the use of BFs was an absolute contraindication for a major dental procedure, while 51.3% expressed the same opinion for denosumab. For minor procedures, 3.2% of those surveyed considered the use of bisphosphonates an absolute contraindication and 27.8% a relative contraindication to carry out the procedure. The percentages for the use of denosumab were 4.2% and 28.3%, respectively (figure 1).

41.2% considered that ONJ risk was the same for those receiving bisphosphonates compared to those receiving denosumab. 45% considered that the risk of developing ONJ is greater if bisphosphonates are administered orally. 50% considered that the risk of ONJ is the same in patients with cancer, compared to patients with osteoporosis receiving bisphosphonates or denosumab. 78% of dentists considered that the risk of ONJ increases with the time of exposure to bisphosphonates, and 50% with the time of exposure to denosumab. Regarding the question of the risk of developing ONJ with bisphosphonate compared to denosumab, 70% were not sure.

70% of dentists reported that less than 25% of patients in their clinical practice diagnosed with osteoporosis and 57.8% of these patients being treated with bisphosphonates or denosumab. Of the dentists surveyed, 76.4% have not dealt with any case of ONJ, and of those who had, only 16.9% were associated with osteoporosis. 41.6% are unaware of any document for diagnosing and managing osteonecrosis of the jaw.

Algorithm 1. Selection of surveys of dentists



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Evaluation of clinical decisions

The hypothetical cases used and the clinical decisions that dentists would make in that situation are presented in table 2. Of the reported decisions, the following stood out:

For case 1 (65-year-old woman with hip fracture and osteoporosis treated with denosumab for a year who required an extraction), only 1.37% considered carrying out the extraction and 74.66% considered it necessary to request an extraction authorization from the referring physician in order to perform the procedure.

For case 2 (53-year-old man with a history of rheumatoid arthritis managed with methotrexate and leflunomide who requires endodontics), 42.47% would request an opinion from the referring physician.

For case 3 (60-year-old woman with osteoporosis managed with alendronate and pending dental implant), 3.42% considered carrying out the treatment without suspending the bisphosphonate and 43.84% would request authorization from the referring physician to endorse the procedure.

For case 4 (64-year-old woman with osteoporosis undergoing treatment with zoledronic acid who required tooth extraction), 4.1% considered carrying out the procedure without suspending the bisphosphonate and 62.3% would request authorization from the referring physician.

For case 5 (Patient with osteoporosis who is being managed with denosumab, of which he has received 3 doses, with telopeptide C levels at 0.05 ng/mL), 26.03% would postpone the procedure while waiting for the decreased levels of telopeptide C.

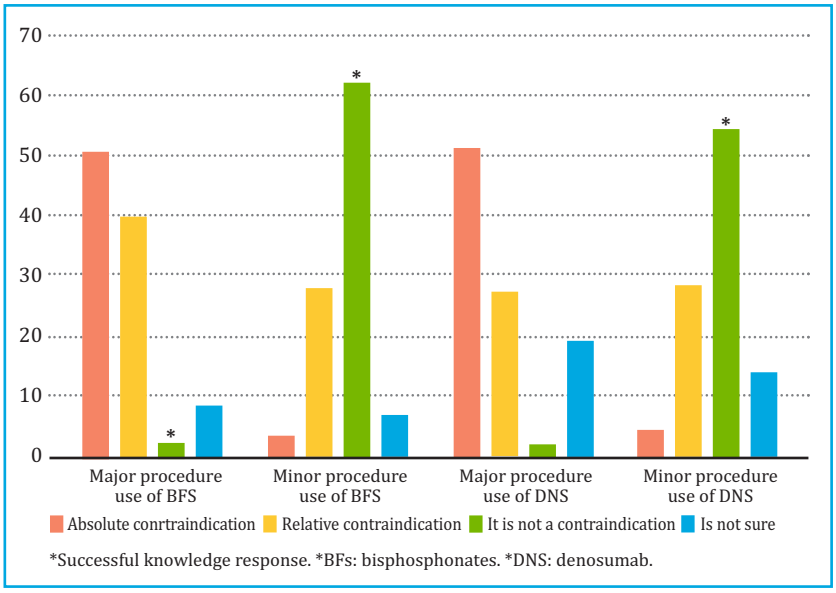
The subgroup analysis showed that a lower proportion of professionals with postgraduate studies considered the use of bisphosphonates a relative contraindication for carrying out minor procedures (43.4 vs 54.9%, p 0.021) (table 3). For the other clinical decisions, no significant differences were found, regardless of the years of experience, the level of education (complete undergraduate vs. postgraduate) or the city where the professional practice was carried out.

Table 1. Demographic characteristics of the dentists surveyed

Characteristics	n (187)
Age, years, median (IQR)	42 (39-45)
Female gender, n (%)	132 (70.2)
Town, n (%)	
Bogota	105 (56.1)
Cali	20 (10.6)
Medellin	21 (11.2)
Cartagena	3 (1.6)
Bucaramanga	4 (2.1)
Others	30 (16.0)
Studies, n (%)	
Full undergraduate	111 (57.4)
Postgraduate in dentistry	76 (42.6)
Years of practice, n (%)	
Less than 10 years	72 (38.9)
More than 10 years	115 (61.1)

IQR: interquartile range.

Figure 1. Knowledge of Colombian dentists about the use of bisphosphonates or denosumab due to the risk of osteonecrosis of the jaw, during major and minor dental procedures



*Successful knowledge response. *BFs: bisphosphonates. *DNS: denosumab.

Discussion

Colombian dentists' knowledge and attitudes regarding the risk of developing ONJ with the use of bisphosphonates and denosumab, in treating osteoporosis, were analyzed in our study. We found a high proportion of professionals had limited knowledge of the ONJ risk associated with bisphosphonates and denosumab. In this sense, they would make incorrect decisions regarding the scheduling time of major and minor procedures.

Our findings are similar to those reported in other countries, where a low level of knowledge regarding the subject was reported. A study published by R Al-Eid et al. shows the results of a survey of 74 dentists in Saudi Arabia in which 39.2% of the respondents were not familiar with the term ONJ and 54% had no knowledge regarding the diagnosis and treatment of ONJ; 44% were unsure whether to discontinue bisphosphonate therapy prior to tooth extraction[8]. A 2017 survey of Mexican dentists by Vinitzky-Brener et al. showed that only

Table 2. Clinical cases concerning decisions taken by Colombian dentists about the use of bisphosphonates or denosumab due to the risk of ONJ, during major and minor dental procedures

Evaluation of clinical decisions. Cases/Responses	Would request a concept by the referring rheumatologist, to define ONJ risk and guarantee the dental procedure	Would postpone the extraction until the effects of the medication wear off (six months)	Would instruct the patient to the use of denosumab is an absolute contraindication for this type of dental procedure and will not be performed	Would carry out the extraction as there is no contraindication for the procedure
Case Nº 1: Woman, 65 years old, hip fracture and osteoporosis, with denosumab. Consultation due to the appearance of a dental lesion that you consider requires an extraction, considering the risk of developing ONJ, would you propose	74.6%	16.4%	7.53%	1.3%
Evaluation of clinical decisions. Cases/Responses	Wouldrequest a written opinion from the referring rheumatologist, in which define the risk of ONJ and whether or not the dental procedure is authorized	Would not carry out the procedure since the patient is receiving treatment with leflunomide	Would perform the procedure since there is no documented risk of ONJ with the use of these drugs	Would not perform the procedure since he receives treatment with methotrexate
Cas2 Nº 2: A 53-year-old man with rheumatoid arthritis managed with methotrexate and leflunomide and oral calcium. Requires endodontic treatment, taking into account the risk of ONJ and the patient's scenario, would you consider:	42.4%	0.6%	54.7%	2.0%
Evaluation of clinical decisions. Cases/Responses	Would request a written opinion from the referring rheumatologist, in which define the risk of ONJ and whether or not the dental procedure is authorized	Would advise the patient that alendronate use is an absolute contraindication for this type of dental procedure and that it will not be performed	Would explain that it is not a dental emergency and wait 6 months to carry out the intervention	Would recommend suspending the treatment and restarting it according to its clinical evolution (closure of the surgical wound)
Case Nº 3: : A 60-year-old woman with osteoporosis managed with alendronate 70 mg weekly for 18 months. She is scheduled to perform a dental implant and attends her consultation prior to the intervention. Regarding treatment with alendronate you:	43.8%	27.4%	20.5%	4.7%
Evaluation of clinical decisions. Cases/Responses	Would request a written opinion from the referring rheumatologist, defining the risk of ONJ and whether or not the dental procedure is authorized	Would advise the patient that the use of zoledronic acid is an absolute contraindication for this type of dental procedure and that it will not be performed	Would recommend suspending the treatment and restarting it according to its clinical evolution (closure of the surgical wound)	Would perform the dental procedure without stopping zoledronic acid
Case Nº 4: Woman, 64 years old with osteoporosis with zoledronic acid, an extraction will be carried out and she comes to your consultation regarding treatment with zoledronic acid:	62.6%	16.4%	11.4%	4.1%
Evaluation of clinical decisions. Cases/Responses	Would not make any recommendation since I do not know the relationship between C telopeptide levels and complications derived from the procedure	Would postpone the procedure, waiting for a decrease in C telopeptide levels	Would tell the patient not to have the procedure done, due to the low levels of C telopeptide	Would indicate that the procedure not be carried out, since the C telopeptide levels are not a contraindication
Case Nº 5: Patient with osteoporosis with three doses of denosumab scheduled for a dental implant, in previous consultations with two dentists, who have refused to carry out the procedure since the patient has C telopeptide levels of 0.05 ng/mL. Your opinion regarding patient's the clinical case would be:	42.4%	26.0%	27.4%	4.1%

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Table 3. Subgroup analysis regarding clinical decisions of knowledge of Colombian dentists about the use of bisphosphonates or denosumab due to the risk of osteonecrosis

Knowledge evaluation	Years of experience			Applied studies			Town		
	Less than 10 years %	More than 10 years %	p	Under-graduate (%)	Post-graduate (%)	p	Bogota	Others	p
Major procedures with BFs. Absolute contraindication	21.3	29.4	0.15	62.1	37.8	0.345	9.5	21.2	0.09
Minor procedures with BFs. Relative contraindication	11.7	16.4	0.88	54.9	43.4	0.021	14.8	12.7	0.93
Major DNS procedures. Absolute contraindication	18.7	32.6	0.82	63.4	36.2	0.225	28.1	22.8	0.30
Minor DNS procedures. It is not a contraindication	21.3	33.1	0.93	59.7	63.4	0.781	32.9	21.2	0.14

BFs: bisphosphonates; DNS: denosumab.

40.5% were aware of ONJ and only 24.6% were familiar with some type of bisphosphonate[9]. Another study assessing the knowledge of dentists about ONJ associated with bisphosphonates carried out in Korea by Yoo et al., in 2010, reported that only 56.5% of those surveyed knew the term ONJ and 31.4% related it to bisphosphonate use[10]. Similar findings were reported by Alhussain et al., in 2015 in a study conducted with Canadian dentists, where 60% of the respondents did not have sufficient knowledge about ONJ and 50% did not know how to manage it[11].

This is the first study conducted to assess the knowledge and clinical decisions of Colombian dentists. Our study suggests that there is a lack of knowledge regarding the risk of ONJ in treating osteoporosis with bisphosphonates and denosumab. According to the American Association of Oral and Maxillofacial Surgeons (AAOMS) 2014 recommendations and the first Colombian Consensus of ONJ associated with medications of 2019, treatment with bisphosphonates or denosumab is not an absolute or relative contraindication. Furthermore, treatment should not be suspended to perform the dental procedure. However, the committee recognizes that there are limited data to support or refute the pharmacological vacation period for patients with osteoporosis treatment, but vacation therapy may be beneficial after prolonged exposure to treatment[12, 13]. This is based on the very low risk of ONJ in the context of osteoporosis, which is 0.01%, as demonstrated by the FREEDOM study, which evaluated the use of denosumab in 4,550 patients, where there were no ONJ cases. In the HORIZON study with 7,765 patients managed with zoledronic acid and followed up for 3 years, only one case of ONJ occurred[13,14].

50% of the dentists responded that the risk of ONJ is the same in patients with cancer compared to osteoporosis. Studies that have evaluated ONJ risk in both scenarios have shown a large difference in risk, which is 10 to 150 times higher in cancer compared to osteoporosis (0.1-1.5% vs 0.01%)[15,16]. Regarding knowledge of scientific documents for the prevention and management of patients with ONJ, 41% are unaware of document for ONJ treatment. There are two important documents for the diagnosing and treating ONJ, the Guide of the American Association of Oral and Maxillofacial Surgeon (AAOMS) of 2014 and the I Colombian Consensus of ONJ published in 2019[12,17]. In the study by R Al-Eid et al., the authors reported that most respondents were unaware of the AAOMS guidelines[17,18].

In the evaluation of attitudes carried out through clinical scenarios, in clinical cases Nº 1 and 3 of patients with osteoporosis managed with denosumab and bisphosphonates, respectively, 74.66% and 43.84% would request an opinion from the referring physician to authorize the dental procedure. According to the AOMMS recommendations and the Colombian ONJ consensus, an assessment by the referring physician is not required to define dental treatment[12,17].

Furthermore, our study suggests that ONJ is a very low frequency disease in dentistry, as shown by the fact that 76.5% of those surveyed have not had any case of ONJ and 70.2% of dentists respondents have less than 25% of patients in their clinical practice diagnosed with osteoporosis. Of these patients with osteoporosis, 57.9% are treated with bisphosphonates and denosumab. Our study suggests that the respondents lack knowledge for decision-making regarding the risk of ONJ with the use of bisphosphonates and denosumab in treating osteoporosis.

This study has some limitations. The sample size was relatively small, which may not be representative of all dentists in our country. However, the study encompasses the highest number of dentists participating in knowledge assessment, compared with other previous studies that used a similar methodology.

Conclusion

The results of our study suggest that there is limited knowledge regarding the risk of developing ONJ with the use of bisphosphonates and denosumab in the treatment of osteoporosis. This low level of knowledge impacts the dental care of patients with osteoporosis managed with bisphosphonates or denosumab, by suspending therapy or delaying dental procedures. A greater effort is required to educate undergraduate and postgraduate students. Updating educational programs for graduated dentists could identify the actual risk and factors associated with ONJ in patients with osteoporosis treated with bisphosphonates or denosumab.

Conflict of interests: The authors declare no conflict of interest.

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Annex 1
Survey

1. Have you had patients in the last 12 months ?
   a. Yes
   b. No
2. Does your clinical practice work exclusively with children under 18?
   a. Yes
   b. No
3. Gender
   a. Feminine
   b. Masculine
4. Age: years
5. City of clinical practice
   a. Bogota DC
   b. Medellin
   c. Cali
   d. Barranquilla
   e. Other cities
6. Level of study attained
   a. Undergraduate
   b. Postgraduate
7. Years of clinical practice:
   a. Less than 10 years
   b. More than 10 years

Knowledge evaluation:
Note to start the survey: In the following survey, the term bisphosphonate refers to the following drugs: zoledronic acid, ibandronate, alendronate, risedronate. These drugs, such as denosumab, are therapeutic options for osteoporosis treatment.

1. When carrying out **major** dental procedures (exodontics, open-field surgical procedures) in patients with osteoporosis treated with **bisphosphonates,** the use of this type of medication is:
   a. An absolute contraindication for the dental procedure
   b. A relative contraindication for the dental procedure
   c. It is not a contraindication
   d. Not sure
2. When carrying out **minor** dental procedures (root canal treatment, cleaning, prophylaxis, resins, amalgams and crowns) in patients with osteoporosis treated with **bisphosphonates**, the use of this type of medication is::
   a. An absolute contraindication for the dental procedure
   b. A relative contraindication for the dental procedure
   c. It is not a contraindication
   d. Not sure
3. When carrying out **major** dental procedures (extractions, open-field surgical procedures) in patients with osteoporosis under treatment with **denosumab**, the use of this medication is:
   a. An absolute contraindication for the dental procedure
   b. A relative contraindication for the dental procedure
   c. It is not a contraindication
   d. Not sure
4. When carrying out **minor** dental procedures (root canal treatment, cleanings, prophylaxis, resins, amalgams and crowns) in patients with osteoporosis treated with **denosumab**, the use of this medication is::
   a. An absolute contraindication for the dental procedure
   b. A relative contraindication for the dental procedure
   c. It is not a contraindication
   d. Not sure
5. The risk of developing osteonecrosis of the jaw in patients with osteoporosis with the use of bisphosphonates compared to the use of denosumab is:
   a. Higher*
   b. Likewise
   c. Less
   d. Not sure

6. The risk of developing osteonecrosis of the jaw in patients with osteoporosis who receive bisphosphonates according to their route of administration is:
   a. Greater if administered intravenously than orally
   b. Greater if administered orally than intravenously
   c. Same regardless of route of administration*
   d. Not sure

7. The risk of developing osteonecrosis of the jaw in patients with osteoporosis receiving bisphosphonates:
   a. Increases in relation to the time of use of the bisphosphonate*
   b. It is not modified in relation to the time of use of the bisphosphonate
   c. Not sure

8. The risk of developing osteonecrosis of the jaw in patients with osteoporosis receiving denosumab:
   1. Increases in relation to the time of use of denosumab*
   2. It is not modified in relation to the time of use of denosumab
   3. Not sure

9. The risk of developing osteonecrosis of the jaw in patients with osteoporosis receiving bisphosphonates or denosumab compared to patients receiving these same therapies for cancer treatment is:
   a. Higher
   b. Less*
   c. Likewise
   d. Not sure

10. Are you aware of published scientific consensus documents for the prevention and management of patients with drug-induced osteonecrosis of the jaw?
   a. Yes*
   b. No

11. The risk of developing drug-induced osteonecrosis of the jaw in a patient with osteoporosis treated with bisphosphonates or denosumab is:
   a. 1 in 10 patients per year
   b. 1 in 100 patients per year
   c. 1 in 1,000 patients per year
   d. 1 in 10,000 patients per year*

12. Approximately, what percentage of patients in your clinical practice have a diagnosis of osteoporosis?
   a. Less than 25%
   b. From 25 to 50%
   c. More than 50%
   d. None

13. Approximately, what percentage of patients in your clinical practice have been diagnosed with osteoporosis and are being treated with bisphosphonates or denosumab?
   a. Less than 25%
   b. From 25 to 50%
   c. More than 50%
   d. None

14. How many cases of drug-induced osteonecrosis of the jaw have you seen in the last 12 months?
   a. No case.
   b. One case
   c. Between 2 to 5 cases
   d. Between 6 to 10 cases
   e. More than 10 cases

15. Of these seen cases of drug-induced osteonecrosis of the jaw, most were related to:
   a. Cancer treatment
   b. Treatment for osteoporosis
   c. Both alike
   d. Not sure
   e. Not seen any cases

16. Do you know if there is any diagnostic test to assess the risk of osteonecrosis of the jaw in patients receiving bisphosphonates or denosumab?
   a. Does not exist.
   b. Yes, it exists but they are useless*
   c. Yes, it exists and it is useful
   d. No
   e. In case your previous answer was options "b" or "c", please specify which diagnostic test(s) refers

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Clinical cases-clinical decisions

1. 65-year-old woman, history of hip fracture and osteoporosis, treated with denosumab for 1 year (last application 1 month ago). Consultation due to appearance of dental lesion that you consider requires extraction, considering the risk of developing ONJ, you would propose:
   a. Postpone the extraction until the effect of the medication ends (six months)
   b. Carry out the extraction as there is no contraindication for the procedure*
   c. Request a written concept from the treating rheumatologist, in which the risk of ONJ is defined and whether or not the dental procedure is authorized
   d. Advise the patient that the use of denosumab is an absolute contraindication for this type of dental procedure and will not be carried out

2. A 53-year-old man with a history of rheumatoid arthritis managed with methotrexate 10 mg/week and leflunomide 20 mg daily, does not use glucocorticoids. His rheumatologist also prescribed oral calcium at his last visit. He requires endodontic treatment, taking into account the risk of ONJ and the patient's scenario, you:
   a. Would not carry out the procedure as the patient is treated with methotrexate
   b. Would not carry out the procedure as the patient is treated with leflunomide
   c. Would carry out the procedure as there is no documented ONJ risk with the use of these medications*
   d. Would request a written concept from the treating rheumatologist, in which the risk of ONJ is defined and whether or not the dental procedure is authorized

3. A 60-year-old woman with a history of osteoporosis managed with alendronate 70 mg weekly for 18 months. She is scheduled to perform a dental implant and attends her consultation prior to the intervention. Regarding treatment with alendronate, you:
   a. Would perform the dental procedure without discontinuing alendronate*
   b. Would recommend suspending the treatment and restarting it according to its clinical evolution (closure of the surgical wound)
   c. Would explain that it is not a dental emergency and I would wait a 6-month cleaning time to perform the intervention
   d. I would advise the patient that the use of alendronate is an absolute contraindication for this type of procedure dental and will not be performed
   e. Would request a written concept from the treating rheumatologist, in which the risk of ONJ is defined and whether or not the dental procedure is authorized

4. A 64-year-old woman with a history of osteoporosis who has been managed with zoledronic acid 5 mg intravenously every year for two years, is scheduled for the next application in two months. The patient will undergo an extraction and attends your consultation prior to the intervention. Regarding treatment with zoledronic acid you:
   a. Would carry out the dental procedure without stopping zoledronic acid*
   b. Would recommend suspending the treatment and restarting it according to its clinical evolution (closure of the surgical wound)
   c. Would explain to him that it is not a dental emergency and I would wait a year for cleaning to carry out the intervention
   d. Would advise the patient that the use of zoledronic acid is an absolute contraindication for this type of dental procedure and will not be performed
   e. Would request a written concept from the treating rheumatologist, in which the risk of ONJ is defined and whether or not the dental procedure is authorized

5. A patient comes to your consultation who wants a second dental opinion. The patient suffers from osteoporosis and as treatment has received three doses of denosumab in the last two years, she was scheduled to carry out a dental implant, however, in previous consultations with two dentists, they have refused to perform the procedure as the patient presents C telopeptide levels at 0.05 ng/mL. Your opinion regarding the clinical case of the patient:
   a. Would indicate that the procedure be carried out, since the levels of C telopeptide are not a contraindication*
   b. Would tell you not to have the procedure done, due to raised C telopeptide levels
   c. Would postpone the procedure, until C telopeptide levels decrease
   d. Would not make any recommendations as I do not know the relationship between C telopeptide levels and complications derived from the procedure


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