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Respuesta al tratamiento con zoledronato tras la suspensión de denosumab en pacientes con osteoporosis

Protective effects of zoledronate after denosumab discontinuation

Autor/a: Ana Igea García

Director/es: D. José Manuel Olmos Martínez
D. José Luis Hernández Hernández

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1. ABSTRACT

Discontinuation of denosumab results in a marked increase in bone turnover markers (BTM) above baseline, known as “rebound phenomenon”. This is accompanied by a decrease in bone mineral density (BMD) and an increase in the risk of vertebral fractures, particularly multiple vertebral fractures. It has been suggested that the administration of antiresorptive drugs could prevent the turnover rebound. Therefore, we have considered important to verify that the administration of antiresorptives after denosumab discontinuation certainly prevents the rebound of BTM.

We report here a case series of thirteen patients with osteoporosis who had received denosumab for more than four years. Approximately 6 months after the last denosumab injection, they were given a single infusion of zoledronate (5 mg), drug chosen due to its potency and posology. They were followed-up for over a two-year period, in which none of them presented any clinical fractures. Furthermore, no single value of CTX nor lumbar spine BMD surpassed their corresponding baseline at any time.

So, as a result of our study we can confirm that an injection of zoledronate at denosumab discontinuation seems to adequately prevent the rebound phenomenon.

Key words: osteoporosis, denosumab treatment, zoledronate, clinical fractures, rebound.

RESUMEN

Tras suspender el tratamiento con denosumab se produce un aumento de los marcadores bioquímicos de remodelado óseo por encima de los valores de partida, conocido como “efecto rebote”. Este se acompaña de una disminución de la densidad mineral ósea (DMO) y un aumento del riesgo de fracturas vertebrales, especialmente fracturas vertebrales múltiples. Se ha sugerido que la administración de fármacos antirresortivos podría prevenir este efecto rebote. Por tanto, hemos considerado importante verificar que la administración de antirresortivos tras la suspensión de denosumab previene realmente el incremento de los marcadores de remodelado óseo.

Describimos los casos de trece pacientes con osteoporosis que habían recibido denosumab durante más de cuatro años. Aproximadamente 6 meses después de la última inyección de denosumab recibieron una infusión intravenosa de 5 mg de ácido zoledrónico, fármaco escogido por su elevada potencia y su posología. Fueron seguidos durante un periodo de dos años, en el que ninguno presentó fracturas clínicas. Además, ningún valor de CTX ni la DMO en la columna lumbar sobrepasó su nivel basal correspondiente en ningún momento.

Por tanto, como resultado de nuestro estudio, podemos decir que una infusión de zoledronato tras la suspensión de denosumab parece prevenir adecuadamente el fenómeno rebote.

Palabras clave: osteoporosis, denosumab, zoledronato, fracturas clínicas, efecto rebote.

2. INTRODUCTION

2.1. Bone structure

Based on the macroscopic and microscopic structure, we can distinguish cortical and trabecular bone. Cortical bone constitutes the outer part of all skeletal structures and it is dense and compact. This type comprises 80% of the skeleton, but, due to its composition, it only represents 30% of the bone surface. On the other hand, trabecular bone is less dense, more elastic, and has a higher turnover rate. It is found inside the bodies of the vertebrae, in the inner portions of the pelvis and in the large bones' epiphysis (1).

In cortical bone, the lamellae are arranged in concentric cylinders which have a blood vessel as their center, constituting Havers systems. In trabecular bone, the lamellae are arranged parallel to each other in bundles, equivalent to open Havers systems. Both lamellar systems are known as osteons, the basic structural units.

Bone is a specialized type of connective tissue consisting of a mineralized matrix and three types of cells: osteoblasts (OB), osteocytes and osteoclasts (OC). These cells are the responsible for the dynamic processes that take place in bones.

- Osteoblasts (OB) are differentiated products of mesenchymal stem cells, sharing their origin with adipocytes, fibroblasts and myocytes. They can be at rest, flattened on the surface as lining osteoblasts, or producing the bone matrix constituents.

- Osteocytes come from osteoblasts embedded in the matrix that has been produced by themselves. These cells are able to detect both mechanical loading and microinjuries and, in response, they enhance remodeling activity by recruiting osteoclasts.

- Osteoclasts (OC) are giant multinucleated cells that derive from hematopoietic cells of the mononuclear lineage. Their function is to resorb bone, action mediated through hydrogenions and proteolytic enzymes, such as cathepsin K.

Bone matrix has organic and inorganic components. The former mostly consists of type I collagen fibers (95%) and non-collagenous proteins. The inorganic components are mainly made up of calcium phosphate, deposited as hydroxyapatite crystals or in an amorphous form in 99% of the organic matrix. The remaining 1% has no mineral salt deposits and is called osteoid.

2.2. Bone physiology

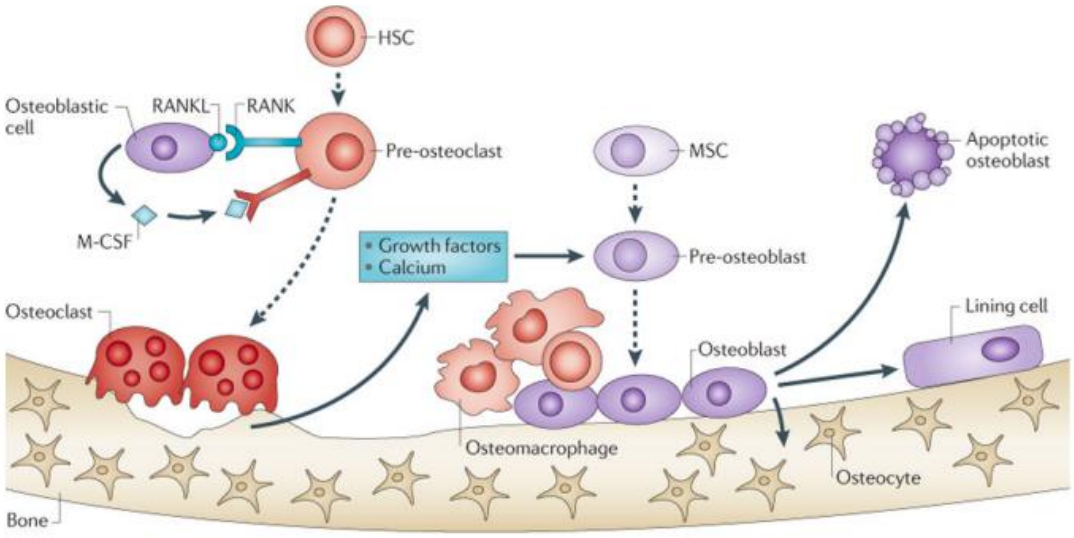
The continuous transformations undergone by the bone can be systematized in two types:

- Modeling: the set of modifications to maintain the morphological characteristics and adapt to mechanical loads. It occurs during growth and fracture repair resulting in bone mass accrual. In this process, the formation and resorption occur independently at different spots.

- Remodeling: the process that continuously renews the bone to prevent the accumulation of fatigue injuries and to adapt to the mechanical needs. It is carried out by the successive action of osteoclasts and osteoblasts at a particular point on the bone

surface (coupling). The newly formed bone is deposited in layers, initially without mineralization (osteoid).

Figure 1. Bone remodeling (2)



Remodeling is a finely balanced, coupled and sequential process (indicated by the dashed arrows). Osteoclasts resorb bone releasing growth factors and calcium. RANKL, presented by osteoblastic cells and M-CSF are critical for their maturation. Osteoblasts replace the voids with new bone, a process that is dependent on osteoblast commitment, proliferation and differentiation paired with osteoblast production of type I collagen and its subsequent mineralization to form the calcified matrix of bone. Osteocytes, sense mechanical strain, signal to osteoclasts and osteoblasts, participating in the remodeling process (3). Bone lining cells have been proposed to form both a canopy over remodeling sites and a layer over bone surfaces, as well as a conduit to communicate with osteocytes (4). The endosteum and periosteum contain a population of tissue macrophages, termed osteomacs (5).

M-CSF, macrophage colony stimulating factor; RANK, receptor activator of NF-κB; RANKL, RANK ligand.

2.3. Regulation of bone remodeling and bone turnover

A large number of factors, both local and general, are involved in the regulation of bone remodeling.

- Local regulation:

Osteocytes detect when a bone multicellular unit needs to be renewed and they send various signals to the surface. Some of them, like vascular endothelial growth factor (VEGF), attract capillaries and, with them, OC precursors. Others, receptor activator of NF-κB ligand (RANKL) and proinflammatory cytokines (IL-1, TNF, IL-6), stimulate these precursors to mature and carry out their function. Once the appropriate amount of bone is destroyed OCs are inhibited by osteoprotegerin (OPG), which binds to RANKL, and TGF-β.

The moment bone resorption is completed, the formation of new bone begins, with the OBs being stimulated by substances. These substances are released from the bone during its resorption (TFG-β) or produced by the OBs (Wnt proteins and bone morphogenetic proteins). The main inhibitory signal to terminate osteoblastic activity is the sclerostin. Sclerostin is produced by osteocytes and inhibits the action of Wnt proteins.

- Systemic regulation:

First of all, the calciotropic hormones have an important role in the bone metabolism. Calcitonin inhibits directly the osteoclasts. The parathyroid hormone and calcitriol increase the RANKL/OPG ratio, stimulating osteoclast activity. Nevertheless, the latter results in a positive balance because it enhances intestinal calcium and phosphorus absorption and diminish PTH secretion.

Estrogens affect inhibiting OC directly and indirectly through OB. Furthermore, they upregulate RANKL and cytokines production by T cells.

Glucocorticoids restrain bone formation, but they also can, temporarily, rise RANKL/OPG fraction, indirectly stimulating OC.

Bone modeling regulators are less well known. The main stimulating factor is mechanical overload, and the hormones accomplishing the same goal in the periosteum are testosterone, GH, IGF-1, Wnt proteins and PTH. Estrogens, on the contrary, have been described as inhibitors.

2.4. Osteoporosis

Osteoporosis is characterized by an increase in skeletal fragility with a tendency to develop fractures, due to a decrease in bone mass and an alteration in bone quality. The decrease in bone mass is established in the trabecular bone by thinning or disappearance of the trabeculae, and in the cortical bone by its thinning and increase in porosity. The quality alteration covers two aspects: the modifications in the bone tissue itself and structural alterations such as a decrease in the connection between trabeculae. These events are asymptomatic until a fracture occurs.

2.4.1. Risk factors and epidemiology

Osteoporosis is a multifactorial disease in which age, along with being a female, are the main risk factors. The reason why women are at a higher risk of suffering from it is explained by the acquisition of less bone mass during development and the estrogen depletion after menopause. The percentage of postmenopausal women who, regardless of having suffered fractures, have bone density values in the osteoporotic range varies from 15% to 30%, depending on whether the measurement is taken at a single site or at several sites.

The turnover increase in the postmenopause is considered to affect fundamentally the trabecular bone (predisposing principally to vertebral fracture), while aging itself would also affect the cortical bone, being more common the hip fracture.

Other important risk factors are personal or family history of osteoporotic fracture and a low body mass index (BMI) (≤ 19 kg/m^2). A sedentary lifestyle, smoking and an excessive alcoholic intake also contribute negatively.

Regarding secondary osteoporosis we can find hyperthyroidism, hyperparathyroidism, hypercortisolism, hypogonadism, myeloma, glucocorticoids, rheumatoid arthritis and other conditions as triggering factors.

2.4.2. Bone turnover markers (BTMs)

BTMs provide a dynamic assessment of skeletal activity and high levels can predict rapid rates of bone loss and an increased fracture risk independent of BMD (6–8). Clinical trials have shown that early changes in BTMs are associated with long-term BMD changes in women taking antiresorptive (9) or anabolic (10) drugs. Moreover, they respond quickly to therapeutic intervention (11).

- Serum C-terminal telopeptide type-1 collagen (CTX): is the preferred resorption marker.

- Serum carboxyterminal propeptide of type-I collagen (PINP): is the favorite to represent bone formation.

- Alkaline phosphatase: is also a formation marker but due to its low sensitivity is only useful in conditions with an intense turnover rate, like Paget disease.

2.4.3. Treatment

Preventive measures

The first step to reduce fracture risk involves fall reduction strategies like establishing muscle and balance training, correcting impaired vision or hearing, diminishing fall risk inducing drugs, smoking cessation and limiting alcohol intake (12).

Moreover, an adequate protein, calcium (1000-1200 mg/day) and vitamin D (800-1000 UI/d) intake through diet, preferred, or supplements, is necessary.

Pharmacological agents

US guidelines recommend pharmacologic therapy for postmenopausal women with (11):

- A bone density T-score < -2.5 standard deviation (SD) in the lumbar spine, femoral neck, total hip, or distal radius.

- A -1.0 SD < T-score < -2.5 SD who have:

    - Suffered a fragility fracture of the hip or spine.

    - A FRAX 10-year probability of ≥3% for hip fracture or ≥20% for major osteoporotic fracture

We can classify osteoporosis drugs by their mechanism of action (13,14):

- Inhibition of bone resorption

- Bisphosphonates carry out their effect by binding to active sites of bone remodeling and inhibiting OC.

Alendronate (oral), risedronate (oral) and zoledronic acid / zoledronate (injectable) have demonstrated to reduce the risk of vertebral, hip and other nonvertebral fractures, while ibandronate (oral) has only been shown to reduce the risk of vertebral fractures.

Adverse effects most commonly seen with oral bisphosphonates are gastrointestinal, like esophagitis and diarrhea, whereas with IV bisphosphonates acute-phase reactions consisting of low-grade fevers, arthralgias and myalgias have been reported. Infrequently, some patients have experienced ocular inflammation. This pharmacologic class is contraindicated in patients with renal impairment (creatinine clearance <35 mL/min).

More serious adverse effects include osteonecrosis of the jaw (ONJ) and atypical femoral fractures (AFFs). ONJ incidence is higher in patients with comorbidity (cancer or immunosuppression) treated with high-dose IV bisphosphonates. Moreover, dental extractions, periodontal infection and denosumab use increase the risk of ONJ. AFFs (3.2-50 cases / 100,000 person-years) occurs more frequently with long-term treatment, raising its incidence to 100 cases per 100,000 person-years.

- Denosumab is a fully human monoclonal antibody (IgG2) with high affinity and specificity for the RANKL. It mimics the action of endogenous osteoprotegerin. This binding prevents the RANKL action on the RANK receptor of OCs which results in a reduction in the number and activity of these cells, and ultimately in a decrease in bone resorption (15).

It is effective against both types of fractures too, being an alternative in patients who have not responded to or cannot tolerate bisphosphonates. A 60 mg dose is injected every 6 months subcutaneously.

Its pharmacokinetics is not linear, and three phases have been observed. One of prolonged absorption at 5 to 21 days after its administration. A prolonged half-life of up to 32 days and a rapid terminal phase when the concentration is less than 1,000 ng/ml (16). It has been estimated that the time it takes to reach the maximum concentration of the drug in the body is 26 days.

Like bisphosphonates, denosumab can lead to the development of ONJ and AFFs, which explains the drug holidays' existence. Its main limitation though, consists in the rebound effect produced after its discontinuation, the cornerstone of this article. However, it can be used in patients with a glomerular filtration rate < 30 mL/min, always monitoring hypocalcemia.

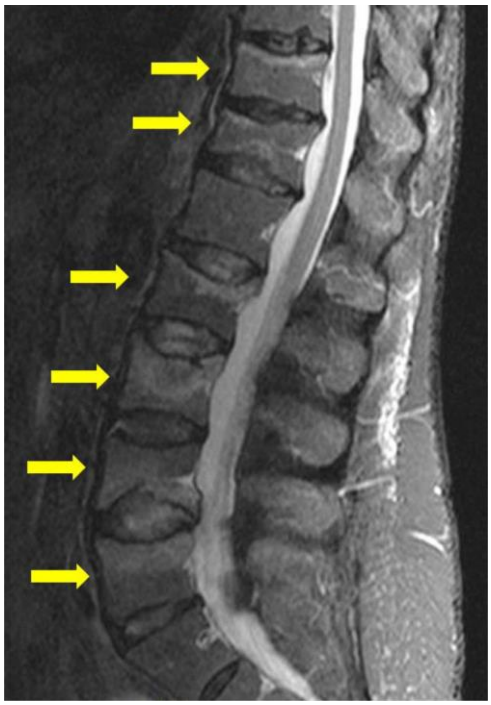


Figure 2. Multiple vertebral fractures in a patient 7,5 months after the last denosumab injection (17).

Indicated by the yellow arrows the fractures located in T11, T12, L2-L5.

- Selective estrogen receptor modulators (SERM) were synthesized to act like estrogens but avoiding their adverse effects, principally cardiovascular disease. There are two SERMs available, raloxifene and bazedoxifene, both useful specifically for vertebral fracture prevention. Also, raloxifene has been shown to reduce the risk of invasive breast cancer.

Women under this treatment can suffer hot flashes, leg cramps and peripheral edema. In addition, like estrogens, they increase the risk for venous thromboembolic events.

- Calcitonin has been removed from the market due to the limited evidence of its efficacy and the doubts about its safety.

- Stimulating bone formation

- PTH analogs increase BMD by stimulating bone formation when administered intermittently, once-daily. Approved drugs include teriparatide, the recombinant 1-34 sequence of human PTH, and abaloparatide, a synthetic analog of human PTHrP. They are not first-line therapy, but they can be used in postmenopausal women at high fracture risk not tolerating or responding to other treatments. In contrast, it is contraindicated in patients with preexisting hypercalcemia, history of bone radiotherapy, bone metastases, or skeletal malignancies. The treatment with these drugs must be discontinued after 2 years but should be followed up by other antiresorptive medication.

Adverse effects include injection-site reactions, nausea, arthralgia (teriparatide), hypotension and tachycardia. Transient hypercalcemia and hypercalciuria can occur.

- Inhibition of bone resorption and stimulation of bone formation

- Romosozumab is a monoclonal antibody that binds to and inhibits sclerostin.

Arthralgia and headache are the most common adverse effects reported. More worrying are the cardiovascular events, who has led to its prohibition in patients who had suffered a myocardial infarction within the previous year.

3. HYPOTHESIS AND OBJECTIVES

Ten-years of therapy with denosumab results in continuous bone mineral density (BMD) gains at the lumbar spine and hip, without reaching a plateau. This leads to a decrease in the incidence of fractures, with a good safety profile (18). But, to prevent ONJ and AFFs, denosumab cannot be taken life-long. Another reason to discontinue denosumab treatment, is an insufficient or even an ineffective response, reflected in BMD, to this treatment.

Unlike bisphosphonates, denosumab does not attach to bone, and therefore its effect on bone resorption ceases when withdrawn. Two studies (19,20) carried out about 10 years ago showed that the discontinuation of denosumab is followed by a marked increase in bone turnover markers (BTM), which rise well above the baseline values (about 60% above baseline level and 120-140% above post-denosumab treatment levels). This increase was described as a "rebound phenomenon". This effect is believed to occur in view of a compensatory upregulation of the RANK receptors on osteoclast precursors while in presence of denosumab. It peaks during the year following cessation of denosumab activity and lasts for approximately two years. BMD decreases at the spine and hip during the first year, so that the increase achieved during denosumab treatment is lost.

More recently, several case reports have pointed out that, in some patients, the discontinuation of denosumab is associated with the development of vertebral fractures, and more specifically of multiple vertebral fractures (21–24). A radiography showing these multiple vertebral fractures can be seen in Figure 2. These fractures occur mainly over a period of one or two years after discontinuation of denosumab, corresponding with the rebound phenomenon. In fact, both the loss in the BMD gained with denosumab treatment and the increase in vertebral fractures are considered to be due to the rebound phenomenon shown by the increase in BTM.

Therefore, it should be expected that, if the rebound phenomenon is prevented, the decrease in BMD and the rise in bone fractures could be avoided or at least reduced. That is the reasoning behind the experts' recommendation regarding administering an antiresorptive upon cessation of denosumab treatment. The ECTS, for instance, has stated that "bisphosphonate therapy should be considered to reduce or prevent the rebound increase in bone turnover" (25). Due to its greater potency and its easy posology (a single injection), zoledronate could be the drug of choice for it.

In accordance with the above, it must be concluded that it is important to verify that the administration of antiresorptives (e.g., zoledronate) after denosumab discontinuation certainly prevents the rebound of BTM. Along with it, we must take note of the changes in the BMD and in the incidence of fractures, although the latter will certainly be more difficult to assess because it requires larger samples. To date, there are very few studies that have analyzed these issues. In the article by Anastasilakis et al (26), zoledronate infusion in 27 women previously treated with denosumab was followed by significant but small increases in serum P1NP and CTX during the first year and stabilization thereafter. However, in 30 women who did not received zoledronate after denosumab treatment, BTM increased markedly at 15 months. BMD decreased in both groups of patients two years after denosumab discontinuation, but the decrease was much

smaller in the zoledronate group. More recently, Everts-Graber et al (27) have shown a similar pattern in BMD changes, but their assessment of BTM is limited and insufficient to enable us to reach a clear conclusion about the zoledronate impact on the rebound phenomenon. Although P1NP and CTX are reported on some 20 patients who received one infusion of zoledronate after stopping denosumab, their baseline levels are not given. Furthermore, neither the time at which the zoledronate was administered, nor the time elapsed between its administration and the measurement of the BTM are specified.

The randomized clinical trial still ongoing by Sølling et al. (28) studies BMD (at the lumbar spine, total hip and femoral neck), CTX and the onset of clinical fractures in 61 patients after denosumab discontinuation. They were randomly allocated to three groups, in each of which patients received the zoledronate infusion at three different intervals after the last denosumab injection. In one group zoledronate infusion was given 6 months after denosumab discontinuation, in another 9 months after discontinuation and the observation group, who received the zoledronate infusion when bone turnover had increased, with the cutoff value being CTX > 1,26 µg/L (50% above the normal range for postmenopausal women and elderly men), no injection was given later than 12 months after discontinuation. The results from the first 12 months after the initial administration of zoledronate suggest that in patients discontinuing denosumab after long-term treatment a single intravenous infusion of zoledronate 5 mg is not sufficient to completely prevent bone loss. Based on the rapid increase in CTX in the 6-month group this seems the most appropriate option and proposes the possible benefit of another zoledronate infusion 3 to 6 months later. Also, the outcomes evoke that the treatment duration with denosumab and the baseline CTX levels are important determinants for the zoledronate efficacy to maintain the suppression of bone turnover.

These are the reasons why we consider it appropriate to add some information on this issue. In the present article we report on the issue of BTM and BMD changes in patients who had been on denosumab for at least two years and were transitioned to zoledronate at the time of denosumab discontinuation.

4. PATIENTS AND METHODS

4.1. Patients

We report here thirteen patients (twelve women, one men) treated in the Bone Metabolism Unit of a tertiary-care center (Hospital Universitario Marqués de Valdecilla) in Northern Spain, who received zoledronate after discontinuing denosumab, and were followed-up for a mean of 20 months. Their mean age was 70±8 years (range, 55-83 years).

Five patients had previous fragility fractures: vertebral (n=3), hip (n=1) and proximal humerus fracture (n=1). Seven patients had previously received bisphosphonates and were shifted to denosumab because the densitometric response was considered insufficient.

Table 1. Characteristics of the patients included in the study.

Case	Age (y)	Sex	BMI (Kg/m^2)	Prior Fx	Prior BP	Months Dmab	Weeks Dmab-Zol	Months post-Zol
1	72	F	31	Yes	No	65	26	21
2	71	F	29	No	No	30	22	21
3	65	F	27	Yes	No	60	25	15
4	66	F	30	No	No	66	25	15
5	66	F	24	No	Yes	56	25	15
6	70	F	23	No	Yes	60	24	15
7	76	F	25	No	Yes	66	24	21
8	83	F	28	Yes	Yes	48	26	20
9	81	F	32	No	Yes	42	26	22
10	55	M	31	Yes	Yes	36	24	22
11	60	F	18	No	No	72	26	23
12	76	F	30	No	No	54	29	12
13	68	F	27	Yes	Yes	30	32	34

F: female; M: male; BMI: body mass index; Fx: fracture; BP: bisphosphonates; Dmab: denosumab; Zol: zoledronate. Months Dmab: months of treatment with dmab; Weeks Dmab-Zol: weeks since a new denosumab injection should be given and the administration of zoledronate; Months post-Zol: months of follow-up after administration of zoledronate.

No patient was taking any drug related to the development of osteoporosis or fragility fractures. Denosumab discontinuation was decided by the attending clinician because the T-score was above -2.5 and there was no recent history of fracture. The number of denosumab injections ranged between 4 and 12, with a mean of 8. The duration of denosumab treatment ranged from 30 months to 72 months.

Zoledronate infusion was given an average of 6.3±0.8 months after the last denosumab injection. All patients received vitamin D supplements to maintain serum 25OHD levels between 20 and 40 ng /mL. A calcium-rich diet was advised, and oral supplements were prescribed (500 mg/d) only when dietary calcium intake was insufficient.

After zoledronate injection, patients were clinically followed for 20±5 months (range 15-34) and questioned specifically regarding the development of clinical fractures. Serum P1NP and CTX levels were determined with automatized chemiluminescence assays (IDS-iSYS Multi-Discipline Automated Analyzer; Tyne and Wear, UK) in the morning after overnight fasting. Sensitivity was 1 ng/ml and 0.033 ng/ml, respectively. Normal premenopausal ranges were 19-76 ng/ml for P1NP and 0.034-0.635 ng/ml for CTX. For postmenopausal women, normal ranges were 21-102 ng/ml and 0.034-1.037 ng/ml respectively. Intra- and inter-assay coefficients of variation were 6% and 8% for P1NP and 7% and 6% for CTX. We considered as least significant change (LSC) a difference of 25% for both markers, as proposed by the International Osteoporosis Foundation (29). BTM were measured before starting the denosumab treatment (T0 or baseline), at the time of transition from denosumab to zoledronate (T1), 6 months (approximately) after zoledronate injection (T2), and at the end of the follow-up period (T3). BMD was measured at the lumbar spine, femoral neck, and total hip by dual-energy X-ray absorptiometry (DXA) using an Hologic device (Hologic QDR 4500, Bedford, MA, USA). In-vivo precision was 0.4%-1.5% at the different measurement sites. Results were expressed as gr/cm2 and T-score. We considered as LSC a change ≥ 5% at the spine and ≥4% at the FN, as proposed by the International Foundation for Osteoporosis (29). BMD was measured at T0, T1 and T3.

4.2. Statistical analysis

Quantitative variables are expressed as mean ± SD, and qualitative variables as numbers and percentages. Wilcoxon signed rank test was used to compare BTM and BMD values at the different time points. A p-value <0.05 was considered statistically significant in all calculations.

5. RESULTS

5.1. Serum P1NP levels

Serum P1NP levels (Figure 3) were 47.6±31.1 ng/mL at baseline (T0). At T1, after denosumab treatment, they had fallen to 12.6±5.5 ng/mL (p=0.002), which means a decrease of 74%. About six months after zoledronate administration (T2), P1NP levels had increased to 21.9±7.5 ng/mL (p=0.011 vs. T1), which still means a 54% decrease from baseline (p=0.015). Individual values ranged from 10 to 33 ng/mL, thus being below the premenopausal range or at the lower values thereof. No individual value was higher than baseline. At the end of follow-up (T3), serum P1NP levels were 44.0±13.8 ng/mL, a value close to baseline (p=0.61). The individual values ranged from 25 to 73 ng/mL without, therefore, any of them exceeding premenopausal normal values. At this point (T3), serum P1NP values were at baseline levels in 4 patients, and above them in 4 others.

5.2. Serum CTX levels

Serum CTX levels (Figure 3) were 0.601±0.223 ng/mL at baseline (T0), and 0.040±0.020 ng/mL at T1 (p=0.002), accounting for a 93% decrease. At T2, CTX levels were 0.145±0.115 ng/mL (p=0.004 vs. T1), which still represents a 76% decrease compared to baseline levels (p=0.002). The individual values were between 0.033-0.470 ng/mL, and none, therefore, exceeded normal premenopausal levels. Finally, at the end of the follow-up period (T3), serum CTX levels were 0.242±0.145 ng/mL (p=0.004 vs. baseline). Although this means an increase with respect to T2 determination, it still represents a 60% decrease compared to baseline levels. Again, no individual values were above premenopausal normal levels, and none exceeded the baseline values either (although in three cases they returned to baseline levels). Therefore, a "rebound phenomenon" of CTXs has not been observed in patients who, after discontinuation of denosumab, receive a zoledronate injection.

5.3. BMD

Regarding BMD, their values at the three locations at times T0, T1 and T3 along with the percentage of change at times T1 and T3 vs time T0, are given in Table 2.

Table 2. BMD at times T0, T1 and T3. BMD changes (in percentage) at times T1 and T3 compared to T0.

BMD (g/cm^2)	T0	T1	△ vs T0; p	T2	T3	△ vs T0; p
Lumbar spine	0.744 ± 0.076	0.845 ± 0.104	13,6%; 0,001	---	0.811 ± 0.091	9.0%; 0,046
Femoral neck	0.661 ± 0.119	0.716 ± 0.112	8,3%; 0,001	---	0.670 ± 0.089	1,4%; 0.55
Total hip	0.766 ± 0.101	0.840 ± 0.092	9,7%; 0,001	---	0.798 ± 0.095	4,2%; 0,51

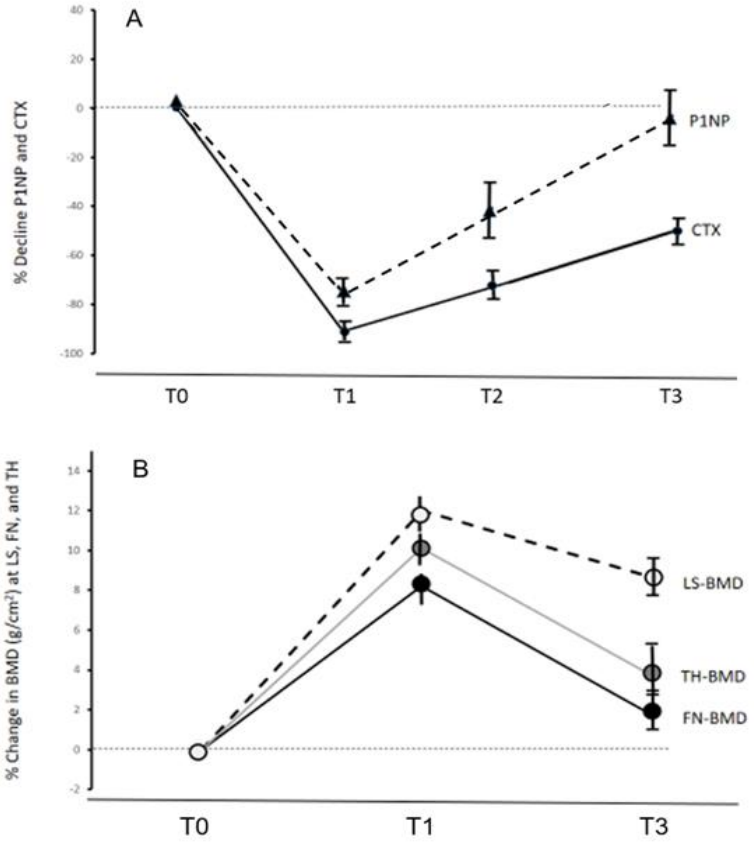
BMD: bone mineral density; T0: baseline (before starting denosumab treatment); T1: time of transition from denosumab to zoledronate; T2: 6 months after zoledronate injection (there are no data in this column since BMD was not measured at this time); T3: end of the follow-up period. □: difference in BMD; p: level of significance.

BMD was not assessed at T2, because only 6 months had elapsed since T1. Figure 3 provides a visual image of those changes. As it can be seen, denosumab treatment was followed by a marked increase in BMD at the lumbar spine, femoral neck and total hip. At the end of the follow-up, the increase in lumbar BMD still represents a significant 9% increase over the baseline value. At the hip, however, mean BMD values (either at the femoral neck or the total hip) were not significantly different from baseline, but nevertheless they were not lower than them.

Figure 3.

A. P1NP and CTX after denosumab discontinuation followed by a 5 mg injection of zoledronate.

B. BMD at lumbar spine, femoral neck and total hip after denosumab discontinuation followed by a 5 mg injection of zoledronate.

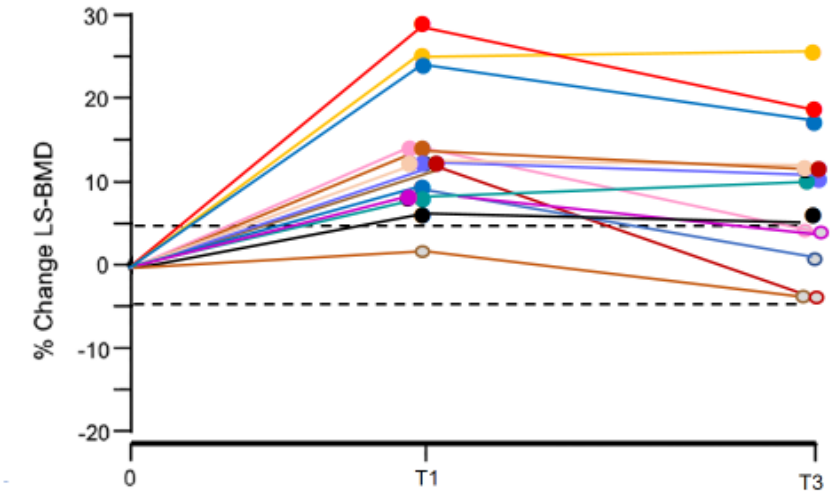


T0: before denosumab administration (baseline); T1: after denosumab administration and before the injection of zoledronate; T2: 6 months (approximately) after zoledronate injection; T3: at the end of the follow-up (20 months on average)

All individual values at lumbar spine (Figure 4) rose above the baseline, with the exception of a single case, whose value remained at baseline levels. At the end of the follow-up (T3), spine BMD values were still above the baseline in 8 patients and had returned to baseline in 5. At femoral neck, all individual BMD values at T1 were above baseline, with the exception of three cases that remained at baseline level. At the end

of follow-up, values were above baseline in 7 patients, at baseline in 5 and below baseline in 1. Finally, at the total hip, all individual values were at T1 above baseline values, except for a patient who remained at baseline levels. At the end of the follow-up (T3), BMD values were above baseline in 8 patients and below in 5.

Figure 4. Individual BMD changes after denosumab discontinuation.



T0: before denosumab administration (baseline); T1: after denosumab administration and before the injection of zoledronate; T3: at the end of the follow-up (20 months on average). Dotted lines represent the limits of the least significant change.

5.4. Clinical fractures

No patient sustained clinical fractures. Since no spine X-rays were performed at the end of the study, the occurrence of morphometric vertebral fractures cannot be ruled out.

6. DISCUSSION

Currently, one of the most important problems when treating osteoporosis, is how to deal with patients who discontinue denosumab. It has been reported that denosumab discontinuation is followed by an increase in BTM well above baseline (the so called "turnover rebound"), along with a loss of BMD and an increase in the risk of fractures, particularly of multiple vertebral fractures. We do not yet know the best approach to prevent this complication. The administration of a potent antiresorptive agent (mainly a bisphosphonate) has been advised by some experts (25) in order to prevent the bone turnover rebound and its consequences. However, the number of studies carried out to assess the precise effect of antiresorptives on the rebound phenomenon have been scarce. We need further studies addressing the issue of whether bisphosphonate administration, in particular intravenous zoledronate (the most potent bisphosphonate), counteracts the increase in BTM, and to what extent it does.

In our case series we have found out that two years after zoledronate administration, given upon discontinuation of denosumab, CTX levels had increased in relation to the values observed at the end of denosumab treatment, but they still remained 60% below baseline levels. What is more, no individual value exceeded the baseline. Therefore, not only did we not observe any rebound effect, but, in addition, the CTX levels were still below those before starting treatment. P1NP levels had also increased in relation to the values observed at the end of denosumab treatment. This increase is greater than that of CTX, so that they do not remain below baseline level, although they stayed close to it. In any case, their mean value did not surpass this baseline. Therefore, again, we can state that there was not a generalized rebound phenomenon.

Nevertheless, the individual values of four patients were above their own baseline levels. This apparent discrepancy in these four patients between the pattern of CTX an P1NP changes (although perhaps only due to analytic variability) could raise the question of whether the higher levels of the P1NP must prevail to decide that there is indeed an increase in bone turnover. The replacement of bone tissue, bone turnover, begins with the phenomenon of bone resorption. Without bone resorption there is no remodeling, and therefore, in mechanistic terms, a resorption marker (CTX) should be a more reliable index of bone turnover than a formation marker. In fact, studies on the effect of antiresorptive drugs on bone turnover markers (30) report early and large change in bone-resorption markers (CTX) compared with bone-formation markers (PINP), therefore CTX reflecting better the effect of antiresorptives on osteoclasts.

7. CONCLUSIONS

We are positive that our findings provide compelling evidence that zoledronate administration after denosumab discontinuation prevents the phenomenon of rebound. In this respect, it is worth emphasising that this phenomenon (a strong increase in bone turnover) should not be confused with the degree of elevation of BTM that follows the shift of denosumab to zoledronate, which is due to the fact that the former develops on osteoclasts a more potent inhibitory effect than the one developed by the latter.

Consistent with BTM results, BMD decreases, but it remains higher than its values at baseline by 9% at the lumbar spine at the end of the follow-up period. As for individual values, five returned to baseline levels, but none fell below this. It seems, therefore, appropriate to conclude that a zoledronate injection adequately maintains BMD values at the lumbar spine for two years. Regarding hip BMD, the mean values at the femoral neck and total hip at the end of the follow-up were similar to baseline values. And as for their individual values, at the femoral neck only one patient showed a BMD value lower than baseline. It is important to bear in mind that this patient had not responded previously to denosumab, since after treatment with this drug her femoral neck BMD was the same as before. With respect to total hip, the individual values of five of the thirteen patients fell below the baseline. This different pattern of BMD changes at the hip compared to the lumbar spine is a finding of interest, because the main type of fracture that has been reported as increased following denosumab discontinuation is vertebral fracture, and not hip fracture. Consequently, the area of the skeleton where the risk of fracture after discontinuation of denosumab is greatest is precisely the one most protected by zoledronate. Such a greater preventive effect on the lumbar spine may take place because the bone turnover is greater in the trabecular bone.

Finally, none of our patients have sustained clinical fractures over the two-year follow-up. It must be taken into account that fractures resulting from denosumab discontinuation are considered to occur between 9 and 16 months after the last injection of the drug. Therefore, the absence of fractures in the two years following withdrawal of denosumab along with a subsequent injection of zoledronate, is consistent with the idea that zoledronate is effective in preventing the increase in the risk of fractures caused by the rebound phenomenon.

The issue of fractures development after zoledronate administration following denosumab discontinuation needs much further research, since very little data have been published on this subject. Anastasilakis et al. (26) have published a series of 27 patients of which only one had vertebral fractures after two years of follow-up. One of these fractures was new and the other consisted in the worsening of a preexisting vertebral deformity. On the other hand, none of the 6 patients reported by Reid et al. (31) nor any of the 22 reported by Lehmann et al. (32) sustained vertebral fractures, after a similar two-year follow-up. More recently, Everts-Graber et al (27) have reported the development of three vertebral fractures (all single) and four non-nonvertebral fractures on 120 patients. One of the vertebral fractures occurred more than two years after denosumab discontinuation and the subsequent zoledronate injection. No vertebral fractures were neither identified in the retrospective case series conducted by Kadaru et al. (33) based on 12 patients. Moreover, 5 of them received a second

zoledronate infusion and in this subgroup BMD levels seemed to suffer a general stabilization.

Considering also our case series, this means a total of 199 patients, of whom only 4 have had vertebral fractures. That would account for about 0,5% multiple vertebral fractures and 1,5% single vertebral fractures. As we still do not know the precise incidence of vertebral fractures in patients discontinuing denosumab without further protection with an antiresorptive drug, we cannot yet conclude whether or not these figures mean a reduction in that incidence. Data from some studies (34) suggest that vertebral fracture incidence when denosumab is discontinued without providing protection with other antiresorptive drug is larger (about 5-10%). This remains an open question notwithstanding.

Our study has some limitations. It is an observational study, and it was conducted on a small number of patients. However, it provides detailed information on the BTM changes in patients who discontinue denosumab switching to a zoledronate infusion. This is a matter of paramount importance, since it provides the pathophysiological basis to establish whether zoledronate prevents or not the rebound phenomenon. In addition, we are also providing detailed evidence on BMD changes, having verified that, although BMD decreases consistently the slight increase in BTM, such a decrease does not imply a return to baseline, in line with the absence of the rebound effect on BTM.

In conclusion, in patients whose denosumab treatment is discontinued, an infusion of zoledronate at the time when a new denosumab injection should be given slows the recovery of bone turnover, this way avoiding the rebound phenomenon. Consistent with this, bone mass is maintained for two years above baseline in most patients, particularly at the lumbar spine. This should reduce the incidence of fractures and, in fact, in our series no patient presented any fracture. Further studies are needed to know whether our results may be generalized.

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