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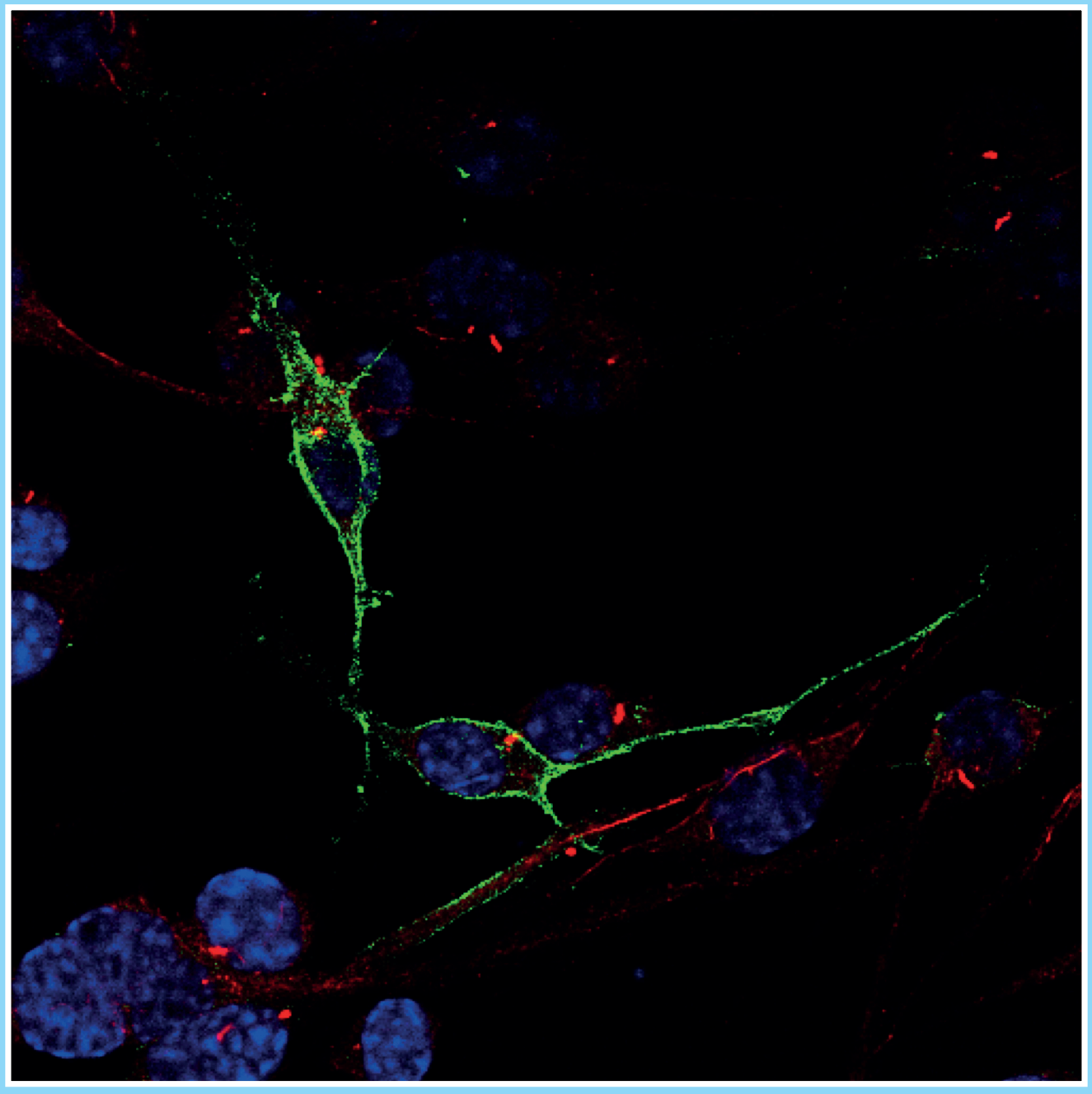

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Sumario

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Original Breve

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Artículo Especial

Imagen de portada:

Osteocitos MLO-Y4 transfectados con el receptor de parathormona (PTH1R) marcado con GFP (verde), cilio primario marcado con alfa tubulina acetilado (rojo) y tinción DAPI los núcleos (azul).

Pablo Orduña. SAI MICROCON. IMMA-Universidad CEU San Pablo. Madrid

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Revista de Osteoporosis y Metabolismo Mineral

Original Breve

Osteoporosis prophylaxis in patients on high-doses glucocorticoids

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Abstract

Background: chronic use of glucocorticoids (GCs) is the most common cause of secondary osteoporosis (OP). However, the prevention of GC-induced OP remains suboptimal despite its inclusion in OP management guidelines.

Objective: to analyze the prophylaxis of GC-induced OP at high doses in clinical practice.

Methods: the dispensation of GCs and concomitant treatment for OP was analyzed in a district with 2 health care areas. Patients older than 50 years who were dispensed ≥ 90 tablets of prednisone 30 mg through a pharmacy were included. The following data were collected from health records and the electronic pharmacy application: age, sex, reason for using GCs, number of prednisone containers dispensed, bone densitometry performed, and any concomitant use of bisphosphonates or denosumab.

Results: a total of 427 patients were included (mean age 66 years M 51 % women). The most frequent body systems involved were respiratory (46 %), cutaneous (10 %), rheumatic (9 %) and neurologic (8 %). OP prophylaxis was dispensed in 59 cases (13.8 %). In the multivariate analysis, prophylaxis was associated with age > 70 years (OR, 4.23; 95 %CI, 2.11-8.49), female sex (OR, 3.15; 95 %CI, 1.47-6.74), having a rheumatic OR, neurologic disease (OR, 5.33; 95 %CI, 2.53-11.23), a bone densitometry assessment (OR, 3.55; 95 %CI, 1.66-7.57) and dispensation of > 120 prednisone tablets from the pharmacy (OR, 2.31; 95 %CI, 1.14-4.70).

Conclusion: in our setting, GC-induced OP prophylaxis was definitely suboptimal. Training sessions are needed for doctors who prescribe high doses of GCs, and electronic prescription alerts should be implemented.

Keywords:
Glucocorticoids.
Osteoporosis.
Prophylaxis.

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INTRODUCTION

The chronic use of glucocorticoids (GCs) is the most common cause of secondary osteoporosis (OP), leading to an increased risk of fracture and a consequent reduction in quality of life. The prevention of GC-induced OP and fractures is included in the clinical practice guidelines of scientific and medical societies (1-3).

In the case of the American College of Rheumatology (1), the most recent clinical practice guidelines identify fracture risk as a determining factor when initiating prophylaxis for osteoporosis, emphasizing that treatment should begin early.

The recommendations of the Spanish Society for Bone Research and Mineral Metabolism (SEIOMM) (2) indicate that postmenopausal women and men older than 50 years on GCs at equal or and higher doses of prednisone 5 mg for more than 3 months should be prescribed OP prophylaxis. In premenopausal women, the indication depends on the dose of GCs (> 30 mg/d) or a history of fracture. SEIOMM recommends bisphosphonates as the first-line therapy of choice, and denosumab if there is a contraindication or intolerance to bisphosphonates.

The recommendations of the Spanish Society of Rheumatology (SER) (3) indicate prophylaxis for all patients on GCs for more than 3 months with a starting dose ≥ 30 mg of prednisone. In cases involving lower doses, a prescription for OP prophylaxis would depend on the history of fracture, the result of the densitometry test and the risk of fracture estimated by FRAX®.

The concept "imminent risk of fracture" includes any recent fracture, patients who have previously fallen and high doses of GC (4). As the term indicates, imminent risk means a very high risk of fracture that may be independent of bone mineral density, such as it is the case with frail, elderly people with frequent falls. Patients on high doses of GCs represent a vulnerable population for which fracture prevention should be implemented, given the imminent risk of fracture.

The incidence rate of fractures increases with GC treatment. In one study, the incidence rate of non-vertebral fractures increased from 1.6 per 100 person-years in the year before starting oral GC, to 2.0 within the first 3 months of treatment (5). In addition, a review of randomized clinical trials found a higher rate of vertebral fracture among GC initiators and a relative decline in the incidence rate of fracture incidence with longer duration of treatment (6).

GCs reduce bone mineral density by increasing the activity of osteoclasts and decreasing the activity of osteoblasts and osteocytes. Impaired bone formation and increased bone resorption seem be the main mechanisms underlying GC-induced bone loss. Clinical patients on GC treatment often have inflammation-related diseases that can impact the effects of GC on bone cells and the progression of OP and the effects of GC on bone cells (7). Furthermore, GCs have effects on various physiological factors, including muscle strength, calcium and vitamin D metabolism, fat metabolism, and sex steroid levels (7).

Various clinical trials and population studies demonstrate that oral bisphosphonates are associated with a significant reduction in the risk of fractures, especially the risk of vertebral fracture in patients on GCs (8). Zoledronic acid and denosumab are also effective in maintaining bone mineral density, both of which are superior to risedronic acid (9). In patients with very high risk of fracture on GC (eg, patients with vertebral fracture) treatment with teriparatide is justified (1).

The objective of our study was to analyze the prophylaxis of GC-induced OP at high doses in clinical practice.

PATIENTS AND METHODS

We conducted a cross-sectional observational study in which OP prophylaxis was assessed in patients treated with GCs at high doses.

The study subjects were selected from the verified electronic prescription dispensation records of the health system throughout 2022 on the island of Gran Canaria (Canary Islands, Spain). In 2022, the island had a total population of 853,262 inhabitants, 338,830 of them older than 50 years.

The following inclusion criteria were applied:
- Age ≥ 50 years.
- Dispensing by a pharmacy of 90 or > 30 mg prednisone tablets.

For each patient, the following variables were collected from the patients' health records:
- Age.
- Sex.
- Underlying reason for the use of high-doses GC.
- The patient's health care area (Gran Canaria is geographically divided into 2 health care areas, North and South).
- Number of packages of prednisone 30 mg (30 tablets) obtained from the pharmacy.
- Any concomitant prescription of oral bisphosphonate (alendronic acid, risedronic acid, ibadronic acid) or denosumab during the period analyzed.

STATISTICAL ANALYSIS

Descriptive statistical analyses were performed, in addition to a bivariate analysis, examining the associations

between the prescription of OP prophylaxis with the pharmacy-dispensed GCs according to sex, age, body system involved and health area. For qualitative variables, contingency tables and the Man-Whitney U test were used. For quantitative variables the Student's t test for unpaired samples was used. Variables with a statistical significance ($p < 0.05$) were included in a binary logistic regression model using IBM® SPSS version 27.

RESULTS

A total of 630 patients were evaluated, 203 of which were excluded due to a lack of data or an inability to access the health history. Therefore, the final sample included a total of 427 patients, 218 women (51.5 %) and 209 men (48.9 %).

By health areas, 238 patients corresponded to the Northern area (55.7 %), and 189 to the Southern area (44.3 %). The mean age was 66.5 years (SD, 10.5; range 50-93), 65.4 for the Northern area and 66.1 for the Southern area ($p = 0.1$). The percentage of women was similar in the Southern area (53.9 %) vs the Northern area (48.7 %; $p = 0.28$).

The distribution of disease groups is shown in table I, with respiratory diseases predominating (46 %); these included asthma ($n = 129$), chronic obstructive pulmonary disease (COPD) ($n = 53$) and interstitial lung disease ($n = 18$). Of the 427 patients, 256 (59.9 %) had been diagnosed before the study period, whereas 171 (40.0 %) received the diagnosis during the study year.

Table I. Distribution of patients by disease type and health area

	Health area		Total
	Nord 238	South 189	*n* 427
Respiratory	96 (46.3)	104 (55.0)*	200 (46.8)
Dermatologic	31 (13)**	12 (6.3)	43 (10.1)
Rheumatic	25 (10.5)	16 (8.4)	41 (9.6)
Neurologic	21 (8.8)	16 (8.4)	37 (8.7)
Digestive	14 (5.9)	15 (7.9)	29 (6.8)
Allergic	15 (6.3)	10 (5.2)	25 (5.9)
Hematologic	17 (7.1)	6 (3.2)	23 (5.4)
Ophthalmic	4 (1.6)	6 (3.2)	10 (2.3)
Others	15 (4.2)	4 (2.1)	19 (4.4)

*Data express n (%). *p = 0.002; **p = 0.02.*

OP prophylaxis was prescribed in 59 cases (13.8 %), corresponding to 39 women (17.8 %) and 20 men (9.5 %) ($p = 0.012$). Figure 1 illustrates prophylaxis by decade of age. The difference in percentage of OP prophylaxis in those > and < 70 years was statistically significant (21.6 % vs 8.7 %; $p < 0.001$).

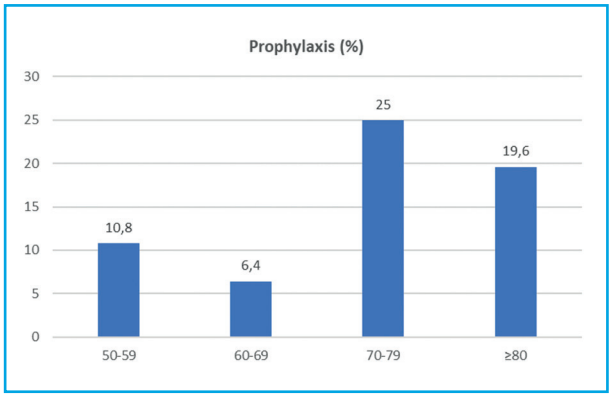

Figure 1. Osteoporosis prophylaxis by decade of age.

Table II illustrates the most significant differences between patients who received OP prophylaxis and those who did not. Patients prescribed OP prophylaxis were older, predominantly women and had undergone bone densitometry testing. When analyzing OP prophylaxis according to the dispensing of prednisone (30 mg packages), the group that had received > 4 packages had been prescribed prophylaxis in 35 % of cases vs 10.0 % of those who obtained < 4 packages ($p = 0.002$).

There were no significant differences in OP prophylaxis depending on the health area or date of diagnosis of the body system involved (12.8 % before the study period vs 15.2 % during the study period).

The diseases with the highest percentage of OP prophylaxis were rheumatic (39 %) and neurological (32 %) (Fig. 2). The rheumatic and neurological diseases with the highest percentage of prophylaxis were vasculitis (66 %) and myasthenia gravis (35 %).

Respiratory, dermatologic, hematologic and digestive patients received prophylaxis at rates ranging between 8 % and 13 %. Among respiratory causes, we observed that prophylaxis was prescribed more frequently for interstitial lung disease (22 %) than for asthma or COPD (7 %) ($p < 0.03$). In the cases of asthma and COPD, there were no differences in prophylaxis rates between patients who had received > 4 packages of prednisone from the pharmacy vs 3-4 packages (8.7 % vs 7 %). The diseases with the lowest percentage of prophylaxis were allergic and ophthalmologic, both measuring 0 %. The difference in

Table II. Characteristics of patients who received osteoporosis prophylaxis vs those who did not

	OP prophylaxis	No OP prophylaxis	*p*	Multivariant
	n = 59	*n* = 368		OR (95 %CI)
Age, mean (SD)	69.5 (11.5)	65.1 (10.2)	0.018	
> 70 years	35 (59.3)	115 (31.2)	< 0.001	4.23 (2.11-8.49)
Sex (women), *n* (%)	39 (66.1)	183 (49.7)	0.012	3.15 (1.47-6.74)
Health area				
North	36 (15.1)	202 (84.9)	0.37	
South	23 (12.2)	166 (87.8)		
Rheumatic disease	16 (27.1)	25 (6.7)	< 0.001	5.33 (2.53-11.23)
Neurologic disease	12 (20.3)	25 (6.7)		
Packages of prednisone dispensed				
mean (SD); median	5.8 (3.8); 5	4.7 (2.6); 4	0.03	2.31 (1.14-4.70)
> 4 packages	28 (47.4)	51 (13.8)	0.002	
Bone densitometry, *n* (%)*				
At any time	31 (53.4)	57 (28.3)	< 0.001	1.86 (0.92-3.74)
During the study period	25 (43.1)	31 (15.4)	< 0.001	3.55 (1.66-7.57)

**Available for 258 patients.*

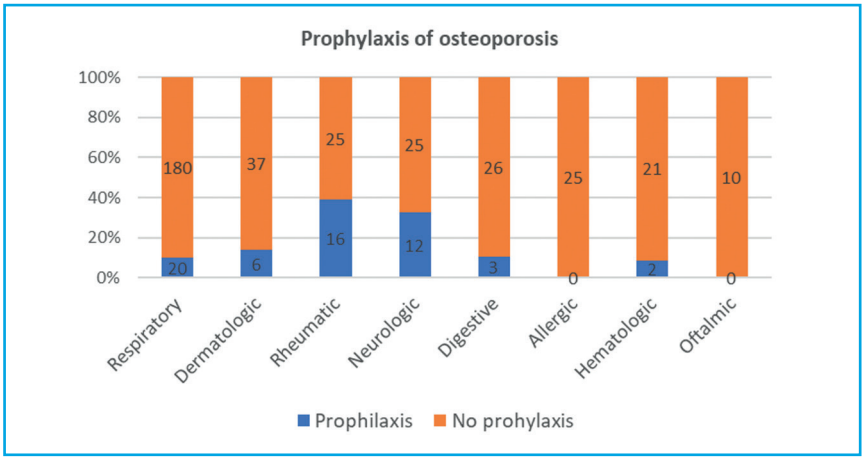

Figure 2. Osteoporosis prophylaxis based on the body system involved. The numbers represent the number of patients, and the size of the bars the percentage of prophylaxis.

prophylaxis rates for rheumatic and neurological diseases vs the other groups was statistically significant ($p < 0.001$). Prophylaxis for rheumatic diseases was higher in the Northern (52.3 %) than in the Southern health area (21.4 %) ($p = 0.08$). Seven out of every 10 women aged > 70 with a rheumatic or neurologic disease were prescribed prophylaxis vs 5 out of every 100 women younger than 70 with other diseases.

In a logistic regression analysis in which the dependent variable was a prescription of prophylaxis for OP and the independent variables were age, sex, the body system involved (rheumatic and neurologic vs others), the performance of bone densitometry and the dispensation of prednisone packages (> 4 vs < 4), the following results were obtained (Table II): all variables were independently associated with the dispensation of OP prophylaxis, with an OR of 5.33 for the underlying rheumatic or neurologic disease and an OR of 4.2 for age > 70 years. When asthma/COPD cases were excluded from the multivariate analysis, all results remained significant with an OR of 3.22 (IC95 %, 1.54-6.72; $p = 0.002$) for rheumatic or neurological diseases [OR, 2.99 (IC95 %, 1.48-6.02; $p = 0.002$)] for age older than

70 years and an OR of 2.89 (IC95 %, 1.42-5.89; $p = 0.003$) for dispensation of > 4 packages of prednisone.

Bone densitometry, performed in 48 patients, was requested in 87 % of cases by a hospital specialty and in 12.5 % of cases by primary care physicians.

Treatment for OP consisted of risedronic acid for 23 patients (38.9 %), followed by alendronic acid for 21 (35.5 %), denosumab for 12 (20.3 %), and ibadronic acid for 3 (5 %). Overall, 37 of the 59 patients (79.6 %) on OP prophylaxis received an oral bisphosphonate. OP prophylaxis dispensations were administered by hospital specialties (87 %) and the GP (13 %). The dispensation of OP prophylaxis was initiated during GC treatments in 26 cases and before 2022 in 33 patients.

DISCUSSION

In addition to healthy lifestyle habits, such as a dairy products-rich diet, regular physical exercise and smoking cessation, patients on high-dose GCs are the primary candidates for co-prescription of a bisphosphonate. As far as we know, our study is the first to specifically focus on OP prophylaxis in patients on high-dose GCs. The results show that prophylaxis of GC-induced OP remains very low (13 %) and clearly does not comply with current recommendations. An approximate estimate based on the age of the patients (without having a FRAX risk scale) would be that between 75 % (ACR) and 100 % (SEIOMM, SER) of patients would be eligible for prophylaxis (1-3). Moreover, management guidelines emphasize the importance of sparing GCs for when indicated and at the lowest possible dose, even with immunosuppressants as GC-sparers, if necessary (3,11).

Therefore, there is a gap between clinical practice guidelines on GC-induced prophylaxis and their effective application. In our study, although the highest percentage of prophylaxis was found in rheumatic diseases (vasculitis, lupus nephropathy, etc.) it did not reach 50 % of patients. In our study, the profile of patients with prophylaxis was that of a woman older than 70 years with a rheumatic or neurological disease. Thus, male patients or those with other conditions younger than 70 years received prophylaxis at very low rates. The snapshot generated by our analysis is very informative in nature to encourage the implementation of training sessions aimed at those specialties that use high doses of GCs in the management of their patients.

Our findings are consistent with other studies. Albaum et al. conducted a systematic review, identifying 29 published studies, and found that < 40 % of patients who chronically used GCs (at different doses) received prophylaxis with calcium, vitamin D or bisphosphonates (11). Thus, in one of the studies with 17,736 patients on chronic GCs, a third with ≥ 10 mg/d of prednisone, the authors found that only 22 % of the new prescriptions included prophylaxis for OP (27 % in the case of patients aged ≥ 70) (12). Just as we observed, this Canadian study found that the patients most likely to receive prophylaxis were women older than 60 years treated by rheumatologists (12). A different registry study conducted in France with 32,812 patients who received, at least, 7.5 mg/d of prednisone for, at least, 3 months, reported that only 8 % underwent bone densitometry and only 12 % had OP prophylaxis with bisphosphonates. Prophylaxis was independently associated with female sex, age older than 55 years, a prescription of GC(s) by a rheumatologist, autoimmune disease, and an order for a bone densitometry (13).

Focusing on local data, a Spanish multicenter study that evaluated OP prophylaxis with GCs in patients with polymyalgia rheumatica (14) found that 69 % of cases underwent densitometry and 46 % were prescribed a bisphosphonate.

Our study has some strengths, such as its sample size, the reliability of the electronic dispensation data in terms of medication dispensed (not just indicated), the sample based in a well-defined territory and data drawn from real-world clinical practice. However, it does have some limitations. It was not possible to precisely identify whether the initial GC prescription was made by the GP or a hospital doctor, in some cases due to lack of information in the health record or electronic prescription. A different limitation to consider is that patients with asthma/COPD may use GCs occasionally during periods of disease exacerbations. Moreover, it is not reliably documented whether patients received uninterrupted treatment for longer than 3 months. However, this does not invalidate our results; patients with asthma who obtained 3 packages from the pharmacy received prophylaxis in 4 % of cases, while those who received > 7 packages benefited from prophylaxis in 10 % of cases. The results indicate that in the respiratory field there is no adequate awareness of the risks of GC, which underscores the need for specific training, especially because respiratory diseases remain the most prevalent disease in our series. The dispensation of zoledronic acid or teriparatide was not assessed in this study. These are infrequently used treatments; in fact, either drug is not indicated as a primary prevention method.

Computer aids such as the health history or electronic prescription alerts when high doses of GCs and other drugs are prescribed could be helpful in enhancing OP prophylaxis (15).

In conclusion, OP prophylaxis in patients on high-dose GCs remains very low; based on our results, specific

training across multiple medical disciplines is highly warranted, both in hospital specialties and in primary care, especially in respiratory diseases, allergology and ophthalmology. Another important aspect that, based on the findings of this study, is to widely disseminate the results in order to sensitize doctors not only to the risks of fracture when prescribing high doses of GCs, but also to the availability of effective treatments for its prevention (8). The implementation of alerts in electronic prescriptions when high doses of GCs have been prescribed should be studied in greater detail as a tool for facilitating OP prophylaxis.

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Original

Predictors of early and late recovery in post-thyroidectomy transient hypoparathyroidism

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Abstract

Purpose: to analyze predictors of early, intermediate, late remission of transient hypoparathyroidism after total thyroidectomy.

Methods: we conducted a multicenter, retrospective, observational study of individuals who developed postoperative transient hypoparathyroidism.

Results: a total of 164 patients with postoperative transient hypoparathyroidism were analyzed. Thyroidectomy was performed in 56 % for benign disease and 44 % for suspected malignancy. Hypoparathyroidism remission occurred early (< 3 months) in 47 % of patients, intermediate (3-6 months) in 23.7 %, and late (> 6 months) in 29.3 %. No differences were found across 3 groups regarding preoperative PTH levels, PTH 24 hours after surgery, or the percentage of PTH decrease. However, we observed higher calcium values 24 hours after surgery, and higher serum calcium and PTH levels at the first outpatient appointment (2 weeks after discharge) in patients with early remission of hypoparathyroidism. In patients with late remission of hypoparathyroidism, a past medical history of surgery for malignancy, the presence of undetectable PTH levels, lower serum calcium levels 24 hours after surgery, and the need for IV calcium treatment were remarkable ($p < 0.05$). This group had lower PTH levels in their 1st and 2nd outpatient visits, and lower serum calcium levels on the 2nd visit ($p < 0.05$).

Conclusions: serum calcium and PTH levels measured 2 weeks after discharge can predict early recovery. Late remission was more common in patients undergoing thyroidectomy for suspected malignancy, those with undetectable PTH 24 hours after surgery, and those requiring IV calcium. Although calcium levels 24 hours after surgery could probably predict hypoparathyroidism remission time, the absence of a unique protocol to guide management at hospitalization does not allow us to draw robust conclusions.

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INTRODUCTION

Calcium homeostasis is a complex process mainly regulated by parathyroid hormone (PTH) and vitamin D, with PTH being the major regulator of calcemia (1,2). Hypoparathyroidism is a disorder of mineral metabolism characterized by hypocalcemia and absent or deficient production of PTH (3).

Surgical hypoparathyroidism occurs after inadvertent trauma, devascularization, or removal of the parathyroid glands during neck surgery. In fact, anterior neck surgery is the most common cause of acquired hypoparathyroidism, especially after bilateral thyroid surgery (4). Postoperative hypoparathyroidism can be transient or permanent. Permanent hypoparathyroidism is most commonly defined as the failure of the parathyroid gland to return to normal function 12 months after surgery (5). The ability to anticipate transient as opposed to permanent hypoparathyroidism after thyroidectomy requires further investigation. Furthermore, since transient hypoparathyroidism requires frequent biochemical monitoring for tapering or discontinuation of calcium and calcitriol due to risk of hypercalcemia and potential kidney damage, the identification of predicting remission parameters is needed to improve follow-up (6,7).

Although numerous authors have sought to predict the development of postoperative hypocalcemia with increasing accuracy, to our knowledge, parameters that predict the timing of recovery in transient hypoparathyroidism have not been studied. The objective of this retrospective, multicenter observational study was to identify predictors of early, intermediate, and late remission of hypoparathyroidism following total thyroidectomy.

MATERIALS AND METHODS

This retrospective analysis enrolled 164 patients older than 18 years from different hospitals in the Community of Madrid (Spain) with experience in thyroid surgery, who underwent total thyroidectomy and developed transient hypoparathyroidism. Remission of hypoparathyroidism was defined as the discontinuation of substitutive treatment and categorized as "early" (< 3 months), "intermediate" (3-6 months), or "late" (> 6 months after surgery). Indications for surgery were thyroidectomy for benign nodular disease; thyroidectomy for Graves´ disease; thyroidectomy for malignant disease; combined thyroid and parathyroid surgery; and re-intervention for thyroidectomy totalization. We included combined thyroid and parathyroid procedures as a single group, as preexisting hyperparathyroidism may alter postoperative calcium metabolism, likely through the influence of hungry bone syndrome.

We included sociodemographic and clinical data (sex, age, presence of obesity, treatment with vitamin D or thiazide). Data on the surgical procedure, changes in parathyroid function, and the management of hypoparathyroidism were reviewed. Intact PTH, alkaline phosphatase, and vitamin D were preoperatively assessed. Serum PTH and calcium levels (albumin-adjusted calcium), and phosphatemia were evaluated at 24, 48, and 72 hours, at the 1st outpatient visit after discharge, the 2nd visit after discharge, and the visit of recovery. Since several hospitals were involved in the study, there was not a unique follow-up protocol: on average the 1st follow-up visit was performed 2 weeks after discharge and the 2nd, 4-8 weeks after discharge. Visits between 4 and 8 weeks, as well as those at the time of recovery, were not systematically recorded but were conducted at the discretion of the treating physician, following the recommendations of the I International Conference on the Management of Hypoparathyroidism (3), which was the prevailing guideline at that time. We also included as treatment data the need for IV calcium or magnesium in the immediate postoperative period. Therapy at discharge included oral calcium carbonate and calcitriol, according to the physician´s clinical criteria, same as the treatment withdrawal.

The descriptive and statistical analysis was performed using the Statistical Package software for Social Sciences 24.0 (SPSS). Qualitative variables are presented with their frequency distribution. Quantitative variables are expressed as mean. Quantitative variables showing an asymmetric distribution are expressed as median. A comparison of sociodemographic, clinical, surgical, and laboratory data based on the time of remission was conducted as well. The association between qualitative variables was assessed using the chi-square test or Fisher's exact test, if > 25 % of the expected values were < 5. For quantitative variables, means were compared using a two-tailed Student's t-test or the Mann-Whitney U test if quantitative variables did not follow normal distribution.

RESULTS

A total of 164 patients were included. There were 144 women (87.8 %) and 20 men (12.2 %) with a mean age of 51 years (range 18-89), and mean vitamin D levels of 23 mg/mL and 17.7 % having received previous vitamin D treatment. A total of 5.5 % of patients were on thiazides. Most patients (72, 43.9 %) underwent a total thyroidectomy for suspected malignancy; 61 (37.2 %) patients for benign nodular disease and 21 (12 %) patients for Graves´ disease. In addition, 6 patients (3.7 %) underwent combined thyroid and parathyroid surgery, and 4 patients (2.4 %) required reoperation for completion thyroidectomy (Table I).

Table I. Patient characteristics and indications for surgery

	Early remission (n = 77)	Intermediate remission (n = 39)	Late remission (n = 48)
Patient characteristics			
Patient age at surgery, mean ± SD	54 ± 15*	46 ± 14*	50 ± 15
Sex n (%)			
Male	9 (12)	4 (10)	7 (15)
Female	68 (88)	35 (90)	41 (85)
Indication for surgery n (%)			
Malignant disease	30 (39)	13 (33)	29† (60)
Benign nodular disease	33 (43)	15 (38)	13 (27)
Graves´ disease	7 (9)	8 (21)	6 (3)
Combined thyroid and parathyroid surgery	5 (6)	1 (3)	0
Completion thyroidectomy	2 (3)	2 (5)	0

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

Hypoparathyroidism remission was early in 47 % of patients, intermediate in 23.7 % and late in 29.3 %. Although no sex-related differences were observed, age-related differences at the time of surgery were identified in the early (p = 0.02) and intermediate (p = 0.021) groups compared with the remaining patients. There were no differences in clinical parameters such as obesity, presence of low vitamin D, serum alkaline phosphatase, or previous treatment with vitamin D or thiazides before surgery. In our study, malignant disease was associated with late recovery (p = 0.003). Regarding biochemical parameters, no differences were found across the 3 groups regarding preoperative PTH values (55 pg/mL, 52 pg/mL, and 53 pg/mL); PTH 24 hours after surgery (10 pg/mL, 9 pg/mL, and 9.5 pg/mL), or the median percentage of PTH decrease (82 %, 80.5 %, and 83 %).

As shown in table II; patients from the early remission hypoparathyroidism group had significantly higher serum calcium levels 24 hours after surgery (8.4 mg/dL, p = 0.001), and only a small proportion of patients had undetectable PTH 24 hours after surgery (6.6 %, p = 0.020) vs other patients. In this group, only 9.2 % (p = 0.000) of patients required IV calcium and 0 % required magnesium. In addition, at the 1st visit, these patients had mean calcium levels of 9.3 mg/dL (p = 0.010) and median PTH levels of 20 pg/mL (p = 0.039), indicating early resolution of hypoparathyroidism and allowing discontinuation of calcium and calcitriol treatment.

In contrast, in patients with late resolution of hypoparathyroidism, calcium levels were < 7.9 mg/dL (p = 0.000) 24 hours after surgery; and 25 % of these patients had undetectable PTH (p = 0.003). In the postoperative period, 43.8 % (p = 0.000) of patients required IV calcium and 6.3 % magnesium. Calcium levels were lower, especially at the 2nd visit (8.8 mg/dL, p = 0.019), and PTH levels were lower at the 1st visit (12 pg/mL, p = 0.000) remaining lower in the 2nd visit (21 pg/mL, p = 0.014). Regarding surgical parameters, 60.4 % of patients in late remission (p = 0.012) underwent total thyroidectomy for malignant disease. After a median 11 months the remission of hypoparathyroidism occurred.

Due to several missing values, phosphorus levels were not analyzed.

Receiver operating characteristic (ROC) curve analysis showed that the only parameter with an adequate area under the curve (AUC) was serum calcium at 24 hours after surgery. The AUC for detecting early hypoparathyroidism remission using 24-hour postoperative serum calcium was 0.645, with the best cutoff value of 8.22 mg/dL (sensitivity, 56 %; specificity, 32 %; positive predictive value, 0.6) (Fig. 1).

DISCUSSION

Hypoparathyroidism is the most common complication of thyroidectomy. Its frequency, if transient, can reach up to 46 %, and, if permanent, up to 3 % in literature (8,9). Early prediction of postoperative hypocalcemia is critical to initiate treatment and avoid potentially life-threatening complications; however,

Table II. Serum PTH, calcium, alkaline phosphatase, vitamin D levels and postoperative treatment

	Early remission (n = 77)	Intermediate remission (n = 39)	Late remission (n = 48)
PTH levels			
Preoperative (pg/mL)	55 (44-75)	52 (40-68)	53 (45-65)
Postoperative (pg/mL)	10 (5-12)	9 (7-12)	9.5 (5-13)
Median % of PTH decrease	82 (77-92)	80.5 (75-90)	85 (80-91)
Patients with undetectable postoperative PTH (%)	5 (6.6)*	4 (10.8)	12 (25)†
1st visit (pg/mL)	20 (12-34)*	19 (15-34)	12 (8-18)‡
2nd visit (pg/mL)	31.5 (20-48)	31.5 (18-41)	21 (12-34)*
Alcaline phosphatase (U/L)	74.4 (63-88)*	71 (56-78)	68.1 (42-76)*
Vitamin D (pg/mL)	23 (14-30)	19 (16-25)	27.5 (20-36)
Calcemia (mg/dL)			
24 after surgery	8.4 ± 0.8†	8.1 ± 0.7	7.9 ± 0.6‡
48 after surgery	8.1 ± 0.9	8.2 ± 0.7	8.2 ± 0.6
72 after surgery	8.3 ± 0.8	8.6± 0.8	8.3 ± 0.7
1st visit	9.3 ± 0.7*	9.1± 0.4	9.3 ± 0.5
2nd visit	9.2 ± 0.9	9.2± 0.4	8.8 ± 1.2*
Treatment			
Treatment with IV Ca (%)	7 (9.2)‡	9 (23.1)	21 (43.8)‡
Treatment with Mg (%)	0 (0)	2 (5.1)	3 (6.3)

**p < 0.05, †p < 0.01, ‡p < 0.001. Data are expressed as mean ± SD or median (IQR) range depending on the type or the data distribution.*

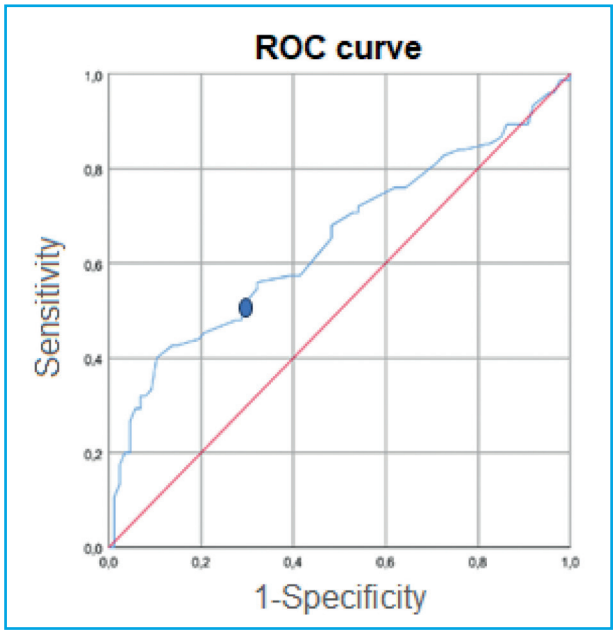

Figure 1. Receiver operating characteristic (ROC) curve analysis, serum calcium levels at 24 hours.

this treatment must be closely monitored since resolution of hypoparathyroidism should be accompanied by a discontinuation of calcium and calcitriol to prevent hypercalcemia (10).

Type and extent of surgery, operative technique, surgeon expertise and cause of disease contribute to the risk of surgical hypoparathyroidism (11-13) and probably to the length of the recovery period. In our study, patients who underwent total thyroidectomy for malignant disease more frequently experienced later remission compared with those who underwent thyroidectomy for other causes, possibly due to a more aggressive surgical approach. Because of the multicentric design of the study, we could not compare surgeon expertise as a predictor of time of remission.

PTH has been shown to be a useful tool in predicting postoperative hypoparathyroidism. Since calcium trends often require sampling over a period of 12 to 24 hours or longer, PTH seems to be a more reliable parameter (12). Although several studies have attempted to validate different PTH thresholds and combinations of PTH and calcium, results have not been consistent (14-18).

The percentage decrease in PTH levels has also been evaluated as a predictor of hypocalcemia. In our series, the median decrease in PTH was of 84.9 %, which is similar to the decline reported by other authors in patients developing hypoparathyroidism. According to clinical practice guidelines (19) postoperative PTH can be used to predict patients who will not develop permanent postoperative hypoparathyroidism. The development of permanent hypoparathyroidism is unlikely when PTH values exceed 10 pg/mL within 12 to 24 hours after surgery; thus, long-term treatment with active vitamin D and calcium supplements beyond the recommended daily allowance is generally unnecessary. Furthermore, according to Yao et al. (20) 3-64 % of patients with PTH values < 10 pg/mL 12-24 hours after surgery may still recover from temporary hypoparathyroidism. In our study the mean levels of PTH 24 h after surgery were 9 pg/mL, while the median levels were 11 pg/mL, thus supporting guidelines assertion. Although we evaluated these parameters as predictors of time of recovery, we did not find significant differences between preoperative PTH, PTH 24 hours after surgery or in the percentage of PTH decrease. Although the use of different PTH assays across centers could explain the lack of significance, it should not affect the percentage of decrease. We found that higher levels of PTH and calcium in the 1st visit (2 weeks after surgery) could predict early remission of hypoparathyroidism while lower PTH levels were associated with late remission. Regarding serum calcium levels at 24, 48 and 72 hours, the absence of a unique protocol to guide management at hospitalization could have influenced these levels, mainly at 48 and 72 hours and, in some cases, at 24 hours. Our results suggest that serum calcium levels 24 hours after surgery could probably predict hypoparathyroidism remission time, since ROC analysis suggested that calcium levels > 8.2 mg/dL 24 hours after surgery are associated with early remission of hypoparathyroidism. In addition, although were found differences between early and late groups, we cannot draw robust conclusions on this regard.

Moreover, we tried to analyze phosphorus levels, but there were considerable missing values, so results turned out biased. Phosphate has gained interest in the evaluation of PTH function, not only for distinguishing hungry bone disease from hypocalcemia due to hypoparathyroidism in the early postoperative period (21) but also as an indicator of the severity of PTH deficiency. In a study of patients with PTH levels < 10 pg/mL on the first postoperative day, serum phosphorus concentration was identified as an independent factor influencing recovery of PTH to normal levels within the first week after surgery (22). Since fibroblast growth factor 23 (FGF23) was not available in routine biochemical test in several hospitals, this value was not considered in this study. As far as we know, only 1 study has ever evaluated FGF23 in postoperative hypoparathyroidism. In this study, the authors found a significant positive correlation between serum phosphate and FGF23 levels. Serum FGF23 was elevated in patients with hypoparathyroidism and hyperphosphatemia and normalized along with normalized phosphate levels after recovery of parathyroid function. The peak level of phosphate always preceded that of FGF23 by several days, suggesting that elevated phosphate is a primary stimulus for FGF23 release (23), which could explain the fact that phosphate changes appear to be slower than that of serum calcium, whose change is rapidly corrected within minutes.

The strength of this study lies in the multicenter recruitment of patients, the number of enrolled patients and the point that it represents real clinical practice involving 2nd and 3rd-level hospitals (Table III). Furthermore, since our study is focused on transient hypoparathyroidism, we believe that our conclusions could bring understanding to the recovery process of parathyroid function. On the other hand, a limitation of our study is that evaluating a more homogeneous population might have yielded more precise data; however, our goal was to provide a broader perspective that encompasses different clinical settings. We did not consider either the effect that the treatment with Iodine-131 had in patients treated due to malignant disease, which could be of interest since it has been described a prolonged recovery of parathyroid function of patients with thyroid cancer and treatment with Iodine-131 treatment after surgery (24). Additionally, we did not include data on lymph nodes dissection, which influences the extent of surgery.

In conclusion, the definition of permanent hypoparathyroidism remains controversial. Since in most cases, parathyroid dysfunction after thyroidectomy resolves a few months after surgery, several authors consider that if parathyroid disfunction persists over 6 months (3), hypoparathyroidism should be considered as permanent. In our study almost 70 % of the patients had remission of hypoparathyroidism within the first 6 months (early 47 %, intermediate 23.7 %) but after that period, a significant percentage of patients recovered the parathyroid function (29.3 %), supporting the recommendation of the II International Workshop for the Evaluation and Management of Hypoparathyroidism (19) to diagnose permanent hypoparathyroidism if the condition persists > 12 months after surgery.

Table III. No. of patients included per hospital

Medical center	No. of patients included *n*; (%)
Hospital Universitario Clínico San Carlos	32 (23)
Hospital General Universitario Gregorio Marañón	39 (27)
Hospita Universitario La Paz	18 (12)
Hospital Universitario Ramón y Cajal	27 (18)
Hospital Universitario Santa Cristina	15 (10)
Hospital Universitario Infanta Leonor	15 (10)

CONCLUSIONS

Two weeks after discharge, serum calcium and PTH levels can predict early recovery. Late remission was more common in individuals undergoing thyroidectomy for suspected malignancy, those with undetectable PTH 24 hours after surgery, and those requiring IV calcium.

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Original

A scoping review on the safety, efficacy, and effectiveness profile of different antiresorptives for the management of patients with secondary osteoporosis due to transplants

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Abstract

Background: transplant-induced osteoporosis is a frequent metabolic complication influenced by chronic glucocorticoid use, pretransplant comorbidities, and immunosuppressive regimens. Its management is complex due to pre-existing bone loss and a high risk of fractures, which vary depending on the type of transplant and postoperative period. All previously published studies investigating bone disease in transplant populations, regardless of the organ, are limited in size and none of them have robust data regarding the effectiveness of osteoporosis medications in reducing fracture risk.

Objective: to synthesize current evidence on the safety, efficacy, and effectiveness profile of pharmacological therapies used in transplant-induced osteoporosis, identifying knowledge gaps and areas for future research.

Methods: following JBI scoping guidelines, we included studies of adult patients with transplant-induced osteoporosis on bisphosphonates, denosumab, and dual-action sclerostin-targeting monoclonal antibodies that both prevent bone loss and stimulate new bone formation, among other therapies. This review included adult transplant recipients treated with bisphosphonates, RANK-ligand inhibitors (denosumab), and dual-mechanism monoclonal antibodies against sclerostin—agents that not only inhibit osteoclastic bone resorption but also actively promote osteoblastic bone formation—alongside other pharmacotherapies. Efficacy was assessed based on fracture risk reduction and BMD improvement, effectiveness in real-world clinical practice, and safety through adverse event incidence. A total of 24 studies on efficacy, 3 on effectiveness, 1 on safety, and 4 evaluating both safety and efficacy were included.

Results: a total of 24 studies on transplant-induced osteoporosis were analyzed. Among bisphosphonates, pamidronate increased lumbar spine BMD (+8.8 %, $p < 0.015$) and femoral BMD (+8.2 %, $p = 0.01$), while alendronate improved lumbar BMD (+4.2 %, $p < 0.0001$). Ibandronate increased total femur BMD (+1.3 %, $p = 0.01$) and distal radius BMD (+0.6 %, $p = 0.039$). Denosumab significantly improved hip BMD (+0.56 g/cm^2, $p = 0.02$) and spine BMD (+0.79 g/cm^2, $p = 0.01$). In terms of safety, pamidronate was well tolerated, with mild hypocalcemia in 8.6 % of cases. Alendronate was associated with dyspepsia in 15 % of patients, while denosumab showed no severe adverse effects. Regarding clinical effectiveness, ibandronate reduced fracture rates (7.4 % vs. 25.8 %, $p = 0.04$).

Conclusion: although bisphosphonates and denosumab are effective in improving BMD, their impact on fracture reduction is variable. The heterogeneity of studies and short follow-up periods limit the generalizability of results. Although the safety profile of these treatments is generally favorable, additional studies are needed to assess long-term effectiveness and outcomes in underrepresented populations, such as lung and intestinal transplant recipients.

Keywords:
Transplant-induced osteoporosis. Bisphosphonates. Denosumab. Bone mineral density. Bone fractures.

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INTRODUCTION

Transplant-induced osteoporosis is a complex metabolic condition frequently associated with chronic glucocorticoid use and factors related to pre-transplant end-stage diseases. While it shares certain characteristics with glucocorticoid-induced osteoporosis (GIOP), this condition is chronic, irreversible, and influenced by a combination of factors, such as pre-existing bone loss, immunosuppressive regimens, and transplant-associated comorbidities (1). Fractures are a common complication in this population, with incidence rates varying depending on the type of transplant and the postoperative period. For instance, in heart and liver transplants, lumbar spine bone mineral density (BMD) may recover over time, whereas fractures are more prevalent during the first years after transplantation (2-5). In kidney transplants, fractures occur more frequently at appendicular sites, related to persistent hyperparathyroidism and cortical and trabecular bone loss.

Regarding bone loss patterns, the first 6 to 12 months post-transplant represent a critical period for BMD reduction. For example, in heart transplants, trabecular bone loss may exceed 6 % in the spine and femoral neck within the first year, later stabilizing with maintenance doses of glucocorticoids. In liver transplants, fractures are common on year 1 (21 %) and may reach 33 % by the year 4 (1).

The pharmacological management of transplant-associated osteoporosis faces multiple challenges, partly due to variability in therapeutic responses. While both IV and oral bisphosphonates have demonstrated efficacy in improving BMD (6), adynamic bone disease remains a major concern. Denosumab has emerged as a promising option, with studies reporting significant increases in hip and spine BMD, along with a sustained reduction in bone turnover markers (7).

This agent may also be beneficial in hematopoietic stem cell transplants, where bone loss is more pronounced at the femoral neck, and fracture rates are significantly higher than in the general population (8,9). However, the available evidence remains limited, particularly in specific populations such as lung and intestinal transplants, where osteoporosis and fracture rates are particularly high (10,11).

This review aims to synthesize the current evidence on the safety, efficacy, and effectiveness profile of different antiresorptive therapies for transplant-associated osteoporosis, identifying knowledge gaps and areas for future research.

METHODS

This scoping review was conducted in full compliance with the Joanna Briggs Institute (JBI) protocol for scoping reviews (12).

POPULATION, CONCEPT, CONTEXT

We applied the PCC framework. The Population included adults (≥ 18 years) diagnosed with transplant-associated osteoporosis (T-score ≤ -2.5) with or without fractures on pharmacological therapies. The Concept included 3 domains: efficacy (trial-condition BMD gains and fracture risk reduction at 12 and 24 months), effectiveness (real-world fracture incidence and BMD changes), and safety (frequency and severity of treatment-related adverse events). The Context spanned hospitalized, emergency, and outpatient settings worldwide, across all ages, sexes, and cultures.

ELIGIBILITY CRITERIA

We included only prospective controlled clinical trials—randomized or nonrandomized with parallel or crossover designs—published from database inception through April 30, 2025. Eligible interventions encompassed:

- *Antiresorptive agents:* bisphosphonates (pamidronate, alendronate, etidronate, zoledronate, ibandronate).
- *RANK-ligand inhibition:* denosumab.
- *Dual-action sclerostin inhibitors:* monoclonal antibodies targeting sclerostin, recognized for their combined antiresorptive and anabolic effects on bone.
- *Selective estrogen receptor modulators:* estradiol and pyridine derivatives.

Anabolic drugs (eg, parathyroid hormone analogs) were explicitly excluded, as no prospective controlled trials of these agents in transplant-associated osteoporosis were identified. Studies were required to confirm osteoporosis by densitometry (T-score ≤ -2.5) and include a comparator arm (placebo, calcium ± vitamin D, or active comparator).

INFORMATION SOURCES AND SEARCH STRATEGY

We searched across Medline (via PubMed), Embase, Cochrane CENTRAL, ClinicalTrials.gov, Scopus, Web of Science Core Collection, Google Scholar, and OpenGrey from inception all the way through April 30th, 2025. No language or publication-date limits were applied. Key terms were: Osteoporosis OR "bone loss" AND Transplantation OR graft AND Drug Therapy OR pharmacotherapy OR medication OR drugs.

All references were imported into Rayyan (2016) for duplicate removal and screening.

("Osteoporosis"[MeSH] OR osteoporosis[tiab] OR "bone loss"[tiab]) AND ("Transplantation"[MeSH] OR transplant[tiab] OR graft[tiab]) AND ("Drug Therapy"[MeSH] OR pharmacotherapy[tiab] OR medication[tiab] OR drugs[tiab]).

Embase and Lilacs strategies were analogous, using their respective subject headings and title/abstract fields.

STUDY SELECTION

Two reviewers (JP, GT) independently screened titles and abstracts in Rayyan, then assessed full texts against inclusion criteria. Discrepancies were resolved by discussion or by a third reviewer (LT).

DATA EXTRACTION

Data from included studies were captured in a standardized Excel sheet: publication details (author, year, country, funding), design, sample size, intervention (agent, dose, duration), comparator, outcome measures (BMD change, fracture incidence rate at 12 and 24 months, adverse events), and follow-up. JP and GT performed independent extraction; LT adjudicated any discrepancies.

Overall, 24 prospective trials evaluated efficacy, 3 assessed real-world effectiveness, 1 addressed safety alone, and 4 reported both safety and efficacy. This rigorous, reproducible approach ensures that our synthesis reflects the highest-quality prospective controlled evidence for transplant-induced osteoporosis.

RESULTS

A total of 24 studies on transplant-associated osteoporosis were analyzed, evaluating various pharmacological interventions in patients with low bone mineral density (BMD). Of these, 19 studies assessed efficacy, 3 analyzed clinical effectiveness, 3 combined safety and efficacy analysis, and 1 focused exclusively on the safety of interventions. Results showed that different interventions, such as Pamidronate, Alendronate, Etidronate, Neridronate, Ibandronate, and Denosumab, had varying effects on improving BMD and reducing fracture risk.

EFFICACY

Several randomized and nonrandomized studies demonstrated that bisphosphonates and related agents significantly improved bone mineral density (BMD) in transplant recipients. In patients on pamidronate (13) a mean increase of +8.8 % in lumbar spine BMD and +8.2 % in femoral BMD is observed vs calcium-vitamin D controls ($p < 0.015$). Additionally, a long-term trial (30) reported that, at four years post-transplant, those without pamidronate prophylaxis lost 12.3 % at the femoral neck ($p < 0.01$), whereas the pamidronate group maintained stable BMD. Etidronate improved lumbar BMD by +4.3 % ($p < 0.03$) and trochanteric BMD by +10.3 % ($p < 0.02$) without affecting femoral-neck density (14). In a head-to-head trial, Jeffery et al. (2003) (15) showed that alendronate increased lumbar BMD by +4.2 % ($p < 0.0001$) and femoral BMD by +3.3 % ($p < 0.001$), whereas the calcitriol group experienced smaller gains. Another study (21) found that combining alendronate with alfacalcidol produced even greater benefits, with +7.9 % in lumbar and +8.0 % in femoral BMD ($p \leq 0.01$ for both). Neridronate delivered monthly intramuscularly achieved +8.6 % in lumbar spine BMD at 12 months ($p = 0.005$) vs placebo (+4.2 %) (17). Zoledronate was associated with +8.6 % ± 7 % in lumbar ($p < 0.01$) and +5.4 % ± 2.2 % in femoral-neck BMD ($p = 0.039$) (18). Although a systematic review and meta-analysis of multiple bisphosphonates suggested a possible clinical effect on lumbar BMD beyond year 1, pooled analyses did not reach significance (SMD, -0.29; $p = 0.22$) (19). Ibandronate produced modest but significant gains of +1.3 % in total femur ($p = 0.013$) and +0.6 % in ultradistal radius ($p = 0.039$) (20). In heart-transplant recipients, both alendronate and calcitriol maintained stable BMD for more than 1 year (16). All these efficacy findings are shown in table I.

SAFETY

Through multiple studies, pharmacological therapies were generally well tolerated. Pamidronate was associated with mild hypocalcemia in 8.6 % of patients, which was effectively managed (31). Clodronate did not produce severe adverse events in heart-transplant recipients (32). Denosumab did not trigger rejection or major events, though it elicited a slight PTH increase ($p = 0.009$) (26). Alendronate triggered no serious adverse effects or renal-function deterioration (23). In kidney-transplant cohorts, 15 % of alendronate recipients experienced transient dyspepsia, whereas none did with pamidronate, and there were no significant differences in creatinine or GFR across treatments ($p = 0.49$ and $p = 0.41$, respectively) (27). A full summary of safety outcomes is shown in table II.

EFFICIENCY

When focusing on bone-loss prevention, pamidronate reduced hip BMD loss to -1.9 % vs -7.3 % in controls ($p = 0.09$) (22) and provided durable protection at 4 years (30).

Table I. Characteristics of the included studies on the efficacy of therapies for transplant-associated osteoporosis

ID	Author, year, design	Population	Intervention (name), dose, *n*	Intervention outcome	Comparator (name), *n*, dose	Comparator outcome
1	Aris RM et al. (2000). Clinical trial (13)	Outpatient adults (men and women, 18-38 years) with CF*, recruited after lung transplantation, with low bone mineral density (T-score ≤ -2.5)	Pamidronate 60 mg single dose. *n:* 16	Change in lumbar spine BMD: +8.8 ± 2.5 %, $p = 0.015$. Change in femoral BMD: +8.2 ± 3.8 %, $p = 0.01$. Type I collagen N-telopeptide levels: Significant drop of 53.7 ± 39 %, $p < 0.001$. Osteocalcin levels: Increase, $p < 0.001$	Calcium (1 g/day) +vitamin D (800 IU/day). *n* = 18 (men, women, aged 18-38 years)	Change in lumbar spine BMD: +2.6 ± 3.2 %, $p = 0.015$. Change in femoral BMD: +0.3 ± 2.2 %, $p = 0.01$. Osteocalcin levels: $p < 0.001$
2	Arlen DJ et al. (2001). Retrospective cohort study (14)	Outpatient adult patients (men and women aged 18 to 85 years) who received a kidney transplant. With femoral osteoporosis (T-score < -2.5)	Etidronate 400 mg/day for 2 weeks every 12 weeks. *n:* 49	Change in lumbar spine BMD: +4.3 ± 6.1 %, $p < 0.03$. Change in trochanteric BMD: +10.3 ± 11.9 %, $p < 0.02$. Change in femoral neck BMD: +3.4 ± 6.5 % (not significant)	Calcium +vitamin D (dose not specified). *n* = 24 (15 men, 9 women, mean age 42 years)	Change in lumbar spine BMD: +0.55 ± 5.3 %. Change in trochanteric BMD: +2.2 ± 5.7 %. Change in femoral neck BMD: +3.2 ± 6.4 %
3	Jeffery JR et al. (2003). Randomized clinical trial (15)	Outpatient adult patients (men and women, mean age 45 years) who received a kidney transplant. With low bone mineral density (T-score ≤ -2.5 in the lumbar spine or femur)	*Alendronate:* 10 mg/day + 500 mg calcium. *n* = 46	– Increase in lumbar spine BMD: +4.2 %, $p < 0.001$. – Increase in femoral BMD: +3.3 %, $p < 0.001$	Calcitriol: 0.25 µg/day + 500 mg calcium. *n* = 51	– Increase in lumbar spine BMD: +2.0 %, $p = 0.002$. – Increase in femoral BMD: +3.3 %, $p = 0.023$
4	Cohen A et al. (2006). Extension study of a Randomized clinical trial (16)	Outpatient adult patients (men and women, mean age 55 years) who received a heart transplant. With baseline bone mineral density (mean lumbar T-score: -0.31 ± 0.2)	*Alendronate:* 10 mg/day + calcium (315 mg TID) and vitamin D (1,000 IU/day). *n* = 34	Stable BMD in the lumbar spine, hip, and distal radius ($p > 0.05$). Increase of 32 % in Bone-Specific Alkaline Phosphatase (BSAP, $p = 0.001$). No significant changes in NTX (bone resorption marker, $p = 0.25$)	Calcitriol: 0.25 µg twice daily + calcium (315 mg TID) and vitamin D (1,000 IU/day). *n* = 25	Stable BMD in the lumbar spine and hip ($p > 0.05$). Increase of 27 % in NTX ($p < 0.001$). Increase of 58 % in BSAP ($p < 0.001$)
5	Giannini S et al. (2021). Randomized clinical trial (17)	Outpatient adult patients (men and women, mean age 49.3 ± 9.1 years) with heart, liver, or lung transplant and osteopenia (T-score < -2.0)	*Neridronate:* 25 mg intramuscular monthly + calcium (500 mg/day) + vitamin D3 (400 IU/day). *n* = 22	Significant increase in lumbar spine BMD: +7.3 % at 12 months ($p = 0.005$). Drop in total alkaline phosphatase (-31.6 %, $p = 0.002$), bone-specific alkaline phosphatase (-49.3 %, $p < 0.001$), and CTX (-62 %, $p < 0.001$)	*Placebo:* Monthly intramuscular isotonic solution + calcium (500 mg/day) + vitamin D3 (400 IU/day). *n* = 17	*Lumbar spine BMD:* +1.7 % at 12 months (not significant). No relevant changes in bone turnover markers (total alkaline phosphatase -1.1 %, CTX -4.6 %)

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Table I (cont.). Characteristics of the included studies on the efficacy of therapies for transplant-associated osteoporosis

ID	Author, year, design	Population	Intervention (name), dose, *n*	Intervention outcome	Comparator (name), *n*, dose	Comparator outcome
6	Tauchmanova L et al. (2006). Randomized clinical trial (18)	Outpatient young female patients (mean age 26 years, mean lumbar BMD: 0.91 g/cm^2, T-score -1.3), recipients of allogeneic stem cell transplants with ovarian failure.	*Risedronate:* 35 mg weekly orally + calcium (1,000 mg/day) + vitamin D (800 IU/day). *n* = 15	Significant increase in lumbar spine BMD: +5.8 % ± 2.1 %, $p < 0.035$. Prevention of femoral neck bone loss: +1.3 % ± 1.2 %, $p = 0.6$	*Calcium:* 1,000 mg/day, orally administered. *Vitamin D:* 800 IU/day, orally administered. *n* = 15	Significant decrease in lumbar spine BMD: -4.3 % ± 2.3 %, $p = 0.046$. Decrease in femoral neck BMD: -4.2 % ± 1.6 %, $p = 0.046$
7	Lip A et al. (2019). Systematic review and meta-analysis (19)	Outpatient adult patients (> 18 years, men and women, *n* = 1,762) who received a kidney transplant, followed > 12 months, with T-score < -1 (osteopenia) or < -2.5 (osteoporosis)	*Alendronate:* 10 mg/day orally. *Pamidronate:* 60 mg IV every 3 months. *Zoledronate:* 4 mg IV every 12 months. *Ibandronate:* 150 mg orally once a month. *Etidronate:* 400 mg/day orally for 14 days every 3 months. *n = 683*	No significant increase in lumbar spine BMD 12-98 months after the transplant: SMD -0.29 (-0.75 to 0.17), $p = 0.22$. *Fractures:* 2.8 % (*n* = 12/683)	*Calcium:* 1,000 mg/day. vitamin D: 400-800 IU/day. *n* = 1,079	No significant improvement in lumbar spine BMD. Fractures: 2.7 % (*n* = 31/1,079)
8	Smerud KT et al. (2012). Randomized clinical trial (20)	Outpatient adult patients (men and women, mean age 51.4 ± 13.8 years), kidney transplant recipients with stable renal function (eGFR ≥ 30 mL/min). Baseline lumbar spine BMD: 1.184 ± 0.171 g/cm^2 (T-score: -0.50 ± 1.36)	*Ibandronate:* 3 mg IV every 3 months + calcium (500 mg b.i.d.) + calcitriol (0.25 µg/day). *n = 66*	Increase in lumbar spine BMD: +1.5 % ± 0.06 %, $p = 0.28$. Significant increase in total femur BMD: +1.3 % ± 0.04 %, $p = 0.013$, and distal radius BMD: +0.6 % ± 0.03 %, $p = 0.039$. Drop in bone turnover markers: PINP: -13.1 ± 56.4, $p = 0.0003$. *Osteocalcin:* -5.5 ± 21.5, $p = 0.0004$	*Placebo:* Isotonic IV solution every 3 months + calcium (500 mg b.i.d.) + calcitriol (0.25 µg/day). *n = 63*	Increase in lumbar spine BMD: +0.5 % ± 0.08 %, $p = 0.33$
9	Trabulus S et al. (2008). Randomized clinical trial (21)	Outpatient adult patients (men and women, mean age 34 ± 10 years), kidney transplant recipients with low BMD (T-score ≤ -2.5)	*Alendronate + alfacalcidol:* 10 mg/day alendronate + 0.5 µg/day alfacalcidol + 1,000 mg/day calcium. *n = 17* Increase in lumbar spine BMD: +7.9 %, $p = 0.006$. Increase in femoral BMD: +8.0 %, $p = 0.01$. Significant improvement in lumbar T-score ($p = 0.003$) and femoral T-score ($p = 0.02$). *Alfacalcidol alone:* 0.5 µg/day alfacalcidol + 1,000 mg/day calcium. *n = 21*	Non-significant increase in lumbar spine BMD: +0.7 %, $p = 0.8$. Non-significant drop in femoral BMD: -1.8 %, $p = 0.4$	*Alendronate alone:* 10 mg/day alendronate + 1,000 mg/day calcium. *n* = 12. Increase in lumbar spine BMD: +4.4 %, $p = 0.2$. Increase in femoral BMD: +6.5 %, $p = 0.09$. Significant improvement in lumbar T-score ($p = 0.009$) and femoral T-score ($p = 0.005$). Control: 1,000 mg/day calcium. *n = 9*	Non-significant increase in lumbar spine BMD: +0.1 %, $p = 0.7$. Non-significant drop in femoral BMD: -2.1 %, $p = 0.8$

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Table I (cont.). Characteristics of the included studies on the efficacy of therapies for transplant-associated osteoporosis

ID	Author, year, design	Population	Intervention (name), dose, *n*	Intervention outcome	Comparator (name), *n*, dose	Comparator outcome
10	Kananen K et al. (2006). Randomized clinical trial (22)	Outpatient adult patients (men and women, mean age 41-46 years), recipients of allogeneic stem cell transplants with baseline lumbar BMD: 0.91 ± 0.14 g/cm^2 (T-score: -1.3 ± 1.3)	*Pamidronate:* 60 mg IV every 1-3 months + calcium (1,000 mg/day) +vitamin D (800 IU/day). *n = 14*	Lumbar bone loss: -0.7 % at 12 months (p = 0.28). Total hip bone loss: -4.6 %, p = 0.008. Significant reduction in PINP (-86.2 %, p = 0.0003)	*Calcium +vitamin D:* 1,000 mg/day calcium + 800 IU/day. Vitamin D. *n = 16*	Lumbar bone loss: -3.5 % at 12 months (p = 0.07). Total hip bone loss: -7.3 %, p = 0.03. Moderate drop in PINP (-38.5 %, p = 0.06)
11	Huang W-H et al. (2012). Case-control study (23)	Outpatient adult patients (men and women, mean age 47 ± 13 years), kidney transplant recipients with baseline lumbar BMD: 0.90 g/cm^2 (mean T-score: -1.53)	*Alendronate (Fosamax):* 70 mg weekly orally + calcium (1,000 mg/day) + vitamin D (800 IU/day). *n = 34*	Significant increase in lumbar spine BMD: +2.2 % (from 0.90 g/cm^2 to 0.92 g/cm^2, p < 0.001). Significant increase in total hip BMD in men (p = 0.03)	Calcium (1,000 mg/day) + vitamin D (800 IU/day). *n = 42*	– No significant changes in lumbar spine BMD (+0.5 %, p = 0.33) or hip BMD. – 14 % of patients experienced bone deterioration
12	Ippoliti G et al. (2003). Randomized clinical trial (24)	Outpatient adult patients (56 men, 8 women, mean age 50 years), heart transplant recipients with osteoporosis (T-score < -2.5)	*Clodronate:* 1,600 mg/day in 2 doses + calcium (2,000 mg/day). *n = 32*	Significant increase in lumbar spine BMD: from 0.77 ± 0.14 g/cm^2 to 0.86 ± 0.16 g/cm^2, p = 0.02. Reduction in bone isoenzyme of alkaline phosphatase: -35 %, p = 0.03	*Placebo:* Isotonic solution + calcium (2,000 mg/day). *n = 32*	Lumbar spine BMD loss: from 0.75 ± 0.12 g/cm^2 to 0.73 ± 0.15 g/cm^2, p = 0.0001. *Fracture incidence rate:* 9.3 % (2 vertebral fractures, 1 hip fracture)
13	Kaemmerer D et al. (2010). Randomized clinical trial (25)	Outpatient adult patients (men and women, mean age 51.7 ± 12.9 years), liver transplant recipients with baseline lumbar T-score: -1.75 ± 1.08	*Ibandronate:* 2 mg IV every 3 months + calcium (1,000 mg/day) + vitamin D3 (800-1,000 IU/day). *n* = 34	Increase in lumbar spine BMD: +4.42 % at 24 months, p = 0.13. Significant drop in fractures: 7.4 % (2 fractures), p = 0.04	*Calcium + vitamin D3:* 1,000 mg/day calcium + 800-1,000 IU/day. Vitamin D3. *n* = 40	Lumbar spine BMD loss: -1.80 % at 24 months, p = 0.13 Fracture incidence rate: 25.8 % (8 fractures).
14	Alfieri C et al. (2021). Prospective observational study (26)	Outpatient adult patients (men and women, median age 62 years), kidney transplant recipients with femoral osteoporosis (T-score < -2.5)	*Denosumab:* 60 mg subcutaneous every 6 months + calcium (1,000 mg/day) + vitamin D (800-1,000 IU/day). *n = 32*	Increase in lumbar spine BMD: +9.7 %, p = 0.01. Increase in femoral BMD: +5.7 %, p = 0.02. Drop in femoral osteoporosis: from 78 % down to 69 %, p = 0.001	No direct comparator	NA
15	Bita Omidvar et al. (2011). Clinical trial (27)	40 kidney transplant patients (27 men, 13 women) with T-score < -2.5 in the lumbar spine, femoral neck, or total hip	*Pamidronate: n = 20,* 90 mg IV from the 3rd week post-transplant for 3 months	Reduction of 1.42 % in femoral neck bone density and 1.40 % in the femur (less bone loss than the Alendronate group, *p = 0.003 and 0.03)*	*Alendronate: n = 20,* 70 mg oral weekly for 3 months	Reduction of 2.03 % in femoral neck bone density and 1.42 % in the femur. *Adverse events:* GI side effects in 3 patients (dyspepsia)
16	Grotz et al. (2001). Randomized controlled clinical trial (28)	Hospitalized post-kidney transplant patients with reduced BMD, some with osteoporosis (variable T-score)	*Ibandronate:* Variable dose. *n: not specified*	Prevention of BMD loss in the lumbar spine (-0.9 % vs. -6.5 %, *p < 0.0001)* and femur (-10.5 % vs. -27.7 %, *p < 0.0001)*	*Control (without Ibandronate): n: not specified*	Greater BMD loss in the control group (-6.5 % in the lumbar spine, -27.7 % in the femur, *p < 0.0001)*

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Table I (cont.). Characteristics of the included studies on the efficacy of therapies for transplant-associated osteoporosis

ID	Author, year, design	Population	Intervention (name), dose, *n*	Intervention outcome	Comparator (name), *n*, dose	Comparator outcome
17	Tauchmanova et al. (2003). Prospective tandomized study (29)	Outpatient post-allogeneic stem cell transplant patients with osteoporosis (T-score -2.5)	*Risedronate:* 5 mg/day for 12 months. *n = 17*	Increase in lumbar spine BMD: *+4.4 % ± 1.6 % at 6 months and +5.9 % ± 1.7 % at 12 months.* Stable BMD in the femoral neck	*Calcium (1 g/day) + vitamin D (800 IU/day). n = 17*	Drop in lumbar spine BMD: *-4.3 % ± 1.5 %*. Drop in femoral neck BMD: *-4.3 % ± 2.1 %*
18	Fan et al. (2003). Clinical trial (30)	Hospitalized post-kidney transplant patients with *T-score < -2.5,* indicative of osteoporosis	*Pamidronate:* 90 mg IV, starting from the 3rd week post-transplant for 3 months. *n = 20*	*Drop in BMD:* Femoral neck: -1.42 % ($p = 0.003$) *Femur:* -1.40 % ($p = 0.03$)	*Alendronate:* 70 mg/week orally for 3 months	Drop in BMD: Femoral neck: *-2.03 % ($p = 0.003$).* Femur: -1.42 % ($p = 0.03$). *Adverse events:* Dyspepsia in 3 patients

Table II. Characteristics of the studies included for safety in transplant-induced osteoporosis

ID	Author, year, design	Population	Intervention (name), *n*, dose	Intervention outcome	Comparator (name), *n*, dose	Comparator outcome
1	Ippoliti et al. 2003, Clinical trial (24)	64 patients (56 men, 8 women) with bone loss post-heart transplant. *T-score:* -1.43 in the lumbar spine and -4.0 in 1/10 of the forearm	Clodronate (oral), *n = 32, 1,600 mg/day* in 2 divided doses + *2,000 mg/day calcium carbonate*	Mild GI effects: *nausea and epigastric discomfort in 22 % of patients. New bone fractures: 0 %*	*Placebo, n = 32 + 2,000 mg/day calcium carbonate*	*New bone fractures: 9.3 %* (2 vertebral fractures, 1 hip fracture). *Persistent bone pain:* Patients continued requiring analgesics
2	Walsh SB et al. 2009. Clinical trial (31)	*Population:* 93 post-kidney transplant patients (46 in the intervention group and 47 in the control group). *Z-score < -2.0*	*Pamidronate, n = 46,* 1 mg/kg IV perioperatively, then at 1, 4, 8, and 12 months	5 episodes of transient hypocalcemia (8.6 %).	*No bisphosphonate, n = 47* (dose not specified)	6 new fractures (12.8 %) in 24 months. 0 episodes of transient hypocalcemia
3	Alfieri C et al. 2021. Prospective observational study (26)	32 kidney transplant patients (KTxps), *21 women and 11 men, median age: 62 years. T-score:* Femoral -3.0, Vertebral 3.0	*Denosumab, n = 32, 60 mg every 6 months for 1 year*	2 cases of new spontaneous vertebral fractures (sVF). 4 UTIs. No hypocalcemia or graft rejection	No direct comparator group.	*NA*
4	Wen-Hung Huang et al. 2012. Case-control study (23)	76 kidney transplant patients. Osteoporosis: T-score ≤ -2.5; *Osteopenia:* between -1.0 and -2.5	Fosamax (Alendronate Sodium), *n = 34,* 70 mg per week	7 patients did not tolerate Fosamax due to side effects (not specified)	Patients without Fosamax, *n = 42*	No significant adverse events reported
5	Bita Omidvar et al. 2011. Clinical trial (27)	40 kidney transplant patients (27 men, 13 women) with *T-score* < -2 in the lumbar spine, femoral neck, or total hip	Pamidronate, *n = 20,* 90 mg IV from the 3rd week post-transplant for 3 months	No adverse events reported	Alendronate, *n = 20,* 70 mg oral weekly for 3 months	Transient dyspepsia in 3 patients

In kidney-transplant patients, alendronate increased lumbar BMD by +0.035 g/cm² vs +0.003 g/cm² in untreated subjects (23). A comparison of IV pamidronate vs oral alendronate showed that pamidronate preserved femoral-neck density (-1.42 % vs. -2.03 %; $p = 0.003$) and total femur (-1.40 % vs. -1.83 %; $p = 0.03$) more effectively (27). Clodronate achieved an +11.7 % increase in lumbar BMD ($p = 0.02$) while placebo produced no changes at all (24). Although ibandronate lumbar gain of +4.42 % did not reach statistical significance ($p = 0.13$), treated patients experienced significantly fewer vertebral deformities, less height

Table III. Characteristics of the studies included effectiveness in transplant-induced osteoporosis

ID	Author, year (design)	Population	Intervention (agent, dose, *n*)	Comparator (agent, dose, *n*)	Vertebral fracture incidence (intervention vs comparator)
1	García-Delgado I et al. (1997) (Randomized clinical trial) (33)	Outpatient heart-transplant recipients; mean age 53; T-score ≤ -2.5	Calcidiol 32 000 IU/week + Ca 1 000 mg/day (*n* = 13)	Calcitonin 100 IU/day intranasal + Ca 1 000 mg/day (*n* = 13)	0 % vs 30.8 %
2	Sánchez-Escuredo A et al. (2015) (Prospective clinical trial) (34)	Kidney-transplant recipients; mean age 63; lumbar T-score -1.7 ± 0.8; femoral -2.1 ± 0.7	Ibandronate 150 mg monthly + Ca 2 500 mg/day + Vit D 800 IU/day (*n* = 35)	Risedronate 35 mg weekly + Ca 2 500 mg/day + Vit D 800 IU/day (*n* = 34)	Not reported
3	Ninkovic M et al. (2002) (Randomized clinical trial) (35)	Outpatient liver-transplant recipients; mean age 53; lumbar T-score -2.0 ± 0.6	Pamidronate 60 mg IV single dose pre-transplant (*n* = 45)	Standard follow-up without pamidronate (*n* = 54)	8 % vs 8 %

loss, and fewer acute-rejection episodes than controls (28); (25). Risedronate increased lumbar BMD by +5.9 % in 12 months and stabilized femoral-neck density vs declines in controls ($p < 0.05$) (29). Regarding clinical effectiveness, calcidiol reduced vertebral-fracture incidence by 30 % ($p < 0.05$) (33); ibandronate and risedronate lowered NTX levels by 34 % and 28 %, respectively ($p < 0.05$) (34); and although pamidronate did not significantly change fracture rates (8 % vs. 8 %; $p = 0.40$) it did mitigate BMD loss (35). These efficiency and fracture-outcome data are shown in table III.

DISCUSSION

This scoping review confirms that transplant-induced osteoporosis (TO) is due to a multifactorial interaction among pre-existing bone health, chronic glucocorticoid exposure, immunosuppressive regimens, and transplant-specific factors. Nearly all studies included chronic glucocorticoid use as an underlying contributor to bone loss, yet only a minority explicitly reported corticosteroid dosing or its direct impact on BMD outcomes. Consistently, bisphosphonates (pamidronate, alendronate, etidronate, zoledronate, ibandronate) and denosumab increased BMD across renal, cardiac, and mixed transplant populations (13-21,26), even though fracture-reduction data remain sparse and variable.

Our efficacy findings are consistent with earlier reports identifying glucocorticoids as central drivers of post-transplant bone demineralization (30,33); which documented up to 12 % femoral BMD loss in renal recipients and high fracture rates in liver transplant patients. However, the wide divergence in fracture outcomes—such as the lower vertebral-fracture incidence reported (25) vs the neutral fracture effect seen (35)—likely reflects methodological heterogeneity (eg, variable glucocorticoid regimens, follow-up durations, and sample sizes). Notably, although most trials acknowledged patients' glucocorticoid burden, few stratified results by steroid dose or duration, underscoring a gap between recognized pathophysiology and published outcomes.

Overall, pharmacological agents exhibited acceptable safety profiles in the context of concomitant glucocorticoid therapy. Although pamidronate was associated with mild, transient hypocalcemia (31), alendronate only caused only minor GI discomfort (23). Although denosumab did not precipitate rejection or serious adverse events, modest PTH elevations warrant monitoring (26). Of note, none of the studies reported glucocorticoid-related exacerbations of adverse effects, suggesting that these antiresorptives can be safely co-administered with glucocorticoids under careful supervision (Table II).

A clear strength of this review is the inclusion of diverse transplant types and pharmacotherapies, offering a panoramic view of current evidence. The rigorous JBI scoping methodology enhanced reproducibility in study selection and data extraction. Conversely, heterogeneity in glucocorticoid dosing regimens, inconsistent reporting of fracture endpoints, and variable follow-up durations limited cross-study comparability. Furthermore, the near-ubiquitous use of glucocorticoids was seldom quantified, impeding nuanced analysis of steroid-specific effects on BMD and fracture risk.

Clinicians should recognize chronic glucocorticoid therapy as a primary risk factor for TO and implement early, individualized bone-preserving strategies. Bisphosphonates remain first-line agents—particularly in kidney and heart transplant recipients—while denosumab offers an alternative for patients that are intolerant of oral bisphosphonates. Routine monitoring of BMD and fracture risk, along with judicious tapering of glucocorticoids when feasible, may optimize long-term skeletal health in transplant populations (36) (Table I).

To address current evidence gaps, future studies must standardize reporting of glucocorticoid exposure and incorporate fracture endpoints alongside BMD. Large scale, multicenter randomized trials with uniform definitions of TO, stratified by steroid dose and type, are essential. Extended follow-up beyond two years will capture delayed adverse events and fracture outcomes, while subgroup analyses of underrepresented transplant types (eg, lung, intestinal) will inform tailored interventions. Cost-effectiveness and patient-reported outcome measures should also be integrated to guide real-world clinical decision-making (37,38) (Table III).

CONCLUSIONS

Althouhg pharmacological therapies for transplant-induced osteoporosis effectively improve BMD in the setting of chronic glucocorticoid and immunosuppressive use, their impact on fracture prevention remains inadequately characterized. Enhanced focus on quantifying glucocorticoid regimens and standardized fracture reporting will be critical to developing evidence-based, patient-centered strategies that mitigate long-term skeletal complications in transplant recipients.

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Original

β-lapachone nanostructured lipid carriers: repolarization of tumor-associated macrophages for osteosarcoma therapy

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Abstract

Osteosarcoma (OS) is the most common primary malignant bone tumor. Tumor-associated macrophages (TAMs) represent up to 50 % of the cells in the tumor and play a significant role in tumor metastasis and tumoral immunosuppression. The modification of TAMs phenotype has been identified as suitable for controlling tumor progression. Nanoparticles are versatile drug delivery systems that allow enhanced drug tumor accumulation through passive and active targeting. Among NPs, nanostructured lipid carriers (NLCs) possess a unique imperfect matrix structure with increased drug loading capacity of highly hydrophobic drugs. β-Lapachone (β-Lap) is a natural naphthoquinone with anti-tumor activity against various cancer cells. The aim of this work is to design and optimize a NLCs formulation loaded with β-Lapachone to be specifically internalized by TAMs for OS treatment. This approach will use hybrid artificial intelligence tools to functionalize the NLCs with the CD206 ligand mannose. To optimize NLCs preparation, various liquid and solid lipids were tested for drug solubility. AI tools were employed to design NLCs with desired properties, resulting in formulations with particle sizes < 100 nm and stable physicochemical properties. Mannose functionalization enhanced macrophage internalization of the NLCs. Moreover, *in vitro* studies demonstrated these NLCs, particularly the mannose-functionalized formulation, induced a further pro-inflammatory M1-like polarization in TAMs, evidenced by increased TNF-α and IL-6 secretion. This TAMs polarization strategy, combined with the localized delivery of β-Lap, offers a promising approach for OS therapy.

Keywords:
Osteosarcoma. Tumor-associated macrophages. Targeted drug delivery. Tumor environment modulation.

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INTRODUCTION

Osteosarcoma (OS) is recognized as the most prevalent bone primary malignant tumor. It shows an incidence peak in the 2nd decade of life associated to an enhanced activity of the metaphyseal plates and affects more than 9 teenagers per million in the range of 15-19 years-old (1). The most prevalent locations for OS are the femur, tibia, and proximal humerus, particularly around the knee (2,3). In approximately 75 % of cases, the tumor is located within the metaphysis of long bones, where it grows rapidly, extending to the bone periphery (4). The typical clinical signs of OS include localized pain followed by swelling and restricted joint movement. Although rare, a pathological fracture can also occur at the site of the disease (5). OS can be categorized into several types based on the predominant produced matrix (chondroblastic, fibroblastic or osteoblastic) or the malignant grade, which aids in assessing the tumor's metastatic potential (4,6). Regarding its physiopathological origin, the main cause is believed to be the accumulation of genetic mutations in mesenchymal stem cells (MSCs) during their differentiation into osteoblasts (7,8). Surgery and radiation therapy and chemotherapy remain the primary treatment options and are frequently combined for metastatic tumors (9). However, conventional chemotherapy presents several limitations such as drug resistance and side effects (10).

The primary histopathological feature of OS is the excessive production of osteoid tissue, the unmineralized organic component of the bone matrix formed before tissue maturation (11). This matrix is produced by malignant cells of the mesenchymal lineage comprising MSCs, osteoprogenitor cells and osteoblasts (11-13). Furthermore, bone mineral metabolism is also altered and is thought to play a role in several aspects of tumor progression (14,15). OS presents an osteoclastic stimulatory environment increasing the activity of these bone-resorbing cells. This effect is mediated by the secretion of soluble factors. Simultaneously, the induced osteolysis promoted the release of tumor growth inductors, creating a cycle of bone destruction and tumor formation (12,14). The final outcome is the establishment of a metabolic state which increases the production of immature, weaker, and disorganized bone matrix together with the hyperactivity of osteoblasts and osteoclasts (12,14,15).

Additionally, tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSCs) amplify the alterations in bone homeostasis (14). The tumoral microenvironment (TME) formed by tumoral cells, immune cells, stromal cells, extracellular matrix and soluble mediators plays a key role in tumor progression and chemotherapy efficacy (16). In this scenario, immune cells can either facilitate or inhibit tumor growth (17). Specifically, tumor-associated macrophages (TAMs) constitute a significant portion of OS mass, representing up to 50 % of the cells (18). They perform essential functions related to bone formation and osteoblast differentiation and have been identified as main players in tumor metastasis and tumoral immunosuppression (19-21). Macrophages can depict opposing phenotypes, with different functions: the inflammatory phenotype or classical (M1) and the anti-inflammatory or alternative phenotype (M2). Overall, M1-TAMs are associated with tumor inhibition and M2-TAMs with a neoangiogenic effect and secrete cytokines such as IL-1β leading to tumor progression and metastasis (22-24). Given the role of TAMs in controlling tumor progression, multiple strategies based on either avoiding their M2 polarization or suppressing the M2-TAMs profile inducing an M1-TAMs have been proposed to control OS (21,25). Clinical trials have shown that the addition of mifamurtide to the standard chemotherapy treatment, results in an increase in 6-year survival rate in OS patients (26). The mechanism of action of this therapeutic molecule is thought to be associated with its ability to *in vitro* switch M2-TAMs towards and intermediate M1/M2-TAMs, proving that polarization to M1-TAMs was key for controlling tumor progression (27). The development of drug delivery systems targeting TAMs could enable the efficient control of macrophage polarization and, therefore, improve the treatment of OS. This approach seeks to selectively control the macrophage population towards a pro-inflammatory response avoiding undesired side effects. Nanoparticles (NPs) are versatile drug delivery systems with improved pharmacokinetic profiles vs free therapeutic molecules. These systems allow for enhanced circulation time and accumulation within the tumor through passive and active targeting (28). In this case, the surface can be modified to incorporate specific moieties with affinity for the bone extracellular matrix or for cell surface receptors. Several targeted NPs loaded with chemotherapy drugs have been developed for OS management including liposomes, polymeric NPs, mesoporous silica nanocarriers, manganese dioxide NPs and iron oxide NPs (29,30). However, these strategies are mainly focused on controlling proliferative cells and, as far as we know, no TAMs targeted NPs have been reported for OS management.

β-Lapachone (3,4-dihydro-2,2-dimethyl-2H-naphtho[1,2-b] pyran-5,6-dione) (β-Lap) is a natural naphthoquinone extracted from the lapacho tree. It exhibits anti-tumor activity against various cancer cells, including breast cancer, leukemia, prostate cancer, bladder cancer, lung cancer, hepatoma, pancreatic cancer, and OS. β-Lap induces a redox cycle mediated by NAD(P)H: quinone oxidoreductase 1 (NQO1) (31,32). During this process, β-Lap is reduced to an unstable semiquinone that undergoes a two-step oxidation process, returning to its stable form and perpetuating a futile redox cycle. The generated redox cycle disrupts intracellular reactive oxygen species (ROS) balance, leading to cell death through apoptosis and necrosis (necroapoptosis).

This mechanism of action confers drug specificity towards hypoxic tumor regions usually highly infiltrated in TAMs (33). Moreover, an upregulated expression of this anti-oxidant enzyme has been reported in anti-inflammatory M2 macrophages (34). Moreover, some studies have shown that ROS play a key role in macrophage differentiation. Specifically, ROS in tumoral environment activates macrophages to a M1 pro-inflammatory and antitumoral state (35).

Despite β-Lap has the potential to modulate tumor microenvironment in OS avoiding tumor growth and metastasis, its poor water-solubility hinders bioavailability (36). Moreover, while it exhibits preferential activity in cells overexpressing NQO1, improving the drug cellular specificity is necessary to avoid undesired side effects. In this scenario, the development of β-Lap loaded TAMs targeted drug delivery systems will allow to specifically modulate the phenotype of TAMs towards a pro-inflammatory and anti-tumoral profile. Among nanoparticulated drug delivery systems, nanostructured lipid carriers (NLCs) possess a unique imperfect matrix structure with enhanced properties, such as increased drug loading capacity of highly hydrophobic drugs, greater stability during storage, and controlled drug release. Moreover, their surface can be modified by incorporating glycoligands such as mannose, fucose, or glucose for targeting specific cell membrane receptors on macrophages. Therefore, NLCs could be adequate to fine-tune nanocarriers for macrophage polarization control (37-41).

The design of drug delivery systems such as NLCs, is a complex process with several variables involved. Computational techniques have been used to model results, make predictions, and even optimize different formulations (42-44). Artificial neural networks (ANNs), an artificial intelligence (AI) technique that mimics the functioning of the human brain, can be integrated with other AI tools, such as Fuzzy Logic (FL) or Genetic Algorithms (GAs) to create hybrid systems capable of modelling complex manufacturing processes. These computational techniques have been used as effective tools for optimizing the development of NLCs. By incorporating variables such as composition and operation conditions, these techniques enable refined control and prediction of particle size, polydispersity index, zeta potential, and drug loading capacity, ensuring the development of robust and reproducible designs (45,46).

The aim of this work is to design and optimize a NLCs formulation loaded with β-Lapachone to be specifically internalized by TAMs for OS treatment. This approach will be performed by using hybrid artificial intelligence tools (AI tools) functionalizing the NLCs with the CD206 ligand mannose. The developed system would induce immune cellular response (M1-TAMs polarization), leading to a decrease in tumor growth by dual cellular and chemical action.

MATERIALS AND METHODS

MATERIALS

β-Lapachone (β-Lap) was kindly donated by the Pernambuco State Pharmaceutical Laboratory, LAFEPE (Recife, Brazil). Miglyol®, Transcutol® CG, Labrasol® ALF, Labrasol® Lipophile WL, Transcutol® HP, selected as liquid lipids (LL), were kindly provided by Gattefossé (France). Oleic Acid was also selected as LL and it was acquired from Merck (Portugal). Compritol® 888 ATO, Precirol® ATO and Glyceryl Tristearate were selected as solid lipids (SL) and kindly gifted by Gattefossé (France). Polysorbate 80 (Tween® 80), purchased from Sigma Aldrich (Germany), and lecithin (Epikuron® 145 V), donated by Cargill (USA), were used as surfactants. Milli-Q® water (Milli-Q® plus, Millipore Iberica, Spain) was used throughout all the experiments. For the functionalization process, stearylamine and D-(+)-Mannose were acquired from Sigma-Aldrich (USA). The acetate buffer was prepared using acetic acid 0.2 M from Sigma Aldrich (USA) and sodium acetate 0.2 M from Scharlab (Spain).

SELECTION OF NLCS COMPONENTS

β-Lapachone solubility in liquid lipids

The solubility of β-Lap in different LL was assessed following a previously described procedure (45). To this end, 200 mg of β-Lap was mixed with 1 mL of each LL. The mixture was stirred at 300 rpm for 48 hours. Then samples were centrifuged at 12,000 rpm and 20 °C for 30 min and properly diluted in acetonitrile. The amount of solubilized β-Lap was quantified by UV-Visible spectrophotometry using an Agilent Technologies UV-VIS 8453 spectrophotometer (USA) at 257 nm, employing a previously validated calibration curve. Solubility studies were conducted in triplicate.

β-Lapachone solubility in solid lipids

The solubility of β-Lap in different LL was also assessed following a previously described procedure (45). To this end, 200 mg of each SL was heated in a water bath at 80 °C (5 °C above its melting point). Once the SL was melted, β-Lap was added in 5 mg increments until a precipitate appeared, indicating the presence of non-solubilized β-Lap.

Miscibility studies of liquid lipids and solid lipids

LL and SL were mixed in various ratios (50:50, 25:75, 75:25). The mixtures were heated in a water bath at 80 °C with continuous stirring for 5 minutes, phase separation was evaluated as indicative of immiscibility.

Experimental design

Dataform® v3.1 software (Intelligensys Ltd., UK) was used to establish a reduced and balanced experimental design for three variables: LL:SL ratio, concentration of Tween® 80 (% v/v) in the aqueous phase, and percentage of lecithin relative to the total lipid phase. The obtained experimental design conditions are shown in table I.

Table I. Reduced experimental design

Formulation	LL:SL ratio	Tween® 80 (% v/v)	Lecithin (% w/v)
1	30:70	2.00	2.0
2	50:50	0.50	1.5
3	10:90	1.25	1.0
4	30:70	1.25	1.5
5	50:50	2.00	1.0
6	10:90	0.50	2.0
7	50:50	2.00	2.0
8	10:90	1.25	1.5
9	30:70	0.50	1.0

NANOSTRUCTURED LIPID CARRIERS FORMULATION

NLCs were prepared using a high-shear hot homogenization method similarly to previously described (46). Based on the solubility of β-Lap in the different LL and SL lipids, Compritol® 888 ATO (COMP) and Transcutol® HP (THP) were selected as lipid components, while Tween® 80 and lecithin were used as surfactants. A lipid blend of 300 mg was prepared containing the LL and SL in the proportions indicated in table I. The drug was incorporated into this phase on the basis of its LL solubility. Separately, an aqueous phase was prepared by adding lecithin to 10 mL of a Tween® 80 solution in milli-Q water as specified in table I. Then, both phases were heated in a water bath at 80 °C for 5 minutes, and the aqueous phase was then added to the lipid blend and homogenized using an Ultra-Turrax T25 (IKA Labortechnik, Germany) for 10 minutes at 14,800 rpm. Finally, the resulting NLCs dispersion was cooled in an ice-water bath for two minutes with gentle stirring. The dispersions were stored at 4 °C until characterization (41,45,47).

NANOSTRUCTURED LIPID CARRIER CHARACTERIZATION

NLCs dispersions were characterized in terms of particle size, polydispersity index (PdI) and surface charge (ZP) using a Zetasizer Nano-ZS (Malvern Instruments, UK). Samples were diluted in milli-Q water (1:10), and polystyrene cuvettes were used for particle size and PdI measurements (DTS0012, Malvern Instruments, UK). Measurements were conducted at 25 ± 1 °C, and NLCs were characterized 15 minutes and 14 days after preparation. Surface charge was determined by measuring particle mobility in an electric field to calculate the Zeta potential (ZP). Samples were also diluted in milli-Q water (1:10), and measurements were performed using a Malvern DTS 1070 cuvette 15 minutes and 14 days after NLC preparation.

NLCs dispersions were also characterized in terms of drug loading (DL) and encapsulation efficiency (EE). To this end, NLCs were purified using cellulose membranes (MWCO: 3.5 kDa from Spectra/Por®). Afterwards, the purified dispersion was dissolved in acetonitrile (1:2 dilution) to release the encapsulated drug and centrifuged at 12,000 rpm, 4 °C for 30 minutes. The encapsulated drug was quantified spectrophotometrically using a plate reader (FLUOstar Omega, BMG Labtech, Germany) at 280 nm. The same procedure was performed for non-purified NLCs to determine the total drug amount (free drug + encapsulated drug). The DL and EE were calculated using equation 1 and equation 2, respectively,

$$DL\ (\%) = \left[\frac{Encapsulated\ drug\ (mg)}{Weight\ of\ NLCs\ (mg)}\right] \times 100 \qquad \text{(Eq. 1)}$$

$$EE\ (\%) = \left[\frac{Encapsulated\ drug\ (mg)}{Total\ drug\ (mg)}\right] \times 100 \qquad \text{(Eq. 2)}$$

NANOSTRUCTURED LIPID CARRIER FORMULATION MODELLING USING ARTIFICIAL INTELLIGENCE TOOLS

INForm® v5.01 (Intelligensys Ltd, UK) is a commercial software that integrates ANN, and GAs, specifically designed for modelling and optimizing pharmaceutical formulations such as NLCs. This software was used to model the generated experimental database (Table II). Three variables were included as inputs: LL/SL ratio, the percentage of Tween® 80 in the aqueous phase, and the percentage of lecithin relative to the total lipid phase. Four variables were included as outputs: particle size, PdI and ZP after 14 days of storage and DL. The modelling was carried out using the default software parameters.

Composition conditions were selected to produce an optimal formulation based on the following requirements: particle size and PdI with the lowest possible values, ZP with the most negative values at 14 days, and maximum DL. The optimal formulation was experimentally prepared. Unloaded (NLC) and drug-loaded formulations (NLC-β-Lap) were prepared to validate the generated model.

NANOSTRUCTURED LIPID CARRIERS SURFACE FUNCTIONALIZATION

Optimal formulations (NLC-β-Lap) were functionalized with mannose (NLC-β-Lap-MAN). To achieve this, stearylamine (2 % w/w) (relative to the total lipid phase) was added to the lipid blend, and the formulation was prepared as described in section 2.4. The formulation was then incubated with a 50 mM D-(+)-mannose solution prepared in acetate buffer at pH 4, and stirred vigorously for 48 hours, similarly to previously described (45,48,49). The formulation was subsequently dialyzed using a cellulose membrane (MWCO: 3.5 kDa) against milli-Q water with agitation for 30 minutes to remove potential impurities and unattached mannose.

IN VITRO CELL STUDIES

In vitro cell studies were conducted in human monocytes derived from acute lymphocytic leukaemia (THP-1) acquired from ATCC (TIB-202) (USA). THP-1 cells were cultured in RPMI 1640 media supplemented with foetal bovine serum (FBS) (10 %), penicillin/streptomycin (1 %) and 2-mercaptoethanol 0.05 mM. Before studies, cells at a density of $2x10^5$ cells/mL were stimulated with 200 nM of phorbol 12-myristate 13-acetate (PMA) acquired from Sigma-Aldrich (USA) in complete RPMI 1640 media for 48 hours to induce their differentiation to macrophages. Finally, cell monolayers were washed with Dulbecco's phosphate-buffered saline (DPBS), and incubated with complete RPMI 1640 media, allowing them to set for 24 h at 37 °C and 5 % of CO_2 before seeding.

Cell viability studies

Cells were seeded in 96-well plates ($2x10^4$ cells/well). After 24 hours, the optimized formulations (NLC, NLC-β-Lap, and NLC-β-Lap-MAN) were added at a final 300 µg/mL (solid mass per volume) concentration and incubated for 24 hours. As control, cells treated with an equivalent amount of milli-Q water were used. After this time, cell viability was evaluated using the "Cell Proliferation Reagent WST-1" kit from Roche Molecular Biochemicals (Germany) following the manufacturer's instructions. A phenol red-free medium from Gibco (USA) was used, and the WST-1 reagent was applied in darkness. The reagent was also added to wells without cells to serve as absorbance blanks. The plate was incubated with the reagent for 1 hour at 37 °C and then shaken for 1 minute. The absorbance at 450 nm was determined on a plate reader (Bio-Rad 680, Barcelona, Spain). Cell viability was calculated using equation 3.

$$\text{Cell viability (\%)} = \left[\frac{\textit{Sample absorbance–Blk}}{\textit{Control absorbance–Blk}}\right] \times 100 \qquad \text{(Eq. 3)}$$

Cell internalization studies

Cells were seeded in 96-well plates ($2x10^4$ cells/well). After 24 hours, fluorescently labelled formulations (NLC-β-Lap and NLC-β-Lap-MAN) prepared by adding coumarine-6 (4 µg/mL) to the lipid blend during the NLC formulation were added at a final concentration of 300 µg/mL (solid mass per volume) and incubated for 2 hours to quantify their internalization using a fluorometric method. An initial fluorescence measurement was obtained with a plate reader (FLUOstar Omega, BMG Labtech, Germany) at excitation and emission wavelengths of 485 nm and 520 nm, respectively. After 2 hours, 3 washes were performed with 20 mM glycine solution from Fluka BioChemika (Switzerland) in DPBS at pH 7.4. Afterwards, 100 µL/well of Triton X-100 (1 %) from Merck (Portugal) was added to induce cell lysis, allowing the internalized formulations to be released. Fluorescence measurements were then taken post-lysis in the same conditions. The percentage of NPs internalization was determined using equation 4.

$$\text{Cell internalization (\%)} = \left[\frac{\textit{Post–lysis fluorescence}}{\textit{Initial fluorescence}}\right] \times 100 \qquad \text{(Eq. 4)}$$

Evaluation of the tumor-associated macrophages profile modification

The capacity of the developed formulations to modulate TAMs phenotype was tested using THP-1 macrophages. Cells were seeded in 96-well plates ($2x10^4$ cells/well) and allowed to attach for 18 h. Monolayers were then stimulated with lipopolysaccharide (LPS) (100 ng/mL) for 24 h to obtain activated macrophages serving as TAMs models. Following stimulation, cells were then treated with the developed formulations (NLC-β-Lap, and NLC-β-Lap-MAN) at a final concentration of 300 µg/mL (solid mass per volume). Stimulated but untreated cells were used as controls. After 24 hours, cell culture supernatants were collected, and the secreted concentrations of various cytokines and matrix degradative enzymes involved in tumor invasion, metastasis and OS osteolysis (TNF-α, IL-6, IL-8, IL-1RA, and IL-13) were determined using a magnetic bead-based multiplex assay (R&D systems, USA) and according to the manufacturer's instructions for use.

STATISTICAL ANALYSIS

The obtained data was analysed with GraphPad Prism 8 software through one-way analysis of variance (ANOVA) followed by post hoc Tukey's Multiple Comparison Test. Results are expressed as mean ± SD. Statistically significant differences were set at $p < 0.05$.

RESULTS

NANOSTRUCTURED LIPID CARRIERS COMPONENTS SELECTION

Although β-Lap is highly insoluble in water, its lipid solubility was unknown. This study evaluated the β-Lap solubility across different liquid lipids (Fig. 1), revealing statistically significant variation. Transcutol® CG (TCG), Labrasol® ALF, and Transcutol® HP exhibited the highest β-Lap solubilization capacity, while Labrasol® Lipophile WL showed the lowest (Fig. 1). Consequently, Transcutol® CG (TCG), Labrasol® ALF, and Transcutol® HP were selected for further development.

Solubility tests in solid lipids revealed β-Lap was less soluble in Precirol® ATO than in the other SL. Therefore, Precirol® ATO was excluded. Additionally, glyceryl tristearate was excluded due to its immiscibility with the selected LL. On the other hand, Compritol® 888 ATO (COMP) mixed with the LL with high β-Lap solubility (Transcutol® CG, Labrasol® ALF and Transcutol® HP) showed no phase separation. Preliminary blank NLC formulations with a 50:50 LL-to-SL ratio, Tween 2 % v/v relative to the aqueous phase, and lecithin 1 % w/v relative to the lipid phase were prepared to determine the most suitable LL/SL combination (data not shown). Based on these results, Transcutol® HP was selected, as it produced NLCs with the smallest and most homogeneous particle size distribution, appropriate ZP values and good stability after 14 days of storage at 4 °C.

NANOSTRUCTURED LIPID CARRIERS CHARACTERIZATION AND OPTIMIZATION

The formulations shown in table I were prepared and characterized in terms of particle EE and DL 15 minutes post-preparation, and again in terms of size, PdI and ZP after 14 days of storage at 4 °C (Table II).

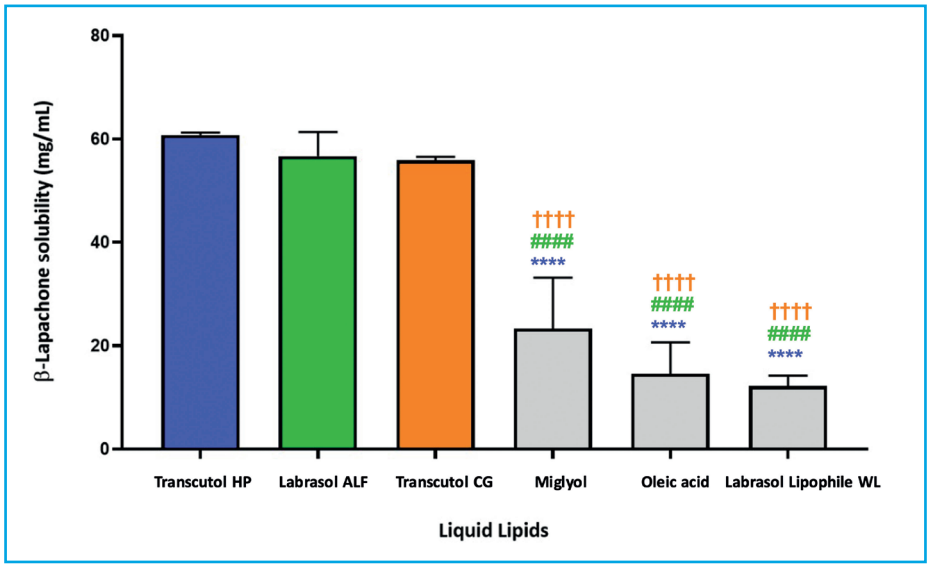

Figure 1. β-Lapachone solubility across different liquid lipids. Values are expressed as mean ± SD ($n = 3$). (****) $p < 0.0001$ vs. Transcutol® HP; (####) $p < 0.0001$ vs. Labrasol® ALF; and (††††) $p < 0.0001$ vs. Transcutol® CG.

Table II. NLCs characteristics 15 minutes and 14 days after their preparation and storage at 4 °C

	NLCs characteristics (t = 15 min)					NLCs characteristics (t = 14 days)		
NLCs	Particle size (nm)	PdI	ZP (mV)	EE (%)	DL (%)	Particle size (nm)	PdI	ZP (mV)
1	90.3 ± 37.9	0.29 ± 0.02	-13.0 ± 6.2	15.0 ± 2.5	0.4 ± 0.2	88.7 ± 39.8	0.24 ± 0.02	-12.2 ± 7.7
2	147.2 ± 76.2	0.34 ± 0.03	-18.9 ± 6.1	14.1 ± 16.8	0.4 ± 1.3	143.3 ± 85.7	0.36 ± 0.01	-19.2 ± 7.4
3	157.7 ± 77.0	0.28 ± 0.01	-15.7 ± 5.0	6.1 ± 0.2	0.1 ± 0.0	156.8 ± 71.6	0.29 ± 0.02	-16.9 ± 5.9
4	104.9 ± 38.9	0.26 ± 0.06	-18.5 ± 6.7	7.5 ± 0.0	0.2 ± 0.1	102.6 ± 38.4	0.26 ± 0.04	-15.4 ± 6.1
5	122.9 ± 52.7	0.23 ± 0.01	-11.5 ± 6.7	15.3 ± 4.0	0.7 ± 0.8	124.2 ± 49.1	0.23 ± 0.02	-11.1 ± 6.2
6	329.9 ± 193.4	0.32 ± 0.03	-18.6 ± 6.6	4.4 ± 1.4	0.1 ± 0.0	316.1 ± 195.1	0.34 ± 0.06	-18.0 ± 4.8
7	97.6 ± 38.4	0.27 ± 0.02	-14.9 ± 5.2	16.9 ± 9.6	0.9 ± 1.4	98.5 ± 38.4	0.26 ± 0.01	-10.0 ± 5.1
8	149.5 ± 67.4	0.31 ± 0.02	-15.9 ± 6.3	5.7 ± 0.3	0.1 ± 0.1	164.5 ± 96.9	0.31 ± 0.01	-15.8 ± 4.7
9	261.6 ± 151.7	0.38 ± 0.05	-18.1 ± 5.8	7.4 ± 6.2	0.2 ± 0.4	251.5 ± 163.2	0.36 ± 0.02	-18.4 ± 5.9

NANOSTRUCTURED LIPID CARRIERS MODELLING USING ARTIFICIAL INTELLIGENCE TOOLS AND FUNCTIONALIZATION

INForm® v5.01 software was employed to optimize the composition for β-Lap-encapsulating NLCs (NLC-β-Lap). The software selected the formulation that meet all pre-defined criteria, specifically, minimizing particle size and PdI after 14 days at 4 °C while maintaining a negative ZP for stability. The optimal formulation consisted of a 50:50 LL:SL ratio, 1.12 % (v/v) of Tween 80 and 1.17 % of lecithin (w/v). Predicted and experimental values for this formulation are shown in table III.

Table III illustrates the model's accurate prediction of particle size, PdI and ZP, with experimental values closely aligned with predictions. The stability of the formulations over 14 days was confirmed by the experimental PdI and ZP values. However, the model overestimated the drug loading capacity (DL) in agreement with former studies, the limited number of loaded formulations within the database limits the accuracy of the model. Consequently, while the model provides reliable initial estimations for particle size, PdI and ZP, further adjustments and experimental validations are necessary to improve its precision regarding DL.

In agreement with previous works (41,45,49), the mannose functionalization of NLCs yielded a shift in the surface charge to positive ZP values for both, blank (NLC) (+ 32.6 ± 5.9 mV) and β-Lap loaded formulations (NLC-β-Lap) (+ 24.1 ± 6.4 mV).

IN VITRO CELL STUDIES

Macrophages were treated for 24 h with NLC, NLC-β-Lap, and NLC-β-Lap-MAN at a final concentration of 300 µg/mL. Untreated cells were used as control. Cell viability is shown in figure 2A. Statistical analysis revealed no significant differences in cell viability among the formulations. Cell viability values were close to 100 % for all formulations, demonstrating high cytocompatibility, regardless of β-Lap incorporation. As expected, blank formulations (NLC) had no adverse effect on cell viability due to the GRAS (Generally Recognized as Safe) status of their excipients.

Since cell viability was consistent across all formulations, subsequent experiments were focused on drug-loaded formulations (NLC-β-Lap and NLC-β-Lap-MAN) (300 µg/mL). Internalization assays (Fig. 2B) demonstrated statistical significantly higher uptake for mannose-functionalized formulations (NLC-β-Lap-MAN) vs non-functionalized ones (NLC-β-Lap), confirming successful mannose coating and enhanced macrophages internalization, which is consistent with previous works (48,49).

Table III. NLCs characteristics predicted by the INForm® software and the experimental results

NLC predicted characteristics		NLC characteristics after 15 minutes		NLC characteristics after 14 days of storage	
Characteristics	Predicted values	Experimental values (NLC)	Experimental values (NLC-β-Lap)	Experimental values (NLC)	Experimental values (NLC-β-Lap)
Size (nm)-day 14	116.6	85.4 ± 33.4	89.2 ± 42.9	76.0 ± 25.3	77.8 ± 28.4
PdI-day 14	0.28	0.28 ± 0.02	0.25 ± 0.02	0.28 ± 0.03	0.22 ± 0.03
ZP (mV)-day 14	-18.5	-18.1 ± 6.6	-14.4 ± 7.9	-14.8 ± 5.3	-14.2 ± 6.0
DL (%)-15 min	0.5	–	0.01 ± 0.00	–	0.14 ± 0.02

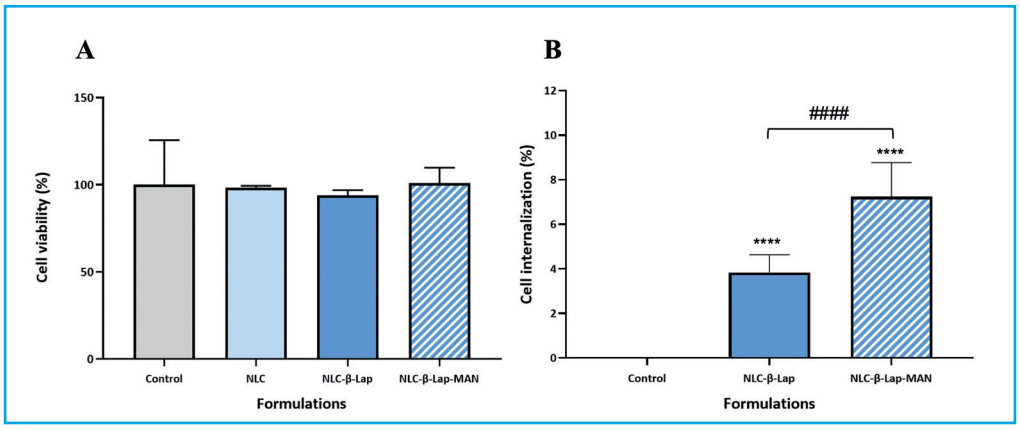

Figure 2. A. THP-1 cell viability after 24 h treatment with formulations (300 µg/mL). B. THP-1 internalization after 24 h treatment with formulations (300 µg/mL). Untreated cells were used as a control. Values: mean ± SD ($n = 6$). (****) $p < 0.0001$ vs. control; (####) $p < 0.0001$ across samples.

The impact of NLC-β-Lap, and NLC-β-Lap-MAN (300 µg/mL) on macrophage phenotypes was assessed by quantifying pro-and anti-inflammatory mediators' secretion from stimulated macrophages (TAMs) (Fig. 3).

The analysis of cytokine profiles (Fig. 3) revealed that both, NLC-β-Lap and NLC-β-Lap-MAN treatments significantly increased TNF-α and IL-6 expression vs LPS stimulated TAMs controls. No significant differences in those parameters were observed between NLCs treatments. Although IL-8 is well known to contribute to TAMs polarization toward an M2 phenotype, no significant differences were observed after treatment with the developed systems. In addition, NLC-β-Lap significantly altered the secretion of the anti-inflammatory cytokine IL-1RA while treatment with NLC-β-Lap-MAN increased the secretion of IL-13.

DISCUSSION

Osteosarcoma (OS) is the most prevalent bone primary malignant tumor where the immune system is intricately involved. Immune cells can either facilitate or inhibit tumor growth (17). Although, currently, chemotherapy is the first-line therapy, it presents several limitations such as drug resistance and associated side effects (10).

Macrophages present a high potential for immunomodulation as they can acquire opposing phenotypes (M1-TAMs or M2-TAMs), with different functions, including bone formation, bone regeneration, and bone homeostasis (50,51). Moreover, β-Lap, a natural naphthoquinone, exhibits anti-tumoral activity against different types of cancer including OS and has demonstrated the ability to modulate intracellular ROS levels and macrophage responses (31-33).

Modulating TAMs profiles with β-Lap treatment may induce a pro-inflammatory phenotype, creating an anti-tumor environment and inhibiting tumorigenic cell proliferation (35). Therefore, β-Lap was encapsulated in nanostructured lipid carriers. To optimize NLCs preparation, various liquid and solid lipids were tested for drug solubility. Based on the solubility studies Transcutol® HP and Compritol® 888 ATO (COMP) were selected (Fig. 1). AI tools (ANN+GAs) were used to establish the optimal NLCs composition of the NLCs based on a previously generated database. The resulting models effectively predicted the nanoparticles physicochemical properties. The developed NLC and NLC-β-Lap formulations exhibited particle sizes < 100 nm and uniform size distributions (PdI < 0.3). Moreover, moderate negative zeta potential (ZP < -15 mV) values, indicative of colloidal stability, were achieved (47). Additionally, no significant differences were observed between the freshly prepared formulations and those stored at 4 °C for 14 days, confirming the absence of aggregation during storage, and therefore, their stability (45). The INForm® v5.01 model accurately predicted particle size, PdI, and zeta potential but overestimated the loading capacity of the systems. Despite the high solubility of β-lapachone in the selected lipids, low DL and EE values were obtained. Despite the limited loading capacity of the developed NLC, the systems exhibited therapeutic effects due to the high potency of

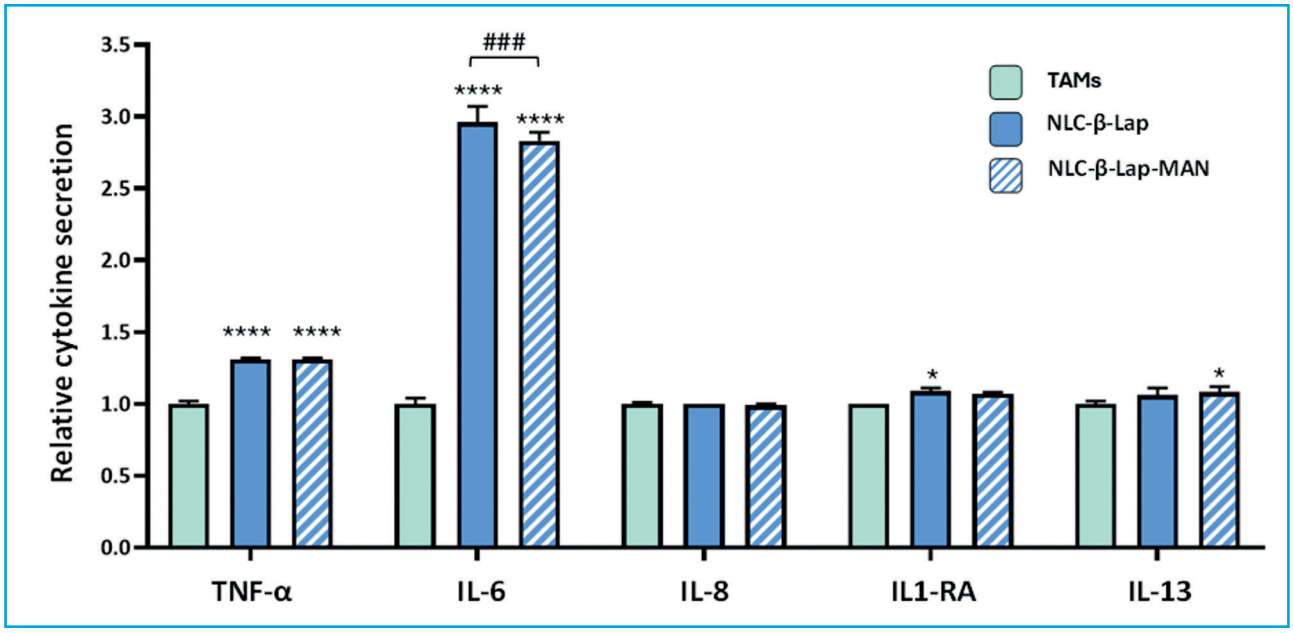

Figure 3. Relative pro-inflammatory (TNF-α, IL-6, IL-8) and anti-inflammatory (IL1-RA, IL-13) cytokines release from TAMs (100 ng/mL) 24 hours post-treatment with NLC-β-Lap and NLC-β-Lap-MAN formulations relative to untreated LPS - stimulated cells. Values: mean ± SD (n = 3). (****) p < 0.0001 vs. TAMs; (*) p < 0.05 vs. TAMs; and (###) p < 0.001 across samples.

the drug. β-Lap is active at low concentrations showing IC50 values in tumor cells between 0.06 μM and 48.94 μM (52,53). The assayed NLCs were able to obtain a drug concentration of 649 μM, that is, between 10,816 and 13.26-fold higher than the required therapeutic concentration. Strategies for enhancing drug encapsulation efficiency could include the use of a blend of different liquid lipids and solid lipids instead of selecting single LL and SL (54). Moreover, the model's capacity to predict the drug loading is restricted by the highly reduced sample number, which is an inherent constraint of the reduced experimental design (45).

Shifting macrophage polarization towards a pro-inflammatory phenotype (M1-TAMs) has the potential to decrease OS tumor growth by cellular strategies (21,25). Macrophages present recognition factors, including mannose receptors, that mediate the uptake and internalization of compounds through receptor-mediated endocytosis (55,56). To exploit this mechanism, the developed NLCs were coated with the monosaccharide mannose.

Mannose functionalization required incorporation of stearylamine (SA), which contains free amino groups that facilitate Schiff base formation ($^{-}N = CH^{-}$) with the aldehyde group of mannose (57). This modification resulted in a shift from negative to positive zeta potential values. Despite mannose inherent negative charge, the excess of unreacted amino groups on the nanoparticle surface led to the observed ZP shift in both blank and β-Lap loaded formulations in agreement with previous works (41,45,49).

All formulations show cell viability values close to 100 %, indicating the high cytocompatibility of the developed formulations. Statistical analysis revealed no significant differences in cell viability among the formulations, concluding this type of NLCs does not negatively affect cell viability.

Internalization assays demonstrated mannose-functionalized NLC-β-Lap-MAN formulations exhibited nearly twice the cell uptake vs non-functionalized formulations (NLC-β-Lap). These results confirm the successful mannose coating and enhanced macrophage internalization after functionalization.

In agreement with these findings Vieira et al. (49) demonstrated a 14.5-fold increase in cellular uptake of mannose-coated vs uncoated NLCs, as determined by fluorescence microscopy and flow cytometry. Despite quantitative differences between studies, the data strongly support the conclusion that mannose functionalization significantly enhances NLCs internalization in macrophages.

Using a tumor-associated macrophage model, the treatment with NLC-β-Lap and NLC-β-Lap-MAN significantly increased IL-6 and TNF-α secretion, indicating a further pro-inflammatory M1-like polarization. However, no increase was observed for IL-8 secretion and a less marked enhancement in IL-1RA and IL-13 secretion was observed, further supporting this M1-like profile. Full confirmation of the macrophage phenotype switch will be performed in future studies using RT-qPCR and immunofluorescence.

Given the stablished role of macrophages in OS tumor metastasis (19-21), repolarizing them towards a pro-inflammatory phenotype or a M1-TAMs phenotype can be an excellent strategy to control OS. The administration of β-Lap loaded NLCs has the potential to enhance drug circulation time and tumor accumulation, facilitating TAMs phenotype modulation and creating an anti-tumoral environment (28). Additionally, the mannose functionalization of the NLCs significantly improve internalization by macrophages, promoting their repolarization toward a pro-inflammatory phenotype (M1-TAMs phenotype).

CONCLUSIONS

NLCs loaded with the anti-tumoral drug β-Lap and surface-functionalized with mannose were successfully developed for targeted drug delivery. AI tools, specifically ANNs, proved effective to accurate predict particle size, polydispersity index, and zeta potential of NLCs. However, drug loading predictions were less accurate, indicating the need for further model refinement or exploration of alternative loading strategies.

Mannose-functionalized NLCs exhibited enhanced macrophage uptake, crucial for targeted delivery to tumor-associated macrophages. Despite suboptimal drug loading, these systems stimulated the secretion of pro-inflammatory cytokines IL-6 and TNF-α, inducing macrophage polarization towards an anti-tumor phenotype. This suggests that even with lower drug concentrations, the targeted delivery and macrophage activation achieved with these NLCs hold significant therapeutic potential for osteosarcoma.

Future studies should be focused on optimizing drug loading efficiency through the screening of other raw materials or loading techniques. Additionally, *in vivo* studies are needed to evaluate the therapeutic efficacy of these mannose-functionalized NLCs in relevant OS models. The successful application of AI tools in predicting key NLC properties underscores their value in accelerating drug delivery system development.

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Artículo Especial

Actualización de las guías de manejo de la osteoporosis de la SEIOMM: abaloparatida*

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Resumen

Introducción: la osteoporosis es un trastorno de elevada prevalencia. La SEIOMM publicó en 2022 unas guías de manejo de estos pacientes. La reciente comercialización en Europa de un nuevo fármaco, la abaloparatida, hace aconsejable considerar su papel dentro de las opciones terapéuticas.

Objetivo y resultados: en este artículo se resume la información existente sobre la eficacia y la seguridad de la abaloparatida y se actualizan los algoritmos terapéuticos propuestos en la guía.

Conclusión: la abaloparatida es un fármaco osteoformador con eficacia y seguridad similares a la teriparatida. Representa una nueva opción en el tratamiento de la osteoporosis grave con muy alto riesgo de fractura.

Palabras clave:
Osteoporosis.
Fracturas.
Abaloparatida.
Guías clínicas.

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***Los miembros de la Comisión de Redacción de las Guías de Osteoporosis de la SEIOMM se presentan en el Anexo.*

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INTRODUCCIÓN

Después de la publicación de las guías de manejo de la osteoporosis de la Sociedad Española de Investigación Ósea y del Metabolismo Mineral (SEIOMM) en 2022 (1), se ha aprobado la comercialización de la abaloparatida en España y otros países europeos. Esto aumenta el número de fármacos osteoformadores disponibles para el tratamiento de la osteoporosis y hace conveniente una actualización de dichas guías. A tal fin, el grupo de trabajo que elaboró las guías ha evaluado los estudios disponibles acerca de la eficacia y seguridad de la abaloparatida y su papel dentro de los esquemas de tratamiento propuestos. Para ello, el comité designado por la SEIOMM revisó la literatura existente sobre la abaloparatida y elaboró un borrador que fue después sometido a la discusión por el resto de la Comisión de redacción de las guías (Anexo). Se exponen aquí sus conclusiones de forma resumida.

La abaloparatida es un péptido sintético análogo de los 34 primeros aminoácidos del péptido humano relacionado con la hormona paratiroidea (PTHrP) que pertenece al grupo de agentes osteoanabólicos. Actúa a través de la activación del receptor 1 de la PTH (PTH1R), favoreciendo la diferenciación de los precursores osteoblásticos e inhibiendo la apoptosis de los osteocitos. La activación del PTH1R en esas células también induce la expresión del ligando del receptor activador del factor nuclear kappa-B (RANKL), de modo que indirectamente estimula la osteoclastogénesis. Desde el punto de vista mecanístico, la abaloparatida se diferencia de la teriparatida en su mayor afinidad por la unión a la conformación R^G del PTH1R, lo que hace que la respuesta intracelular sea menos duradera que cuando la activación se realiza a través de la conformación R^0, que es la que utiliza la teriparatida. Se ha sugerido que ello se traduciría en una menor inducción de la producción de RANKL y resorción ósea por la abaloparatida (2). Este fármaco se administra por vía subcutánea, a dosis de 80 µg/día, durante un máximo de 18 meses.

EFECTO SOBRE LA DENSIDAD MINERAL ÓSEA (DMO) Y LAS FRACTURAS

La eficacia de la abaloparatida en mujeres posmenopáusicas con osteoporosis se evaluó en el ensayo ACTIVE, que comparó el tratamiento con abaloparatida frente a placebo (3). Asimismo, se comparó con una rama abierta de mujeres tratadas con teriparatida. Después de 18 meses de seguimiento, en comparación con el placebo, la abaloparatida redujo el riesgo de fracturas vertebrales (riesgo relativo [RR]: 0,14; IC 95 %: 0,05-0,39). También disminuyó el riesgo de fracturas osteoporóticas mayores (*hazard ratio* [HR]: 0,30; IC 95 %: 0,15-0,60), de fracturas clínicas (HR: 0,57; IC 95 %: 0,35-0,91) y de fracturas no vertebrales (HR: 0,57; IC 95 %: 0,32-1,0; p = 0,049). Por tanto, se considera indicada para el tratamiento de la osteoporosis en mujeres posmenopáusicas que presentan un elevado riesgo de fractura (Fig. 1).

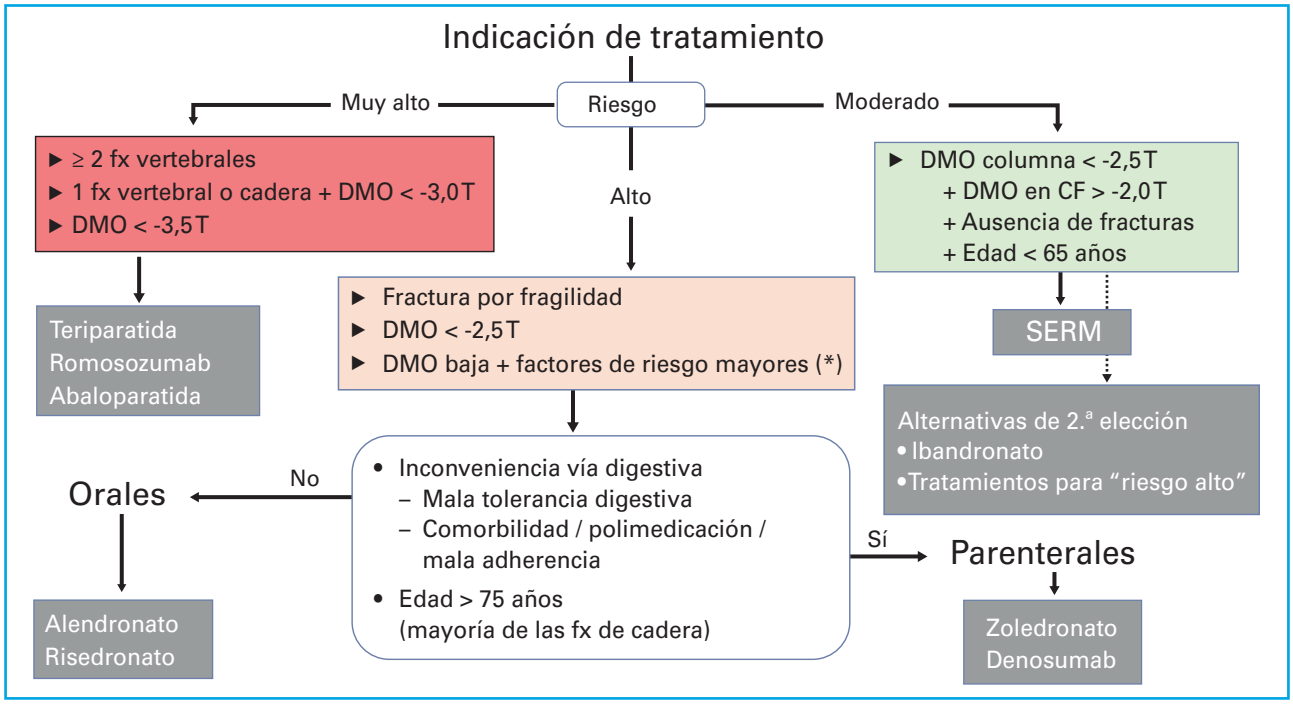

Figura 1. Manejo terapéutico inicial. CF: cuello de fémur; DMO: densidad mineral ósea; SERM: moduladores selectivos de los receptores estrogénicos; T: *T-score*.

Asimismo, la abaloparatida aumentó la DMO en todas las localizaciones, en torno al 8-11 % en la columna y el 3-4 % en la cadera, en comparación con el placebo. El estudio ATOM mostró ganancias en DMO también en hombres (4), pero no cuenta de momento con aprobación para la osteoporosis del varón en Europa. Al igual que con otros osteoformadores, tras finalizar el tratamiento con abaloparatida se aconseja administrar un antirresortivo (un bisfosfonato generalmente), lo que mantiene la reducción del riesgo de fractura (5) (Fig. 2).

EFECTOS ADVERSOS

La abaloparatida es un fármaco generalmente bien tolerado. Los efectos adversos señalados con mayor frecuencia son náuseas, cefalea, artralgias, cambios en la presión arterial (hipertensión y, especialmente, hipotensión ortostática), taquicardia, palpitaciones, hiperuricemia, hipercalcemia e hipercalciuria. No suelen obligar a suspender el tratamiento (6). No obstante, dada la posibilidad de hipotensión ortostática, se recomienda en la ficha técnica que las primeras dosis se administren bajo la supervisión de un profesional sanitario. Por último, este fármaco se considera contraindicado en pacientes con riesgos conocidos de osteosarcoma, como los que han recibido radioterapia, y en los que tienen neoplasias con afectación esquelética.

COMPARACIÓN DE ABALOPARATIDA CON OTROS ANABÓLICOS

En cuanto a la DMO, en el estudio ACTIVE la abaloparatida y la teriparatida tuvieron un efecto similar en la columna lumbar, pero a nivel de la cadera el incremento fue algo mayor con la abaloparatida (diferencia 0,8 % a los 6 meses, $p < 0{,}001$), al parecer a expensas de un mayor efecto sobre el hueso cortical (3).

Al igual que la teriparatida, la abaloparatida induce un aumento de los marcadores de formación ósea. Al cabo de 2-3 meses se observa también un incremento de los marcadores de resorción. El incremento en ambos tipos de marcadores es menor con abaloparatida que con teriparatida.

Solo en el estudio ACTIVE se llevó a cabo una comparación directa de la abaloparatida con otro osteoformador. Cuando se compararon los efectos antifractura

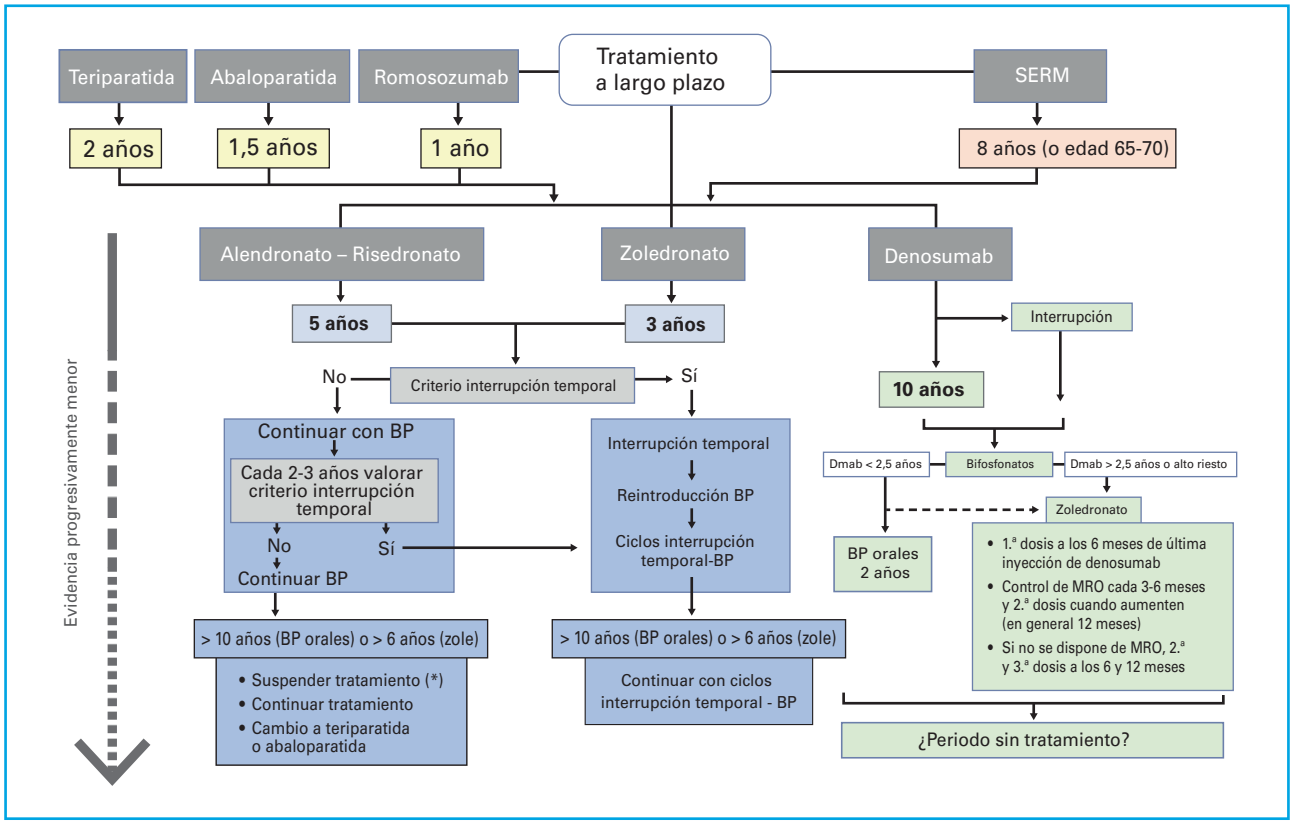

Figura 2. Manejo a largo plazo. BP: bisfosfonatos; DMAB: denosumab; MRO: marcadores de remodelado óseo; SERM: moduladores selectivos de los receptores estrogénicos.

de la abaloparatida frente a la teriparatida, se observó que ambos tratamientos eran generalmente equiparables en la reducción de la incidencia de fracturas vertebrales, fracturas no vertebrales y fracturas clínicas, pero la abaloparatida resultó ser superior en la reducción de fracturas osteoporóticas mayores (1,5 % frente a 3,1 %, p = 0,03) (3). En la misma línea, dos estudios "de vida real" realizados a partir de la misma base de datos americana han sugerido que la abaloparatida reduce el riesgo de fracturas periféricas algo más que la teriparatida. Aunque se hicieron ajustes por diversas características clínicas *(propensity score),* su carácter retrospectivo y no aleatorizado supone una importante limitación (7,8).

Dado que los datos derivados de comparaciones directas son muy limitados, varios metaanálisis "en red" han tratado de comparar los efectos de diversos osteoformadores. Los resultados no revelan de manera consistente diferencias entre los efectos de teriparatida, abaloparatida y romosozumab sobre el riesgo de fractura (9-13).

En cuanto a los efectos secundarios, en el estudio ACTIVE, abaloparatida y teriparatida mostraron un perfil similar. Si bien la frecuencia de hipercalcemia fue algo mayor con teriparatida (6,4 % frente al 3,4 %), la frecuencia de efectos adversos que llevaron a la suspensión del tratamiento fue algo mayor en el grupo tratado con abaloparatida (9,9 frente al 6,8 %).

Por tanto, la abaloparatida y la teriparatida son fármacos con perfiles de eficacia antifractura y seguridades similares. Aunque pueden ocasionar cambios en la presión arterial en algunos pacientes, no se asocian a efectos graves y ambos se consideran seguros desde el punto de vista cardiovascular (14). Además de las contraindicaciones específicas, debe valorarse cuidadosamente su empleo en pacientes con historia de tumores (como los de mama, pulmón o próstata), con elevada tendencia a metastatizar en hueso.

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CONFLICTO DE INTERESES

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