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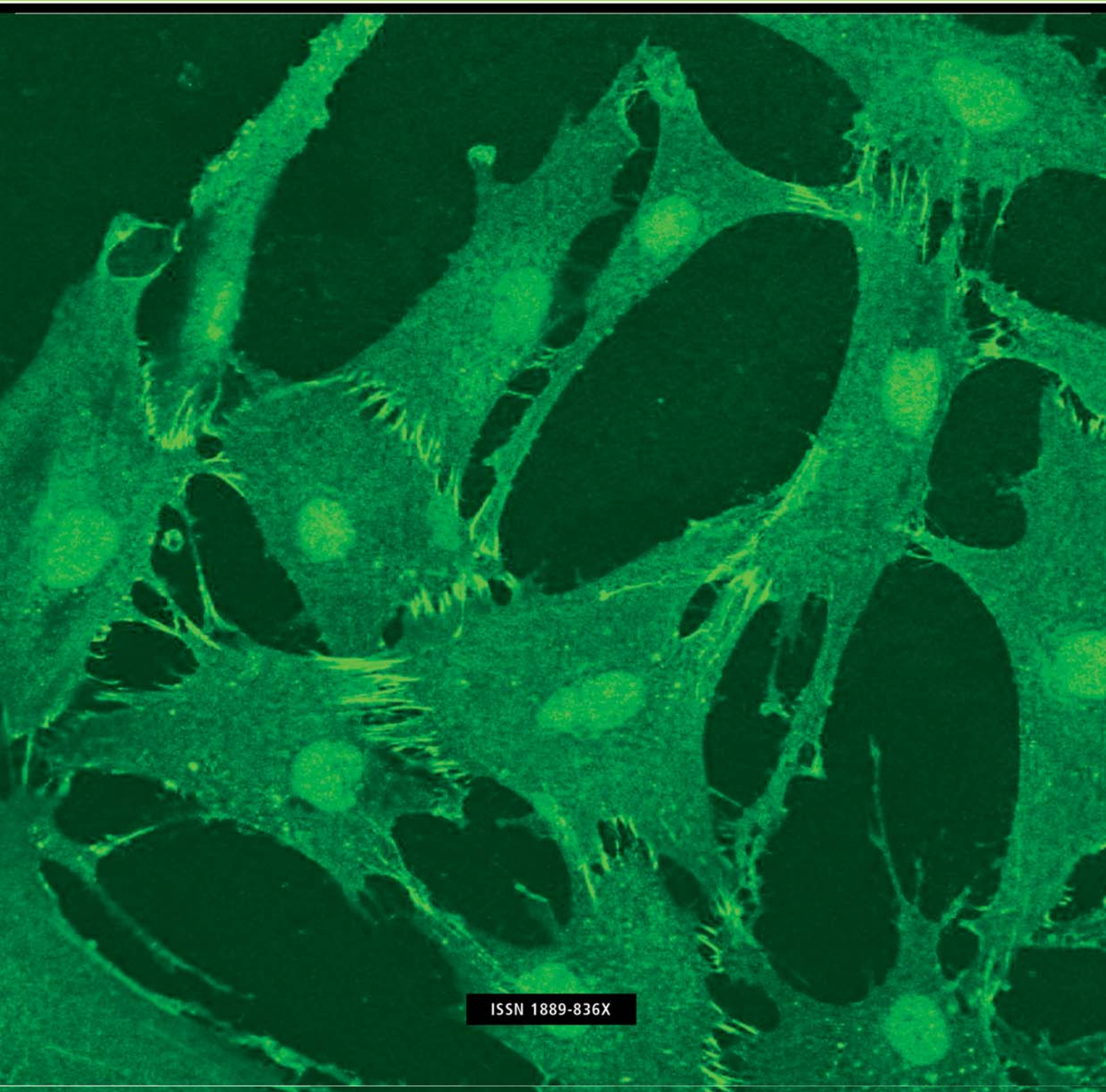

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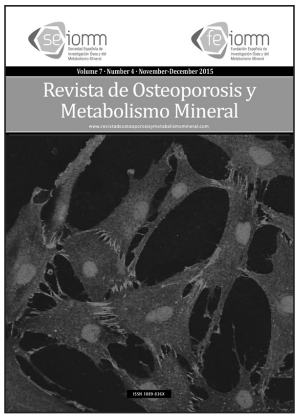

Our cover
Human osteoblasts in culture. Labeling with an anti-betacatenine antibody reveals the presence of this in the nuclei and junctions

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SUMMARY

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Gastric protection or bone protection? The dilemma of proton-pump inhibitor

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The arrival of H2 inhibitors and later the proton-pump inhibitor (PPI) changed the clinical course of gastroesophageal disease, greatly reducing the rate of peptic ulcers and their complications. At present they are used in a high proportion of patients with diverse clinical situations[1]. They are recommended to treat gastro-esophageal reflux, Helicobacter pylori, Zollinger-Ellison syndrome, duodenal ulcer, gastric ulcer and NSAID-induced peptic ulcer. Their proven benefits in preventing ulcers and encouraging good tolerance have led them to be considered as a popular, safe "gastric protector", with little adverse effects and used in many situations without indication.

But do not forget that the blockade of acid secretion by PPIs is the cause of some undesirable effects[2]. Increased intestinal and systemic infections have been attributed to decreased gastric acid secretion and their bactericidal capacity. Other infections, such as pneumonia, are also more common among patients treated with PPIs. B_{12} production capacity and intestinal absorption may be reduced by malabsorption. A reduced of antiplatelet effect of clopidogrel therapy has also been described. Some cancers, especially colon cancer, could be more frequent. Finally, it is worth noting the increased risk of fracture in patients treated with long-term PPI. In this issue, a study by Vera Rodríguez et al.[3] on its possible association with increased fractures in the population of the Canary Islands is presented, confirming the increase in non-traumatic fractures in patients over 50 years undergoing long-term PPI treatment compared to those who have never taken these medications.

PPIs' link to fractures has drawn attention in the past 10 years, long after they came on the market. Several observational studies have shown a relationship between PPI consumption and the presence of osteoporotic fractures of the hip, vertebrae and wrist. Generally, they are associated with high-dose treatments or periods longer than 12 months. The results are contradictory as not all studies have confirmed these findings. The studies are methodologically heterogeneous and have numerous confounding variables, which explained the lack of consistency in the findings. Unfortunately, there are no controlled studies, since this side effect was not detected in clinical trials on long-term use of PPIs, mainly because they were not designed to assess fragility fractures.

In 2011, some meta-analysis based on epidemiological cohort and case-control studies linked chronic use of PPIs to increased fragility fractures in postmenopausal women and older men. Given this evidence, drug regulatory agencies, including the FDA (Food and Drugs Administration) and the EMA (European Medicines Agency), decided to issue warnings about this risk.

In the meta-analysis by Ngamruengphong et al.[4], 10 observational studies (4 cohort and 6 control) with a population of men and women among which more than 200,000 fractures were included. In patients who had used PPIs fracture risk it was increased, with an OR of 1.25 (confidence interval (95% CI: 1.14 to 1.37) for hip fracture, 1.50 (CI 95% CI 1.3 to 1.72) for vertebral fracture and 1.09 (95% CI. 0.95 to 1.24) for the wrist. These studies' heterogeneity limited the analysis of the influence of other factors related to PPI treatment. The results of a second meta-analysis coincided with those of previous two items were included[5] and also analyzed studies with patients treated with H2 receptor inhibitors (IRH2). Their results were consistent with the meta-analysis of Ngamruengphong et al., and also found increased risk of fracture, of similar magnitude in patients with PPI The OR for vertebral fracture was 1.5 (95% CI: 1.32 to 1.72) and hip fracture of 1.23 (95% CI: 1.11 to 1.36), while for the total fractures of 1.20 (95% CI: 1.11 to 1.30). However, it was confirmed that there was no association between treatment with IRH2 and fractures.

In both studies the increase in fractures is proportionally discrete after adjusting for various risk factors. However, to extrapolate these results to the general population, in which the use of PPIs in the population at risk of osteoporosis and fracture is common, the weight of this adverse effect is important. These studies had considerable heterogeneity in relation to risk intensity and duration of treatment until the appearance of fractures, and some variability in relation to confounding factors for which the results were adjusted. Despite these limitations, the occurrence of fractures has been observed especially in patients at higher doses, greater grip and longer duration of PPI therapy. Overall, this increased fracture rate is independent of other risk factors of fracture, including bone mineral density. But some authors have found that in addition to chronic use of PPIs, the use of tobacco is a risk factor for hip fracture in postmenopausal women[6].

Subsequent data have reinforced this evidence. In recent years, several prospective cohort studies have been published. Including a Canadian cohort study (CaMos) including men and women which found that, after 10 years of follow up, patients who used PPIs presented an increase of non-traumatic incidental fractures independent of many known risk factors. The risk of fracture was calculated by hazard ratio (HR) at 01.75 (95% CI: 1.41 to 2.17) for total fracturas[7]. After adjusting for various risk factors, including bone mineral density of the femoral neck, the link remained significant, with an HR of 1.40 (95% CI: 1.11 to 1.77). In another cohort study in postmenopausal women (Australian Longitudinal Study on Women's Health) data from 4,432 women followed over more than 10 years were evaluated. They found an increase in fractures associated with osteoporosis (HR=1.29 95% CI: 1.08 to 1.55). In this study, this association was evaluated according to the drug used, finding that the risk was increased with the use of any PPI, especially esomeprazole (HR=2.06, 95% CI 1.37 to 3.10)[8]. In a case-control study of more than 6,500 males over 45 years treated with PPIs the relationship with increased risk of fracture, especially those whose long-term consumption was confirmed[9].

The active mechanism by which PPIs increase fracture risk is unknown, but some hypotheses have been proposed decreased intestinal absorption of calcium and vitamin B_{12} or direct action of PPIs on the proton pump of osteoclasts. Decreased intestinal absorption of calcium and other minerals is based on experimental and human studies. Intestinal calcium carbonate absorption is pH-dependent and some studies indicate that in patients with hypo- or achlorhydria, such absorption is reduced, especially in fasting elderly women[10]. This alteration of calcium absorption is unproven in men below 50 years[11]. Inhibition of gastric acid secretion could help reduce vitamin B_{12} absorption and thus facilitate CBS deficiency. Reducing homocysteine could hinder incorporation into bone collagen and therefore facilitate fractures.

Some experimental data indicate that the action of PPIs could have direct influence on bone cells. Here, osteoclastic action may suffer due to the inhibition of proton pump available to osteoclasts. This would lead to limited metabolic remodeling. There is no information on what the actual clinical significance of this action may be. Furthermore, the action of PPIs in the bone could be related to an increase of histamine, as the H1 receptor blockade prevents the increased risk of fracture induced by PPI[12].

However, the increased fracture risk observed in patients treated with these drugs does not seem linked to reduced bone mineral density or bone loss acceleration. Some studies have found that patients starting treatment with PPIs have low bone mineral density, but this does not change significantly during their treatment[13].

In view of all these data, the EMA and the Spanish Agency for Medicines and Health Products (March 2012) issued alerts about the safety of PPIs, which warned of the increased risk to the hip, spine and forearm in patients having long-term treatment (over 1 year) with PPI.

The benefits of PPIs in treating problems associated with gastrointestinal reflux and peptic ulcer disease in general are proven. In these cases, the risk-benefit ratio is favorable to the latter. However, as noted by Vera Rodriguez et al. in their paper[3], in a high proportion of patients treated although treatment indication is not clear. In many cases, it has been assumed that the risk of peptic ulcer prevention in patients treated with NSAIDs could be extrapolated to patients with polypharmacy, but this possibility is not proven. In patients for whom the benefits are uncertain, fracture risk, however small, is not acceptable.

One might suppose that the risk induced by PPIs could be controlled with such anti-fracture medications as bisphosphonates (BSF). However, the available data do not support this hypothesis. In a recent meta-analysis, Yang et al. analyzed four studies involving over 57,000 patients, noting the potential benefits of the combination of PPI and BSF. Contrary to expectations, this combination has more risk of fracture than PPIs alone (OR=1.52). The risk of vertebral fracture has an OR of 1.60 (95% CI: 1.13 to 2.26)[14].

With all these data, it seems reasonable to assert that PPI therapy presents a risk factor of fracture that is increased by between 9-75%. This undesirable effect is a classic effect because it is maintained when the different PPIs are analyzed separately. This risk may not be annulled by the concomitant use of BSF. There is no contraindication to the use of PPIs in patients with osteoporosis or at risk of fracture. However, in view of the available data, we should act wisely when deciding whether to recommend the blocking the production of gastric acid secretion that PPI use implies, especially in postmenopausal women and men over 50 years.

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Factors related to bone forming inadequate response to treatment (teriparatide/PTH 1-84) in patients with severe osteoporosis. Preliminary results

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Summary
The aim of this study was to evaluate the long-term bone mineral density (BMD) response rate to osteoanabolic treatment in patients with severe osteoporosis and the factors related to "inadequate" response (IR).
Methods: 49 patients (46F:3M) with a mean age of 69.5±11.1 years treated with teriparatide (41) or PTH1-84 (8) during 18/24months were included (84% had vertebral fractures and 84% had previously received bisphosphonates). Previous skeletal fractures and antiosteoporotic treatment, risk factors and cause of osteoporosis were recorded in all patients. Bone turnover markers (BTM) and 25-OH vitamin D (25OHD) levels were assessed before and at 3, 6, 12 and 18/24 months. Lumbar and femoral BMD and spinal X-ray were assessed at baseline and at 12 and 18/24 months. IR was defined by a lumbar BMD change <3% at 18/24 months.
Results: 29% of patients showed IR to therapy. No significant differences were observed in age, baseline BMD and BTM and 25OHD levels between patients with or without IR. 92% of IR patients had been previously treated with bisphosphonates (vs 79%, p=0.34) during 7±4.8 years (vs 4.9±4.2 years, p=0.19). No significant differences were observed between groups in the magnitude of changes in BTM throughout the study.
Conclusions: 29% of patients with severe osteoporosis presented IR to osteoanabolic therapy. Although no predictive factors related to this finding were identified, previous prolonged therapy with bisphosphonates may play a role.

Key words: *osteoporosis, adequate response, teriparatide, PTH, bone metabolism, bone turnover markers.*

Introduction

The therapeutic effectiveness of most anti-osteoporosis treatments recommended in clinical practice guidelines and in clinical trials is high, especially when evaluated by measuring bone mineral density (BMD). However, in routine clinical practice, therapeutic failure is relatively common, particularly when assessed individually. In this sense, between 18 and 35% of patients treated with antiresorptive medications, mainly bisphosphonates (depending on the criteria) have a failure response and/or inadequate response to treatment[1-3]. Although non-adherence is often one of the main causes of inadequate response, other factors such as comorbidities, previous osteoporosis treatment, disease severity or vitamin D deficiency, among others, may influence response to antiosteoporotic treatment[1-3].

Similarly, bone forming treatment with parathyroid hormone (PTH) and/or teriparatide has been associated with a marked increase in BMD, as high as 10.5% at the lumbar spine after 18 months of treatment, and a decrease in the incidence of vertebral fractures (65%)[4-6]. It noteworthy that in this study (baseline testing), which included only patients with severe osteoporosis, teriparatide treatment was associated with a significant BMD increase in the lumbar spine (>3%) in 94% of patients at 18 months of treatment. Therefore, this agent is especially recommended for patients with severe osteoporosis and multiple fractures and/or inadequate response to other treatments[7]. However, as with antiresorptive treatments in clinical practice, some patients present a lack of response to this treatment, with figures ranging from 8% to 32%[8-10]. While the cause of this poor response to bone forming treatment is unclear, factors such as bone turnover and BMD baseline value, previous use of bisphosphonates and the initial response of bone turnover markers to treatment, have been linked to the magnitude of the BMD response to this long-term therapy[8,9,11,12]. In fact, some authors recommend quantifying the change in PINP values (a marker of bone formation) after starting teriparatide treatment. This indicates that if there is an increase of >10 ng/ml after 3 months of starting treatment, BMD will increase significantly in the long term[13-16].

Currently, both the incidence and factors related to inadequate response to bone forming treatment are poorly understood. Given the specific indications for this type of treatment, especially in patients with severe osteoporosis and multiple fractures, it is necessary to identify factors that may affect its therapeutic efficacy.

Therefore, the aim of this study was to analyze the long-term evolution of BMD after bone forming treatment (teriparatide or PTH 1-84) in patients with severe osteoporosis, and determine the frequency and factors associated with an inadequate response to treatment.

Patients and methods

Study Population

This retrospective study included all patients who followed a bone forming treatment (teriparatide or PTH 1-84) for 18 or 24 months in Bone Metabolism Unit of the Rheumatology Ward of the Clinic Hospital of Barcelona. Patients treated from 2006 to January 2014. All patients treated with teriparatide or PTH followed a clinical protocol that involved:

- *Analysis of risk factors for osteoporosis:* a family history of hip fracture, personal history of fractures, tobacco and alcohol consumption, dietary calcium intake (mg/day) and a history of kidney stones.

- *Assessment of the cause of osteoporosis, comorbidities and concomitant therapy:* including glucocorticoid treatment, and the presence, type and duration of the osteoporosis treatment previously received. Any patient treated for more than 5 years was considered to have undergone prolonged osteoporosis treatment.

- *Analytical determinations:* blood was taken between 8 and 10 am, after an overnight fast, at baseline (before the start of treatment) and at 3, 6, 12 and 18 or 24 months of treatment, performing biochemical profile including calcium, phosphate, creatinine, total alkaline phosphatase (FAT), levels of 25-hydroxyvitamin D (25OHD) and PTH, determined by standard techniques.

Also, the following biochemical markers of formation were determined: bone alkaline phosphatase (bone FA, IDS, Vitro) and aminoterminal propeptide of type I procollagen (PINP, automated method Cobas E411, Roche), and bone resorption: carboxy terminal telopeptide of type I collagen (CTx, automated method Cobas E411, Roche) and amino-terminal telopeptide of type I collagen (Osteomark NTxUrine, ELISA).

- *Quantification of BMD:* BMD of the lumbar spine and proximal femur (hip and femoral neck) was measured in all patients by dual X-ray absorptiometry (DXA, Lunar Prodigy, Radiation Corporation, Madison, Wisconsin, USA) at baseline and at 12 and 18 or 24 months of treatment. Densitometric risk categories (normal BMD, osteopenia and / or osteoporosis) were defined according to WHO criteria[17].

- *Radiological study:* X-rays of dorsal and lumbar spine (anteroposterior and lateral) were performed at baseline and at 12 and 18 or 24 months after commencing treatment. Baseline vertebral fractures were identified during follow-up, according to Genant criteria[18].

The study was carried out with the approval of the Hospital Ethics Committee.

Statistical analysis

"Inadequate response" was defined as a decrease or an increase in the lower lumbar BMD 3% at 18/24 months after treatment[9]. In addition, the percentage of patients with increased values of PINP >10 mg/ml at 3 months after starting bone forming treatment was analyzed[13]. Only patients who continued treatment with teriparatide vs PTH over 18/24 months were included.

The results were expressed as mean ± standard deviation of the mean (SD). Differences between

means of continuous variables were analyzed using the Mann-Whitney non-parametric test, and differences between proportions by Fisher test. The Wilcoxon test was used for comparison between paired variables. Pearson correlation coefficient was used to assess the association between variables. The p value <0.05 was considered statistically significant. Statistical analysis of the data was performed using SPSS software (version 18.0, Chicago, USA).

Results

Figure 1 shows the patients' flowchart. In all, 63 patients were included, of which 49 completed the 18/24 months of treatment. The clinical characteristics of the patients included are shown in Table 1. Briefly, 46 females and 3 males with a mean age of 69.5±11.1 years; 41 were treated with teriparatide and 8 patients with PTH 1-84 for 18 or 24 months. 84% of patients had previous vertebral fractures, with an average of 5 fractures per patient, and 84% had received previous treatment with bisphosphonates. The main cause of postmenopausal osteoporosis was in 32 patients (65%), followed by glucocorticoid osteoporosis (n=11; 22%) and miscellaneous in 6 patients.

In all, 29% of patients presented an inadequate response to treatment within 18/24 months. As shown in Table 1, there were no differences in age, baseline in BMD and markers of bone formation and resorption. No differences in 25OHD values were reported between patients with and without adequate response to treatment. Although there was no significant difference in the percentage of patients previously treated with bisphosphonates or in previous duration of the treatment between the two patient groups. Patients with inadequate response to bone forming treatment had previously undergone bisphosphonate therapy (92% vs . 79%, p=0.34) over a longer period (7±4.8 vs 4.9±4.2 years, p=0.19) (Table 1).

As expected, significant differences in the evolution of BMD between the two groups of patients (Figure 2) were observed. Thus, patients with inadequate response to treatment showed a loss of BMD in both lumbar spine (lumbar BMD: -2.7% at 12 months, p=0.646; 3.4% at 24 months, p=0.021) and proximal femur (BMD total femur. -2.8% at 12 months, p=0.261; 0.6% at 24 months, p=0.475 femoral neck BMD: -1.7% to 12 months, p=0.477; -3.12% at 24 months, p=0.333), whereas patients with adequate response showed a significant increase in lumbar spine BMD (12 months 9.4%, p<0.001 ; 24 months: 12.8%, p<0.001) and femoral BMD (total femur 12 months: 4.02%, p=0.008; 24 months: 4.5%, p=0.001; femoral neck 12 months: 2.7%, p=0.049; 24 months: 6.8%, p<0.001).

No significant differences were observed in the evolution of the markers of bone formation and resorption between both groups of patients throughout the study (Table 2). No differences were observed in the percentage of patients with increased PINP values >10 ng/mL at 3 months treatment in both groups of patients (inadequate response: 82% adequate response vs 91%, p=0.422), or the evolution of values 25OHD during follow-up (Table 2).

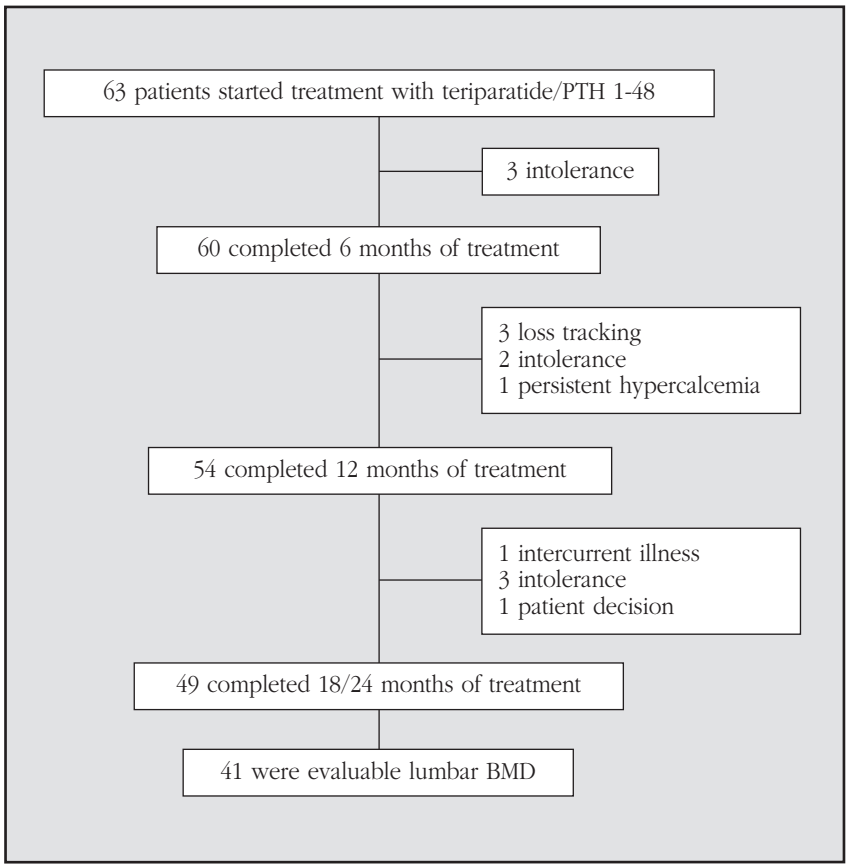
The incidence of fragility fractures (≥2) was similar in both groups of patients (14% vs 16%, p=ns). The change in lumbar spine BMD and/or femoral to 18/24 months was not associated with changes in the value of markers of formation and/or resorption at the start of treatment (3 and 6 months of treatment) (data not shown).

Discussion

Preliminary results of this study demonstrate that a relatively high percentage of patients with severe osteoporosis present an inadequate response to bone forming treatment when analyzed by measuring BMD. Thus, about 30% of patients included in this study had an inadequate response, with no significant increase in BMD at 18/24 years of treatment. However, it should be noted that these patients had multiple fractures associated with osteoporosis and also had undergone previous treatment with bisphosphonates for several years. Although we found factors related to inadequate response to bone forming treatment, patients with this type of response had a tendency to have had prior bisphosphonate treatment more often and longer, with an average of 7 years of treatment.

However, in most patients (70%) a marked increase in BMD at 18/24 months of treatment, around 12% at the lumbar spine and proximal femur 4.5% was observed. This outcome is similar in scale to data published previously[4,6], but differs in the incidence of inadequate response compared to those studies. Thus, only 6% of patients included in the study of Neer et al.[6] had an inadequate response to teriparatide treatment, a finding that was observed in about 30% of our patients. In addition, this group of patients not only experienced an increase in BMD after bone forming treatment, but presented a loss of bone mass in the lumbar spine and proximal femur of -3.4% and -0.6% respectively. Although the causes of this increased incidence of treatment failure remain unclear, it is possible that the severity of the disease and previous treatment with bisphosphonates have contributed in part to these results. In this sense, our patients had a more severe osteoporosis, with an average of 5 vertebral fractures per patient, double the baseline studies, and often (>80%) had undergone previous treatment with bisphosphonates, a fact that was observed in only 14-16% of patients in reference studies 6. In fact, recent studies also indicate an increased incidence of inadequate response after bone forming treatment, rising to 32% of patients in the study by Chen et al.[9]. This suggests that baseline BMD and bone remodeling (especially low PINP values) and previous treatment with bisphosphonates could influence the magnitude of therapeutic response to these agents[8-11,18]. In our study, although differences in the rate and duration of prior bisphosphonate treatment between both groups of patients were not significant, over 90% of patients

Figure 1. Flow chart of patients included



with inadequate response to treatment had had prior bone forming treatment with bisphosphonates for an average 7 years, suggesting a possible inhibitory effect of this type of treatment.

As noted previously, several studies point to the role of bone remodeling markers[8,9,11,12,15,16,19] to predict the level of response to long-term bone forming treatment[9,12,19], especially when the PINP bone formation marker is used[12,16]. In a post-hoc study, Eastell et al.[13] note that by quantifying the change in PINP at 3 months of starting teriparatide treatment allowed for the indentification of long-term patients who presented a significant BMD increase, particularly if the increase was greater than 10 ng/mL[9,12-16]. In our study, we observed a marked increase in the values of all bone remodeling markers after starting bone forming treatment. The magnitude of the increase was similar in both groups of patients during follow-up. We did not detect significant differences in the percentage of patients who experienced an increase of serum PINP (>10 ng/mL) after starting the bone forming treatment between both groups of patients.

Some of this study's limitations include the small number of patients and its characteristics as a retrospective study. However, as initially indicated, this preliminary analysis includes a sample of patients treated in a specialized, bone metabolism unit. All patients were evaluated based on a standardized therapeutic protocol in which serial determination of biomarkers and densitometric, radiological and clinical monitoring was carried out, allowing close monitoring of the clinical course in this group of patients.

In conclusion, although these are preliminary results in a small number of patients, our study shows that about 30% of patients with severe osteoporosis presented an inadequate response to long-term bone forming treatment. Although this study has not identified predictors of this type of response, it is possible that prolonged prior bisphosphonate therapy may be related to the findings. These results indicate the need to analyze this in a greater number of patients.

Conflict of interest: No conflict of interest by the authors.

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Table 1. Baseline characteristics of patients included

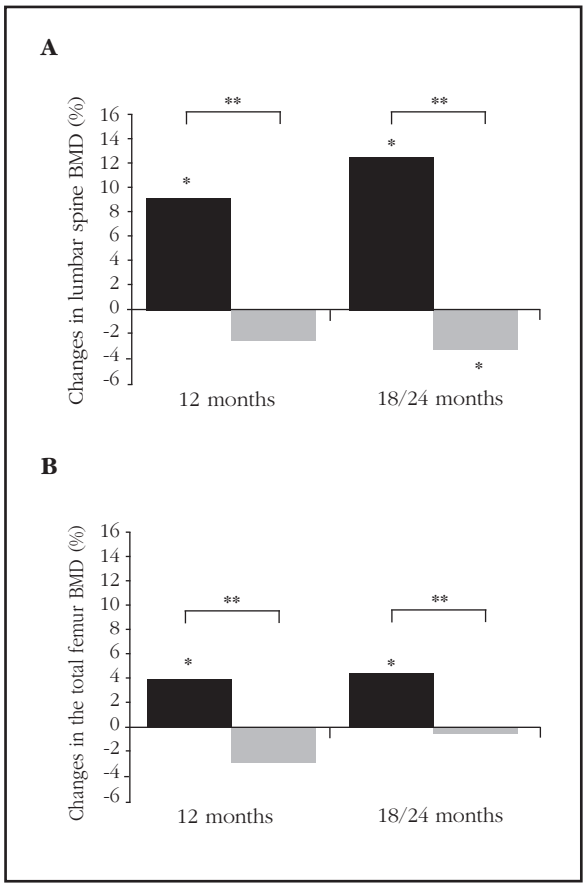
	All patients (n=49)	Appropriate response (n=29)	Inadequate response (n=12)	p
Women/Men (n)	46/3	27/2	12/0	0.351
Age (years)	69.5±11.1	68±11	70±11	0.641
BMI (kg/m^2)	26±5	25±5	27±5	0.483
Treatment				
Teriparatide/PTH 1-84 (n)	41/8	25/4	10/2	0.813
Duration of treatment (months)	21±3	21±3	20±3	0.47
Skeletal fractures				
Previous vertebral fractures (%)	84	86	75	0.386
Number of prior vertebral fractures (n)	5±4	5±4	4±4	0.832
Baseline BMD				
Lumbar (g/cm^2)	0.775±0.161	0.733±0.146	0.812±0.166	0.197
Total femur (g/cm^2)	0.701±0.121	0.674±0.087	0.701±0.157	0.474
Prior osteoporosis treatment				
Patients with previous BF (%)	84	79	92	0.339
Duration of previous BF (years)	5.8±4.5	4.9±4.2	7±4.8	0.195
BF discontinuation time (months)	3.2±7.3	2.8±6.8	3.1±8.6	0.635
Comorbidities				
Glucocorticoid treatment (%)	22	21	17	0.767

BMI: body mass index; BMD: mineral bony density; BF: bisphosphonates.

Table 2. Evolution of biochemical parameters and bone turnover markers at 3 and 6 months of bone forming treatment in patients with adequate response (RA) and inadequate (RI) treatment

	Basal		3 months		6 months	
	RA	RI	RA	RI	RA	RI
Calcium (mg/dL)	9.7±0.6	9.8±0.4	9.9±0.7	9.5±1.8	9.9±0.7	9.9±0.5
AP bone (ng/mL)	14.9±7.4	14.1±4.7	23.3±13.7	23.7±13.8	34.5±36.2	45.3±43.3
PINP (ng/mL)	45±42	36±33	132±140	126±156	184±127	191±227
CTx (ng/mL)	0.42±0.38	0.24±0.07	0.85±0.76	1.06±1.72	1.02±0.5	1.16±1.14
NTx (ng/mL)	55±43	41±24	98±82	98±99	107±63	111±72
25OHD (ng/mL)	32±17	27±10	22±11	22±5	25±12	23±4

Figure 2. Changes in BMD in the lumbar region (A), and total femur (B) in patients with adequate response (black bar) and inadequate response (gray bar) at 12 and 24 months into the treatment



*p<0.05 compared to baseline value.
**p<0.05 comparing both groups of treatment response.

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The VEGF (VEGFR2) 2 receptor and PTH (PTH1R) 1 receptor act as mediators in the anti-apoptotic response to mechanical stimulus in MLO-Y4 osteocyte-like cell

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Summary
Mechanical stimulation plays a crucial role in bone mineral maintenance. This stimulation prevents osteocyte apoptosis by a mechanism that involves β-catenin accumulation and nuclear translocation of extracellular-signal-regulated kinases (ERKs). The vascular endothelial growth factor (VEGF) and parathyroid hormone-related protein (PTHrP) modulate bone formation, although their interaction with osteocytes is unknown. In this paper we have considered the possible role of VEGF (VEGFR2) 2 receptor and PTH (PTH1R) type 1 receptor in the anti-apoptotic response to mechanical stimulation of MLO-Y4 osteocyte-like cells. The cells were subjected to mechanical stress by laminar fluid flow (10 min, 10 dinas/cm^2) or hypotonic shock (240 mOsm, 1h), or stimulated with $VEGF_{165}$ or PTHrP (1-36). We also compared the effects of overexpressed VEGFR2 and mechanical stimulation of these cells. Mechanical stimulation, $VEGF_{165}$ or PTHrP (1-36) stimulated cellular viability and β-catenin stabilization in a similar manner, associated with its localization in the membrane. Mechanical stimulation increased PTH1R presence in the membrane. VEGFR2 inhibition as well as the PTHrP (7-34) antagonist reduced these effects. On the other hand, VEGFR2 overexpression in MLO-Y4 cells mimicked the mechanical stimulation effect on β-catenin and cellular viability. Our findings support a functional role for both systems, VEGF/VEGFR2 and PTHrP/PTH1R, in the early response to mechanical stimulation in promoting osteocyte-like viability.

Key words: *PTH1R, VEGFR2, mechanical stimulation, β-catenin, apoptosis..*

Introduction

The skeleton adapts its mass, macro and micro architecture to changing mechanical forces[1]. Physical activity increases bone formation while immobilization increases bone resorption[2-4]. Osteocytes, mostly bone cells, are differentiated completed osteoblasts that are embedded in the mineralized matrix which form a mechanosensitive network. Transgenic mice with ablation of the osteocytes present trabecular and cortical bone loss.5 This is consistent with the bone cells' ability to detect changes in mechanical loading and response, coordinating osteoblast and osteoclast function[6-8].

Accumulating evidence indicates that mechanical forces regulate the viability of osteocytes by ill-defined mechanisms. *In vivo* studies in rodents and *in vitro* in cultured osteocyte-like cells demonstrate that physiological levels of mechanical loading reduce osteocyte apoptosis, whereas the lack of mechanical stimuli promotes it[4,9,10]. Activation of the Wnt/β-catenin pathway, an important regulator of osteoblastic proliferation and differentiation[11], is essential for increased bone formation in response to mechanical loading[12,13]. Mechanical stimulation of osteocytes in the mouse ulna causes rapid activation of this pathway[13] associated with a reduced expression of Sost/sclerostin, an inhibitor of bone formation[14]. The role of prostaglandin E2 and NO has been suggested and the phosphatidylinositol 3 -kinase/Akt pathway in stabilizing β-catenin and cell survival by mechanical stimulation in osteocytes[15,16]. Recent findings indicate that mechanical stimulation promotes the formation of a signaling complex consisting of integrins, Src kinase, focal adhesion kinase and caveolin-1, resulting in the phosphorylation and nuclear translocation of extracellular signal-regulated kinase (ERK)[17,18].

The anabolic action of parathyroid hormone (PTH) has been shown to depend largely on its anti-apoptotic effect through the type 1 PTH receptor (PTH1R) in osteoblasts and osteocytes[19,20]. Mice heterozygous deletion of the gene for PTH-related protein (PTHrP), local counterpart bone, osteoblasts show osteopenia associated with decreased survival of osteoblasts and osteocytes[21]. Furthermore, mice with conditional suppression of PTH1R osteocytes specifically exhibit altered homeostasis of calcium and osteopenia[22]. In contrast, mice with constitutive overexpression of this receptor in the osteocytes show increased periosteal bone formation, associated with activation of the Wnt pathway and decreased osteoblastic apoptosis[23]. PTH1R's possible mediating role in the maintenance of bone mass by mechanical stimuli is, nevertheless, unknown. In this respect, there appears to be a synergistic effect of the mechanical load and the anabolic action of PTH on bone formation and resistance in the long bones of rats[24]. *In vitro*, the fluid flow has been shown to alter the conformation of PTH1R in osteoblastic cells MC3T3-E1[25]. Moreover, also *in vitro*, mechanical stimulation induces gene expression of PTHrP local ligand of PTH1R in the bone, in osteoblastic cells and osteocytes[26]. Furthermore, the vascular endothelial growth factor (VEGF) is an important angiogenic factor, modulator of bone formation and repair, mainly through its receptor 2 (VEGFR2)[27]. VEGF / VEGFR2 system is an important mediator of proliferation, survival and differentiation of osteoblasts and osteoclasts[28,29]. The VEGFR2 mediates the actions of PTHrP on the differentiation and apoptosis in osteoblasts[29-31]. In endothelial cells, this receptor is activated by mechanical stimuli in a manner independent of ligand VEGF[32].

In the present study, we evaluated the possible involvement of PTHrP/PTH1R and VEGF/VEGFR2 systems in the survival of MLO-Y4 osteocyte cells promoted by mechanical stimulation.

Material and methods

Cell cultures: The MLO-Y4 and MLO-Y4-GFP cells, kindly provided by Dr. Lynda Bonewald (University of Missouri, Kansas City, Missouri, USA) and Dr. Teresita Bellido (Indiana University, Indianapolis, Indiana, USA), respectively were grown in culture medium α-MEM supplemented with fetal bovine serum (FBS) 2.5% calf serum (CS) 2.5% and 1% penicillin streptomycin in a humidified atmosphere of 5% CO_2, at 37°C. Cells were cultured at a density of 20,000 cells / cm^2 in culture dishes or glass slide both coated with collagen (FlexCell, Hillsborough, NC, USA); the next day fresh medium was added for 24 h. Then the cells were subjected or not (controls) to mechanical stimulation by shear stress or by laminar fluid flow or by several exposures to hypotonic medium, as described below. Cells were pre-incubated with PTHrP (1-36) (100 nM), generously supplied by Drs. A. F. Stewart and A. Garcia Ocana (Faculty of Medicine, University of Pittsburgh, Pennsylvania, USA.), or $VEGF_{165}$ (6 ng / ml) (Calbiochem, Darmstadt, Germany) as agonists, or the following antagonists and inhibitors: [Asn^{10}, Leu^{11}, D-Trp^{12}] PTHrP (7-34) amide [PTHrP (7-34)] (1 M) and JB 4250 (1 μM)[6]; VEGF neutralizing monoclonal antibody (0.1 mg/ml) (R & D Systems, Minneapolis, Minnesota, USA); or SU5416, an inhibitor of phosphorylation of VEGFR2 (1 M) (Calbiochem). These agents are added 30 min -1 hour prior to mechanical stimulation.

Mechanical stimuli: Cells were subjected or not (control) to fluid flow at a rate of 10 dynes/cm^2, 8Hz, for 10 min in a Flexcell® Streamer® Shear[7] stress device. Osmotic shock was carried out by replacing the culture medium in the cell culture plate by a hypotonic solution (240 mOsm) for 1 h. Cell exposure to the isotonic solution (317 mOsm) was used as control. After mechanical stimulation, protein extracts were collected and the cells were incubated with pro-apoptotic agent (etoposide) for 6 h.

Immunocytochemistry: Cells were fixed with 2% p-formaldehyde and permeabilization treatment with 0.1% Triton in phosphate buffered saline

(PBS). Non-specific binding was blocked with bovine serum albumin 5%, followed by overnight incubation with primary polyclonal anti- -catenin rabbit (Abcam, Cambridge, Massachusetts, USA) in a cold, humid chamber. Cells were washed with 0.1% Triton-PBS before incubation for 1 h with anti-rabbit IgG conjugated with Alexa Fluor 546 (Invitrogen, Groningen, Netherlands). The micrographs were obtained using a fluorescence microscope.

Cell Transfection: Cells were transfected with a plasmid expressing a dominant negative VEGFR2 (dnVEGFR2), a plasmid overexpressed VEGFR2 (provided by Dr. Alex Ullrich, Max-Planck Institute of Biochemistry, Martinsried, Germany) or empty vector (pcDNA, Invitrogen) using Lipofectamine LTX Plus (Invitrogen) following the manufacturer's instructions.

Assays of cell death/apoptosis: The MLO-Y4 cells were exposed to etoposide (50 μM) for 6 h to induce apoptosis after mechanical stimuli. Cell viability was determined by trypan blue exclusion and apoptosis in MLO-Y4-GFP cells was assessed by visualizing chromatin condensation and / or nuclear fragmentation. The percentage of nonviable cells to total cell number was calculated in each case. Etoposide induced cell death in these cells represented 13.6±0.8% or 30.3±0.4%, by trypan blue exclusion or nuclear morphology respectively. These values were normalized to 100% in Figs. The corresponding values of untreated cells with etoposide were 1±0.5 and 1.2±0.5% respectively.

Western blotting: Analysis of sub-cellular fractionated samples (Pierce, Rockford, IL) was used to obtain extracts of membrane and nuclear protein. These extracts (25-30 g) were then separated by SDS-PAGE (8-12% polyacrylamide) and transferred to nitrocellulose membranes (GE-Amersham, Pittsburgh, Pennsylvania, USA). The membranes were blocked with 2.5% skimmed milk in 0.1% Tween-PBS at room temperature for 1 h and subsequently incubated overnight at 4C with the following rabbit polyclonal antibodies: anti-β-catenin (Abcam); anti-PTH1R (Ab-IV, Covance, Berkeley, California, USA); and anti-ERK1/2 (Cell Signaling, Beverly, Massachusetts, USA). As loading controls, the following antibodies were used: goat polyclonal anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, California, USA) or monoclonal mouse anti-α-tubulin (Santa Cruz Biotechnology). Then the corresponding secondary antibody coupled to horseradish peroxidase (Santa Cruz Biotechnology) was added. Detecting the luminescent signal in the membranes was performed with the ECL system (GE-Amersham) and band intensities were quantified using densitometry.

Statistical analysis: Results are expressed as mean ± SEM. Statistical analysis between two groups was performed using the Mann-Whitney. A $p < 0.05$ was considered significant.

Results

Treating MLO-Y4 osteocyte cells with two different methods of mechanical stimulation was found to protect etoposide-induced cells from death (Figure 1). Mechanical cell stimulation by fluid flow for 10 min at 10 dynes/cm^2 protected from apoptosis induced by etoposide exposure for 6 h (Figure 1A). This protective effect was blocked by cell pretreatment with a selective inhibitor of VEGFR2, SU5416 (1 μM). Mechanical stimulus protection was reproduced by the pre-treatment of cells with 6 ng/ml of VEGF (Figure 1A). Furthermore, cells were submitted to mechanical stimulation by exposure to hypo-osmotic buffer for 1 h, which also induced protection against etoposide. This protective effect was blocked by pretreatment with PTH1R inhibitor, PTHrP (7-34) (Figure 1B). As observed previously with VEGF, PTHrP (1-36) pre-treatment reproduced the protective effect of osmotic shock (Figure 1B).

Translocation of ERK to the nucleus is a requirement for survival induced by mechanical stimuli. Thus, we observed that stimulation by fluid flow (10 min, 10 dynes/cm^2) induced an increase in ERK in the nucleus of MLO-Y4 cells (Figure 1C). This effect was blocked by pretreatment with an anti-VEGF antibody, as well as inhibitors of VEGFR2 and PTH1R, SU5416 and JB4250, respectively (Figure 1C). It is also known that the Wnt/β-catenin pathway is involved in mechanotransduction in osteocytes. Observed by immunocytochemistry and Western that mechanical stimulation of MLO-Y4 cells induced rapid translocation of β-catenin to the cell membrane (Figures 2A and 2B) transfer; an effect blocked by antagonists VEGFR2 and PTH1R, SU5416 and PTHrP (7-34), respectively, and by an anti-VEGF antibody. Similarly, osmotic shock induced translocation of β-catenin membrane (Figure 1C). The involvement of VEGF/VEGFR2 system in mobilizing the β-catenin membrane by mechanical stimulation was also analyzed by transfection of MLO-Y4 cells with a plasmid that overexpresses VEGF or dnVEGFR2. Overexpression of VEGF in these cells reproduced translocation of β-catenin membrane; whereas this mobility induced by mechanical stimulation did not occur in cells with dnVEGFR2 (Figure 3).

Furthermore, we wanted to study if mechanical stimulation modulated the location PTH1R in these membrane osteocytic cells. We note that both osmotic stress treatment and PTHrP (1-36) increased protein levels exogenous receptor in the membrane of MLO-Y4; while antagonists PTH1R, PTHrP (7-34) and JB 4250, blocked osmotic shock effects (Figure 4).

Discussion

Osteocyte viability, essential for the maintenance of bone mass and strength, is compromised in situations of osteopenia/osteoporosis[33,34]. Under physiological conditions, the viability of osteocy-

tes remains critical levels of mechanical loading through poorly defined mechanisms[33]. *In vitro* studies in cells of MLO-Y4 have shown that stretching induces cellular anti-apoptotic response through a mechanism involving a complex signaling related to nuclear translocation ERK[4,18]. It has also been shown recently that the viability of MLO-Y4 cells induced by mechanical stimulation is modulated by the interaction between the pathways of caveolin-1/ERK and Wnt/β-catenin[18]. In this study we observed that both systems, PTHrP/PTH1R and VEGF/VEGFR2, are involved in protection against cell death by apoptosis which give the osteocytic cells two different mechanical stimuli, and osmotic shock fluid flow.

It has previously been shown to express the PTH1R osteocytes and respond to stimulation with PTH[35], an important calciotropa hormone responsible for calcium homeostasis in physiological conditions. Recent studies in genetically engineered mice indicate that the action of PTH requires a functional PTH1R in osteocitos[22]. From a pharmacological perspective, intermittent administration of PTH in mice attenuates rapidly osteoblast apoptosis in the vertebrae; this effect appears to be only a consequence of direct hormone action on osteoblasts, but also indirectly through its inhibitory effect on the expression of Sost/sclerostin in osteocytes[20,36,37]. Furthermore, in these cells PTH1R appears to play a key role in bone anabolic response to mechanical loading[38]. In this sense, described in rodents induced bone anabolism intermittent administration of PTH is enhanced by mechanical stimulation[25,39]. The functional interaction between mechanical stimulation and PTH is supported by *in vitro* studies using primary cultures of osteocytes[32]. Thus, the present data suggest that the osteocytes PTH1R integrates mechanical and hormonal for coordinated regulation of bone formation signals.

Moreover, our results indicate that VEGFR2 is critical for both the translocation of β-catenin to the cell membrane and for ERK to the nucleus. The system of VEGF is involved in the mechanisms of survival in various cell types including osteoblasts[29,30,40]. This growth factor promotes survival of endothelial cells by stimulating the formation of a multi-transmembrane protein complex that includes VEGFR2, VE-cadherin and β-catenina[40]. Our results demonstrate that, immediately after stimulation by fluid flow, the β-catenin was translocated to the membrane of MLO-Y4 osteocyte cells associated with VEGFR2 activation. The possibility that this may occur *in vivo* mechanism to explain the observed in response to mechanical stimulation requires further studies in animal models for osteocyte survival.

In summary, our *in vitro* results support an important role both for VEGFR2 and PTH1R as mechanisms that promote the viability of osteocytes after mechanical stimuli.

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Conflict of interest: The authors declare no conflicts of interest.

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Figure 1. Changes in apoptosis (A) and cell viability (B) caused by mechanical stimulation in MLO-Y4, pre-incubated with cells or PTHrP (1-36) or $VEGF_{165}$, or with antagonists, PTHrP (7-34) (PTH1R) or SU5416 (VEGFR2), followed by incubation with 50 μM etoposide for 6 h. Values are mean ± SEM of 3 independent experiments in triplicate. *p<0.05 vs basal in static control condition; #p<0.05 vs basal low fluid flow; ap<0.05 vs isotonic basal medium; $p<0.05 vs basal in hypotonic medium. (C) nuclear ERK expression was assessed by western blot after fluid flow or static control, after pre-incubation with the indicated agonist or antagonist for 30 min. Load control was verified by Ponceau S staining (not shown). The results of a representative experiment are shown

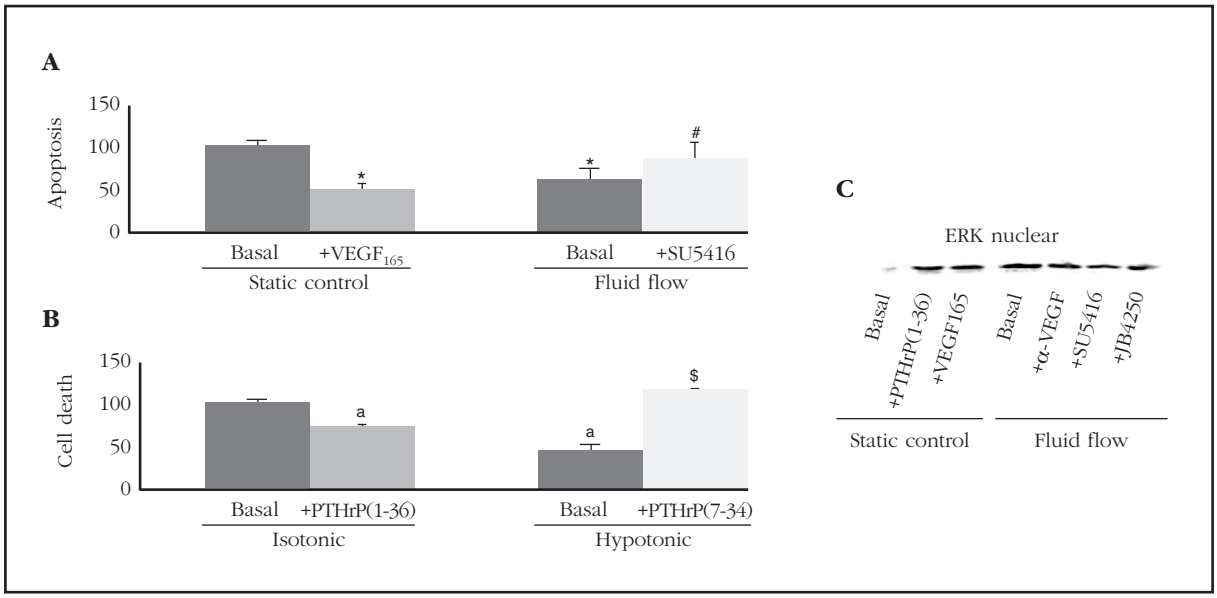

Figure 2. Changes in β-catenin induced mechanical stimulation or after incubation with $VEGF_{165}$ in MLO-Y4 cells, in presence or absence of antagonist SU5416 (VEGFR2), anti-VEGF antibody (α-VEGF) or PTHrP (7 -34) (PTH1R) as cited in the bottom of Figure 1. Changes in the β-catenin were evaluated by immunocytochemistry (A) or Western blotting in extracts of cell membrane after mechanical stimulation fluid flow [control loading was verified by Ponceau S staining (not shown)] (B) or hypotonic shock (C). Representative autoradiograms are shown in each case

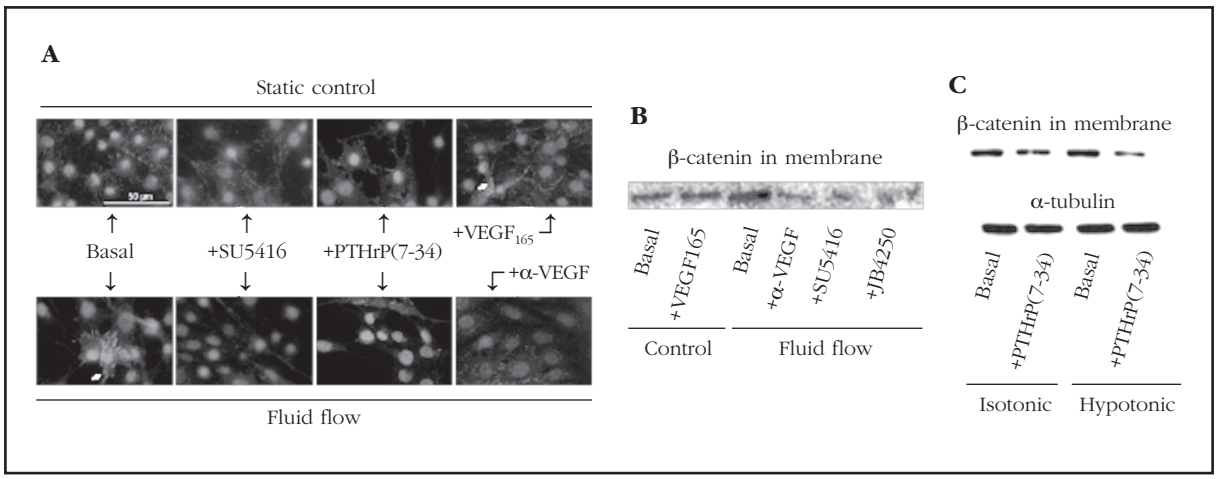

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Figure 3. Changes induced in β-catenin after fluid flow in the MLO-Y4 cells with altered expression of VEGFR2. Immunocytochemistry was carried out with β-catenin after fluid flow in these cells transfected with a plasmid over-expressing VEGFR2, the dnVEGFR2 plasmid or empty plasmid

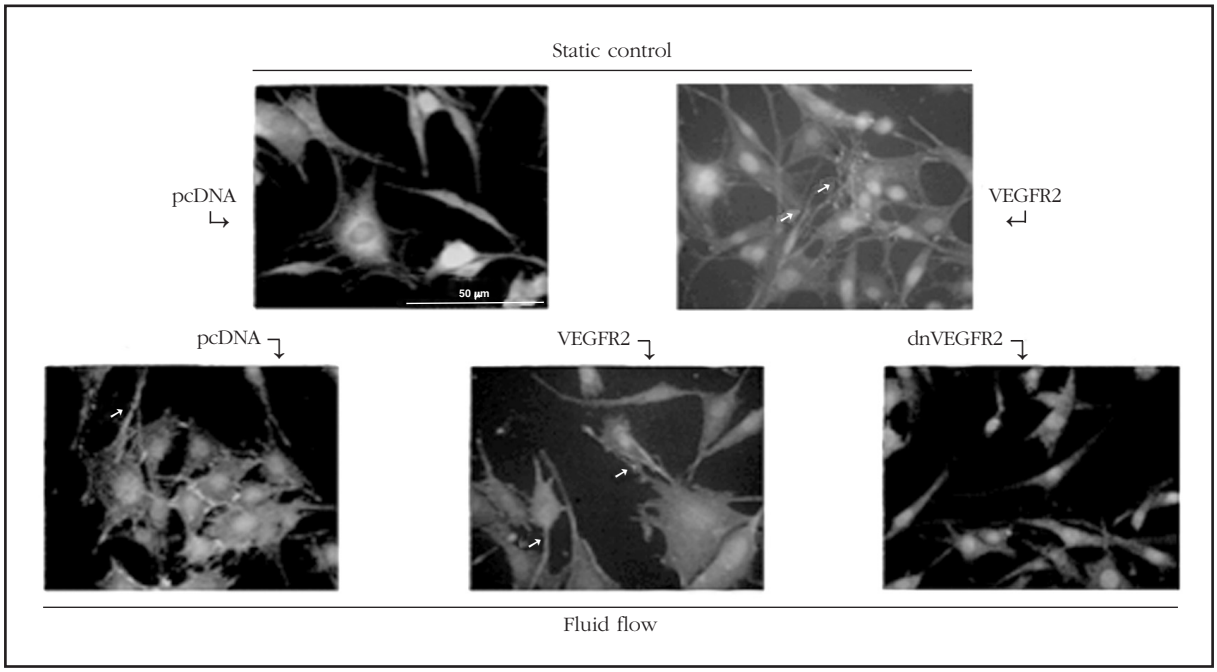
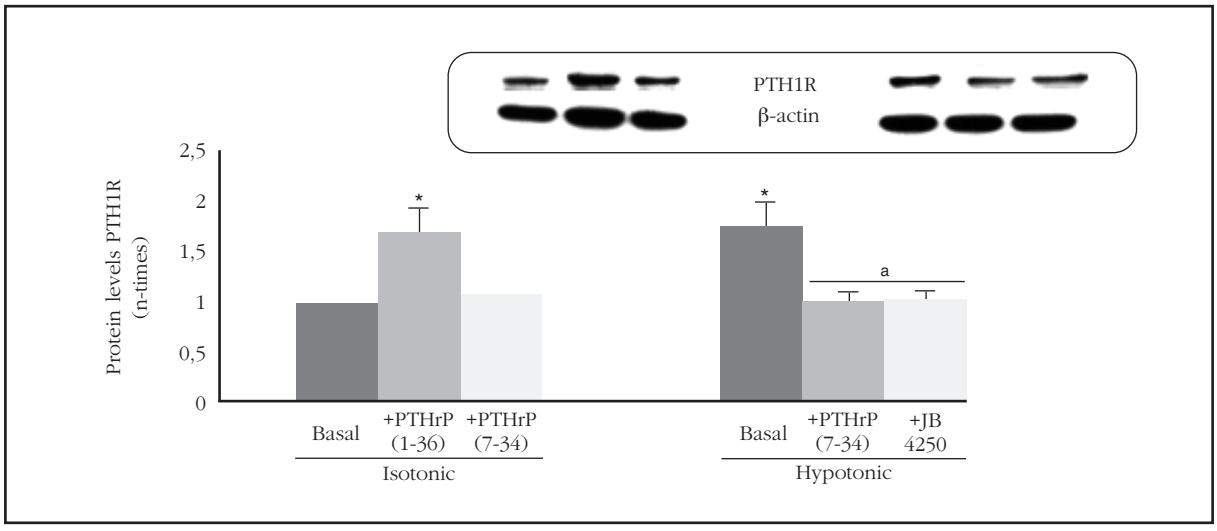


Figure 4. Changes in the membrane PTH1R of MLO-Y4 cells after mechanical stimulation. Protein levels of this receptor were assessed by western blot on cell membrane extracts of MLO-Y4 cells after pre-incubation with PTHrP (1-36), PTHrP (7-34) or 4250 JB for 1 h followed by hypotonic (or not, isotonic) shock. A representative autoradiogram is shown. Densitometric relative values are mean ± SEM of two independent experiments in triplicate. *p<0.01 vs basal and PTHrP (7-34) (isotonic condition); [a]p<0.05 vs corresponding baseline



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BMD evolution during treatment with aromatase inhibitors and its relation to the CYP11A1 gene: prospective study in the B-ABLE cohort

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Summary

Objectives: The aim of this study was to analyze bone mineral density (BMD) changes throughout aromatase inhibitor (AI) treatment in clinical cases and also consider its association with the CYP11A1 gene and the BMD variation after treatment.

Material and methods: The B-ABLE cohort is a prospective study of postmenopausal women with breast cancer, in AI treatment. BMD variation was analyzed during AI treatment, as well as the differences those patients who were treated and not treated previously with tamoxifen (TMX). Three polymorphisms (rs4077581, rs11632698 and rs900798) of the CYP11A1 gene were genotyped for their association with BMD variation.

Results: TMX-treated patients presented more rapid BMD loss than those who did not undergo prior TMX treatment (60% less in spine and 46% in femur at 2 years and 70% less in the spine and 63% in the femur at 3 years). However, no significant BMD loss was detected after treatment in either group. The 3 CYP11A1 gene polymorphisms were significantly associated with BMD variation in the femur at the end of the treatment.

Conclusions: BMD was reduced more rapidly in patients with prior TMX treatment than in those who only received AI, although no significant differences were detected after treatment. The 3 CYP11A1 gene polymorphisms were associated with BMD variation in response to AI treatment.

Key words: *aromatase inhibitors, bone mineral density, CYP11A1, genetic polymorphisms, tamoxifen.*

Introduction

Aromatase inhibitors (AI) have become the accepted adjuvant therapy for postmenopausal patients with breast cancer with hormonal receptor expression[1]. AI brought about a marked reduction in estrogen levels through inhibition of the aromatase enzyme[2] whose activity is relegated to peripheral tissues during menopause[3]. The American Society for Clinical Oncology (ASCO) recommends using the AI for 5 years, or for 2 or 3 years, after previous therapy with tamoxifen (TMX)[4], where the latter option is prescribed for pre/perimenopausal women[5].

However, reduced estrogen levels increase bone resorption and raise the risk of fracture that occurs after menopause[1,6-9]. Clinical guidelines for the management of bone loss associated with AI (AIBL: Aromatase Inhibitor associated Bone Loss) recommends a strict monitoring of bone mineral density (BMD) and other risk factors to assess the need for treatment with anti-resortive therapies[10].

Despite existing data, most of which based on randomized clinical trials (RCT), there is little information on the effect of AI therapy in routine clinical practice, where patient characteristics and adherence to therapy may differ from what is observed in restrictive RCT conditions.

The study data presented are taken from the B-ABLE cohort, a prospective clinical cohort in postmenopausal women with early stage breast cancer receiving adjuvant AI therapy. A recent study[11] in this cohort reported a large inter-individual variability in the change of BMD during AI treatment: at 2 years of therapy, more than 40% of patients experienced more than 3% BMD loss, while 20% of women did not present significant losses or even gained BMD. Moreover, in this same study, an association was found between CYP11A1 gene polymorphisms and bone loss after 2 years of AI treatment[11], thus demonstrating that the observed variability among patients presenting AIBL 2 years after treatment could be partially determined by genetics. The study aimed to describe BMD changes over the entire treatment, up to completion, and to assess the possible association between the CYP11A1 gene and AIBL after treatment.

Material and methods

Study Population

Details of the study design, methods of recruitment and population study[12] have been previously described and here are set out briefly.

B-ABLE is a prospective, observational clinical cohort study, initiated in January 2006 and currently with open inclusion. The women included presented postmenopausal breast cancer with hormone receptor expression, candidates for AI treatment and attending the Outpatient Breast Cancer Unit of the Hospital del Mar (Barcelona, Spain). Exclusion criteria are any history of bone disease, rheumatoid arthritis, endocrine and metabolic diseases or use of oral corticosteroids or any other drug with bone action, except Tamoxifen.

Procedures

Participants were treated with AI (Letrozole, Exemestane or Anastrozole) for 5 years, or alternatively after 2 or 3 years of Tamoxifen treatment (3 and 2 years of AI, respectively), according to ASCO recommendations[4] of starting within 6 weeks after surgery or 1 month after the last cycle of chemotherapy.

All participants received calcium and vitamin D (1,000 mg 800 IU daily), and those with vitamin D deficiency at baseline (<30 ng/ml) received an extra dose of 16,000 IU of Cholecalciferol oral every 2 weeks.

Measurements

Bone Mineral Density

At baseline and annually until the end of treatment, BMD was measured in lumbar spine (LS; L1-L4), femoral neck (FN) and total hip using the Densitometer X-ray Absorptiometry (DXA) SL® QDR 4500 (Hologic, Waltham, Massachusetts, USA), following our unit's standard protocol. In our department, the coefficient of variation for this technique ranges between LS 1% to 1.65% in FN. The images were scrutinized rigorously, especially in the interpretation of tracking scanners. Those who presented artifacts in the image, causing possible erroneous BMD increase (degenerative disc disease with bone spurs, arthritis with hyperostosis of the facet joints, vertebral fractures and / or aortic calcifications) were excluded from the analysis, in accordance with the description of Blake et al.[13].

Other determining

Information of a large number of clinical variables was collected at the time of recruitment, including age, age at menarche and menopause, nursing time, parity, previous chemotherapy and radiotherapy, adjuvant treatments, weight, height, serum levels of 25-hydroxyvitamin D (25 (OH) D), calcium intake and smoking.

Selection of candidate genes and polymorphisms

To study the association with AIBL at the end of treatment, we selected rs4077581 single-nucleotide polymorphisms (in the promoter region), rs11632698 (in intron 2) and rs900798 (in the 3'UTR) of the CYP11A1 gene, which have been previously associated with AIBL after 2 years of treatment[11].

DNA extraction and genotyping polymorphisms

DNA extraction was carried out on peripheral blood in LGC Genomics Units. Polymorphism genotyping was carried out using the Kaspar Genotyping System v4.0, at LGC Genomics. To verify quality of service, polymorphisms were also genotyped in a plate control consisting of a random sample containing 5% of total samples. The results showed 100% concordance.

Declaration of Ethics

Study protocols were approved by the appropria-

te ethics committee (Ethics Committee for Clinical Research of the Parc de Salut Mar [CEIC-Parc de Salut Mar]). Approved protocols for obtaining DNA from blood samples were explained, patients signed an informed consent before being included in the study.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was calculated by the online tool Tufts University Somerville/Medford, Massachusetts, USA [Http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20%20HW%20calculator.xls]. The outcome variable was BMD loss, calculated as the cumulative percentage change in BMD in LS and FN at each follow-up visit until the end of treatment (three or five years of treatment, as they had received tamoxifen previously). The patient group completing AI therapy after 2 years is not taken into account on an isolated basis when assessing BMD development, as the limited number of patients would not allow for statistical inference in this subset. BMD changes from baseline were assessed using Student's t-paired samples.

The association between polymorphisms and AIBL elected at the end of treatment. It was analyzed using multiple linear regression models contemplating heritage dominant, recessive and additive genetic. Analyses were adjusted for age, index body mass, chemotherapy and/or prior radiotherapy, tamoxifen therapy prior, initial BMD and years of treatment with AI. Furthermore it was also studied potential confusion about the levels of 25 (OH) D at the beginning and by the type of AI. For will minimize false findings because of multiple comparisons was used the FDR[14] correction, accepting those predictions with q <0.05 significant. Statistical analyzes were performed using R for Windows Version 2.13.2 (Packages: SNPassoc, foreign, multtest, boot and ggplot2).

Results

Baseline characteristics of patients and study AIBL

A total of 529 women were recruited from March 2006 to February 2013, of which 24.2% had a normal T-score, 57.3% were osteopenic and the remaining 18.5%, osteoporotic. A total of 388 (73.3%) patients did not receive bisphosphonate treatment, and thus were selected for analysis. A 40.5% of these patients (157) had received prior therapy with tamoxifen (TMX Group), while the remaining 60.5% were not treated with any prior hormonal therapy (NO-TMX Group). Clinical baseline features of the participants according to previous treatment with tamoxifen are shown in Table 1. Significant differences were detected between groups in age, BMI (body mass index) and type of AI.

Figure 1 shows the number of patients with available data for LS and FN in each of the follow-up times. Patients with devices in the lumbar scanner and/or scoliosis (n=97) and those with artifacts in the hip scanner and/or bilateral prosthesis (n=14) were excluded for analysis for BMD of LS and FN, respectively. Of the 388 patients included in the analysis, 18 were reclassified as osteoporosis by decreasing BMD during treatment (7 in the first year, 8 during the second year, 1 during the third year and 2 in the fourth year) which were immediately implemented bisphosphonate therapy. From that point, their data were excluded from analysis.

Table 2 shows the absolute values of baseline BMD and end of treatment (3 or 5 years in the TMX and TMX NO-group, respectively). At baseline the TMX patients showed a higher BMD in CF patients that NO-TMX (+0,021 g/cm^2 [95% CI 0.004 to 0.038]; p <0.05). No significant differences were detected in the initial LS BMD. No significant differences were detected in FN or LS BMD between 3 years or values between the values after treatment (3 years, 5 years vs the TMX group TMX NO-group).

Figure 2 shows the cumulative change in BMD from baseline to the end of treatment. The TMX group showed a more accelerated BMD decrease. So, after 2 years of treatment, patients lost TMX 60% in LS (p<0.001) and 46% in FN (p<0.001) than patients NO-TMX. These differences are maximum 3 years, at which time the TMX patients completed treatment, having lost 70% in LS (p<0.05) and 63% in FN (p<0.01) compared to group NO-TMX after 3 years of therapy. However, the TMX group experienced a decrease in BMD of 5.28% in LS and FN 3.66% in the end of treatment (3 years). For its part, the BMD TMX individuals NO-LS were reduced by 3.99% and 3.43% in FN after 5 years AI therapy. No significant differences were detected in BMD loss at the end of treatment.

AIBL genetic association after treatment

All polymorphisms were genotyped in the Hardy-Weinberg equilibrium. Genotyping efficiency was higher than 97%. Table 3 shows the results of analysis of association of polymorphisms of YP11A1 gene with cumulative BMD loss in FN and LS at the end of treatment. After the FDR correction significant results were obtained for the 3 polymorphisms the CYP11A1 gene with AIBL of CF (q<0.02). No significant results were obtained for LS.

Discussion

This prospective study provides information about the variation in BMD during AI treatment in patients with breast cancer in general clinical practice. The results show that BMD decrease is more accelerated in patients who have received prior therapy with tamoxifen but no significant differences were detected after treatment with respect to those who only received AI. Furthermore, previously a large variability in BMD loss was shown in response to AI treatment[11]. In this study a statistically significant association was detected between decreased BMD at the end of AI treatment and some polymorphisms of the CYP11A1 gene.

Regarding patients who had received prior therapy with tamoxifen, more marked differences in

Table 1. Baseline characteristics of patients according to pretreatment with TMX

Characteristic		Group NO-TMX n=231	Group TMX n=157
Age (years), mean±SD		63.4±7.4	58.2±9.0***
BMI, mean±SD		30.2±5.2	28.7±5.5**
Age onset of menopause (years), mean±SD		49.8±4.6	48.8±4.3
Age at menarche, median (RI)		12 (3)	13 (3)
Feeding time (months), medium (RI)		3 (10.5)	4 (12.0)
Number of children, median (RI)		2 (1)	2 (2)
Prior chemotherapy, n (%)		234 (60.3%)	234 (60.3%)
Aromatase inhibitor, n (%)	Letrozol	227 (98.3%)	35 (22.2%)#
	Exemestane	-	122 (77.7%)#
	Anastrozole	4 (1.7%)	-

TMX: tamoxifen; SD: standard deviation; IR: interquartile range; BMI: body mass index.
In t-test compared to the group without tamoxifen: **p<0.01; ***p<0.001.
#: indicates significant difference in the proportion of patients with and without prior tamoxifen.

decreased BMD appear at 3 years treatment, in the TMX group lost 70% more in LS and 63% in FN. Tamoxifen acts as an antagonist competitive estrogen receptor in breast tissue, but, in turn, has partial agonist actions in other tissues, such as bone. There is evidence of its beneficial effects in reducing resorption and stimulation of bone formation in postmenopausal women with breast cancer[15]. However, this analysis concurs with some studies indicating that prior tamoxifen therapy considerably increases the effects of AI in bone remodeling, resulting in a further decrease in BMD[16]. One possible explanation for this phenomenon is the "rebound" effect, that is, the positive influence of tamoxifen not only ceases to finish its therapy[16], but also causes a marked reduction in BMD when AI changes. Thus, they increase their resorptive osteoclast action after inhibited state. Tamoxifen is the preferred peri-menopause treatment for women[5]. This would explain the difference observed both in age and initial BMD FN patients with and without previous TMX.

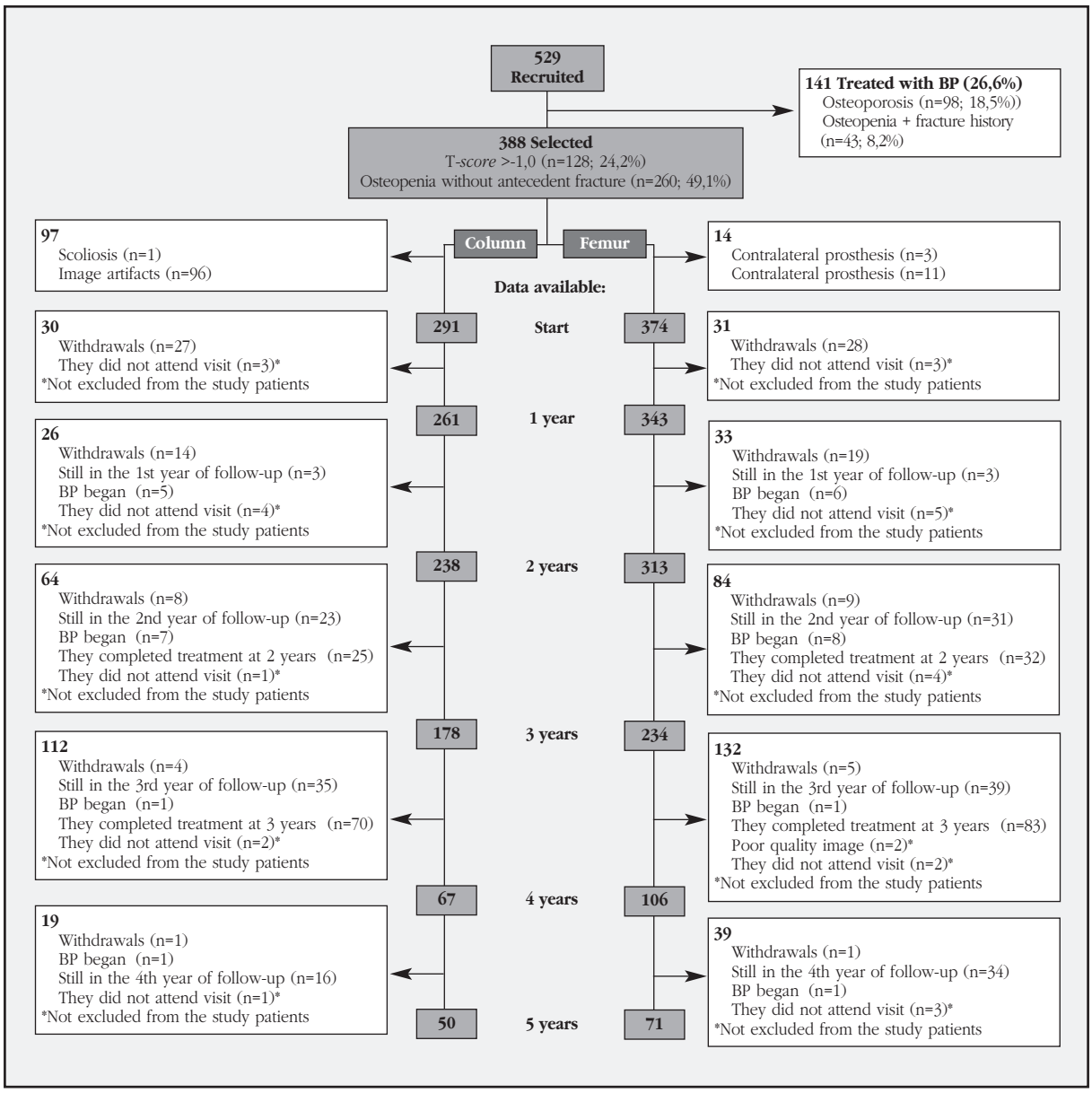
Despite the above, after 5 years of AI therapy, the NO-TMX group was equal to the final BMD loss after 3 years of TMX group [-3.66% vs -5.28% in LS; (P=0.1) and -3.43% vs -3.99% in FN; (P=0.7)], so that no statistically significant differences were detected between groups in BMD values after treatment. It is noteworthy that the rates of BMD loss in LS were at all times higher than FN. In this regard, it is known that trabecular bone is weaker than the cortical in response to AI therapy[17].

Overall, the patients in the cohort B-ABLE lose less BMD compared to previously reported by FFS. For example, the ATAC[18] trial reported losses of 6.08% and 7.24% LS total hip patients treated with anastrozole for 5 years. Patients without bisphosphonates the ABCSG-12[8] trial suffered losses of 7.8% and 4.1% in LS and FN, respectively, at the end of treatment, even registering decreases of 13.6% in LS at 2 years and 7.3% in FN at 3 years. The MA-17[7] study, meanwhile, analyzed patients who received tamoxifen before describing loss at 2 years of 5.35% in LS and 3.6% in total hip.

Differences in some features, such as initial BMD values, may contribute to this result. In this regard, most RCTs mentioned showed higher BMD values than those observed in our cohort, leading to bias by regression to the mean. Furthermore, we have found that the prevalence of vitamin D deficiency among Cohort B-ABLE patients is 88.1%[12] at the time of initiating therapy with AI, regardless of the season, which would explain in part the low BMD values. Cohort B-ABLE is subject to a strict evaluation not only of BMD but also the levels of vitamin D and calcium. Vitamin D status has been linked to BMD[19], and most trials have shown that vitamin D supplements are protective against fractures[20] and falls[21]. The patients in our study received supplemental vitamin D in much higher quantities than recommended by the IOM (Institute of Medicine), so that after 3 months of supplements improving levels of 25 (OH) D were achieved, preventing further bone loss[22].

In the present study, an association between BMD loss after AI treatment and polymorphisms in the CYP11A1 gene was detected. The CYP11A1 gene encoding the side chain cleavage enzyme of cholesterol (Alternative: P450scc) that catalyzes the first step and limits steroidogenesis, converting cholesterol to pregnenolone. In addition to cholesterol, may also P450scc hydroxylating vitamin D2, D3 and precursors[23-25], suggesting a broad

Figure 1. Diagram showing the number of patients with BMD data available at the lumbar spine and femoral neck at each time follow up. Abbreviations: BP (treatment with oral bisphosphonates)



spectrum of functions in metabolism cell. This enzyme is a mitochondrial membrane bound protein expressed mainly in adrenal cortex, ovary, testes, and placenta[26]. In addition, its expression has also been shown at the RNA level and protein in bone tissue and in osteoblasts[11], suggesting a role for this enzyme in bone metabolism.

In this study, polymorphisms in the CYP11A1 gene: rs4077581 (in the promoter region), rs11632698 (in intron 2) and rs900798 (in the 3 'UTR) were associated with BMD loss at the femoral neck after AI treatment. A statistically significant association was not observed with spinal BMD loss. All DXA study images were carefully analyzed to exclude those devices and/or structural changes (such as osteophytes) that might lead to false elevations in BMD. This procedure has consequences for all spinal results, since degenerative changes in this region can significantly increase BMD. Consequently, the number of patients for this determination was reduced which could explain the lack of statistical significance obtained in LS. In this regard, in a study prior to our group, a similar trend was observed associating these polymorphisms with BMD loss at 2 years of treatment, nominally obtaining significant results for spinal BMD[11].

CYP11A1 gene variants may alter the expression or activity, determining the levels of sex hormones in a tissue, and therefore, be responsible for different phenotypes. This hypothesis would be supported by the fact that other polymorphic variants in this gene have been previously associated with the susceptibility of endometrium[28] and breast cancer[27] as well as to polycystic ovary syndrome[29].

Table 2. Absolute values of BMD during treatment with AI as TMX previous

Time of follow up	TMX previous	Site	N	BMD (g/cm²) Mean±SD
Basal	Yes	Column	129	0.953±0.096
		Femur	151	0.760±0.081
	No	Column	162	0.969±0.116
		Femur	223	0.739±0.088*
Year 3	Yes	Column	75	**0.904±0.084**
		Femur	87	**0.730±0.080**
	No	Column	103	0.926±0.112
		Femur	147	0.728±0.089
Year 5	No	Column	50	**0.939±0.116**
	No	Femur	71	**0.725±0.090**

AI: aromatase inhibitors; BMD: bone mineral density; TMX: tamoxifen; SD: standard deviation.
In t-test compared with patients who have taken prior tamoxifen: *p<0.05.
Bold values end of treatment of patients who have highlighted AI over 3 or 5 years.

Thus, CYP11A1 activity may play a central role in local synthesis of steroid hormones, being partly responsible for AIBL. Our study has several limitations. First, evaluation of adherence to AI and tamoxifen was found only by a direct question made by the doctor. Second, the exclusion of patients receiving bisphosphonate treatment provides a selection of women with healthier bone, possibly causing a bias in the results. Third, the loss of patients during follow-up causes a decrease beyond 3 years of treatment. However, the study design is closer to the conditions of routine clinical observation. In addition, the implementation of a specific protocol of management of bone health in these patients showed better results in routine oncology practice.

In conclusion, in the CYP11A1 gene polymorphisms are associated with BMD response to treatment with AI. In our opinion, the study of B-ABLE cohort to conclude that the specific control and bone health treatment with calcium and vitamin D in all patients are interventions required during AI therapy, as they have an influence on direct changes in BMD and probably also translate into decreased risk of fragility fracture.

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Figure 2. Relative cumulative change in BMD up to completion IAI treatment in patients with (A) and without (B) prior tamoxifen. In t-test paired samples compared to the previous period: *p<0.05; **p<0.01; ***p<0.001

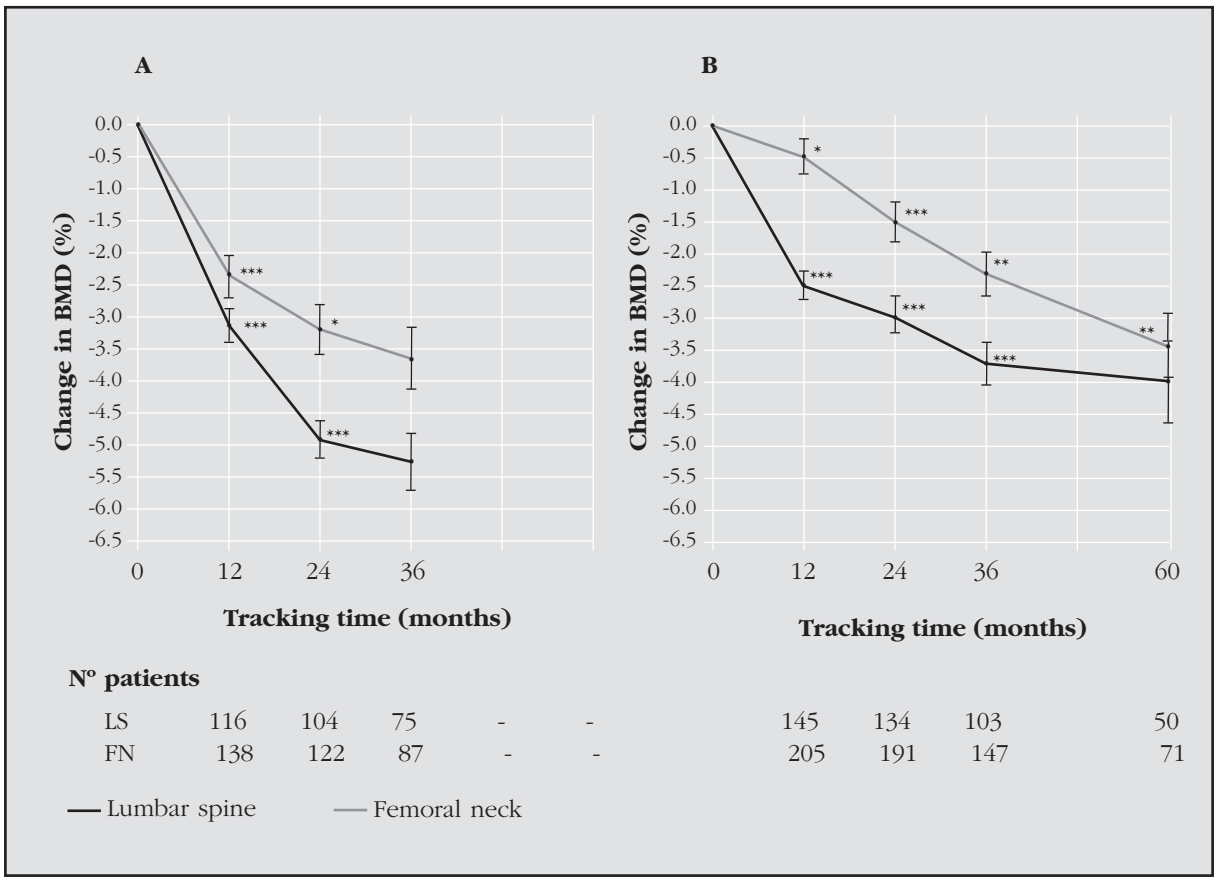

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Table 3. Association between polymorphisms and CYP11A1 gene variation of BMD after AI treatment

Locus	ID	Site	Phenotypic groups	N	Beta coefficient§ [95% IC]	p nominal value*	Value q FDR
CYP11A1	rs4077581	Column	T/T-T/C	116	0.88 [-0.86 a 2.62]	0.324R	0.603
			C/C	24	-	-	
	rs900798		G/G-T/G	113	0.71 [-0.94 a 2.36]	0.402R	0.603
			T/T	28	-	-	
	rs11632698		G/G-G/A	101	0.19 [-1.30 a 1.69]	0.796R	0.796
			A/A	37	-	-	
	rs4077581	Femur	T/T-T/C	151	2.25 [0.51 a 3.99]	0.012R	**0.021**
			C/C	31	-	-	
	rs900798		G/G-T/G	148	2.10 [0.43 a 3.76]	0.014R	**0.021**
			T/T	35	-	-	
	rs11632698		G/G-G/A	135	1.69 [0.19 a 3.20]	0.028R	**0.028**
			A/A	45	-	-	

AI: aromatase inhibitors; A: recessive model; § adjusted for: age, BMI, pretreatment with chemotherapy and/or tamoxifen, initial BMD and years of AI therapy.
In bold those significant p values are highlighted after correction for multiple comparisons (FDR). *The results show the values of p lowest obtained for each of the hypotheses using linear regression.

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Inappropriate use of proton-pump inhibitors and fragility fracture risk. A preliminary study

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Summary

Introduction: Proton-pump inhibitors (PPIs) are widely used drugs, though it should be noted that excessive use is not in line with the accepted indications in Spain and throughout Europe. Furthermore, some authors have established a possible PPI link to the risk of fracture. In this paper, we make an initial approach to knowledge into PPI consumption and analyze what indication is prescribed. We also studied the drugs' possible association with increased risk of fragility fracture in users.

Material and method: An observational, transversal, open and descriptive study in which a number of randomly-chosen patients were interviewed. These patients had been treated in outpatient, emergency and primary care centers. Some had also been treated in hospital wards.

Results: Of the 411 patients interviewed, 54% received PPIs. The average age was 63.3 years, compared with 46% that did not take them and who were younger presenting a mean age of 50.9 years. Gender distribution was similar. PPIs were mainly used as a "gastric protector", in 39.8% of the patients, with no indication appearing in the technical specifications for this group of drugs. Consumers of PPIs presented a higher prevalence of all fragility fractures.

Conclusions: More than half of the population surveyed consumed PPI. Of this group, about 40% did so without proper medical advice. Therefore, in addition to the higher prevalence of fragility fractures that suggest a possible increased risk of fracture among its users, we consider the need for a more rational use of these drugs. These preliminary findings point to a need for further studies to confirm the relationship between PPIs and the risk of osteoporotic fracture.

Key words: *omeprazol, proton-pump inhibitors, abuse, side effects, osteoporosis, fracture.*

Introduction

The proton-pump inhibitors (PPIs) are a group of drugs whose main action is a prolonged decrease in the stomach's hydrochloric acid secretion[1]. They are quite safe and widely used by the public, but not without side effects[2]. It has been reported that consumption of PPIs could be related to an increased risk of fragility fractures. There are published studies in the literature that support this[3-6] and others that deny it[7].

Moreover, PPIs have been used for many years as a stomach protector that may be caused by taking certain medications. This use is not stated in the therapeutic indications registered by the European Medicines Agency[8] and summarized in Table 1. There are no studies or scientific evidence to support its use for this purpose. Conversely, administration of PPIs in conjunction with other drugs may sometimes be counterproductive, as, for example, calcium carbonate, which requires an acid medium for optimal absorption[9], absorption would be inhibited, therefore, with simultaneous administration of PPI.

We conducted a study in a group of patients randomly recruited from different healthcare areas of the Insular University Hospital of Las Palmas, Spain, in order to collect initial data on the prevalence of PPI and the reasons why it is prescribed, and to study the prevalence of fragility fractures in these patients and possible links to PPI use.

Material and methods

To carry out this work, we designed a questionnaire composed of 10 items that were presented to a group of 411 randomly-chosen patients of both sexes. Those interviewed were treated in various health centers: emergency department, hospital internal medicine outpatient, primary care consultation and patients admitted to hospital on the wards. The minimum age for inclusion was 18 years, with no upper age limit. There was no choice in the type of patients in any working environment, whether in the hospital and health centers. Five doctors participated in the data collection. The questionnaire results were entered into a database designed ad hoc and consisting of a total of 20 items related, for the most part, to the use of PPIs.

The statistical study consisted of a descriptive analysis, using mean and standard deviation for the quantitative variables and percentage for categorical variables. To compare categorical variable tests Chi-square and Fisher were used. The Student t-test or Mann-Whitney U test were used to compare quantitative variables, depending on whether or not the variables followed a normal distribution. Variable normality was analyzed using the Kolmogorov-Smirnov. All results were adjusted for age. The significance level was set at 5% ($p < 0.05$).

Results

Table 2 shows the characteristics of the patients included in the study. A total of 411 patients were interviewed, more than half (54%) were receiving PPIs during the field-work survey period, while 46% did not take them. The average age of patients receiving PPIs was greater than those not receiving it (63.3 and 50.9 years, respectively). The age range was 18 to 95 years. The gender distribution of patients in both groups showed no statistically significant differences.

Table 3 shows the indications for which patients received PPIs. Overall, the most common indication was as proton pump inhibitors, which was obtained in about 40% of patients with hiatal hernia as a second cause, which was found in 10% of the users of PPIs. In this respect, no statistically significant differences between men and women were obtained.

The most commonly used PPIs were omeprazole (72.6%), followed by pantoprazole (13.4%). The use of lansoprazole, esomeprazole, and rabenprazol was more limited. The minimum length of treatment with PPIs was 1 month, which was observed in 8 cases, and the maximum 204 months, obtained in 2 patients.

Table 4 shows the prevalence of fragility fractures in the participants. Patients taking PPIs at the time of the survey had a higher prevalence of fragility fractures than those not taking the drugs (12.6% vs 2.6% respectively), with an OR of 5,284, the difference being statistically significant. The prevalence of all different types, vertebral, non-vertebral and hip were higher in patients receiving PPIs ($p=0.003$).

Discussion

Our study shows that there is a significant consumption of PPIs among patients who may not be prescribed them. In our series, 54.1% of patients reported habitually taking PPIs at the time of the survey, similar to the results described elsewhere. In a population of elderly women in Australia, in the so-called Australian Longitudinal Study on Women's Health, with a sample size of 4,432 women, 52.5% received PPI4. In the hospital setting, in a sample of 834 admitted patients, 58.7% were taking PPIs, and "reviewing their indications" they were correct in only 50.1% of the patients[10]. In another study of hospitalized patients in a ward of respiratory diseases, 44% were receiving PPIs, of which 68% did not have a correct indication[11].

By far the most common reason for PPI use in our patients was as a stomach "protector" against other drugs (almost 40% of the total). We would point out that this indication does not exist in any PPI specifications sheet8, and there are no studies indicating that these drugs are effective for this. However, there is a widespread notion in the medical profession that it is prudent to administer various drugs, even when they may be gastro-erosive. The PPI should be added as a "protective" effect only described as effective and indication in the product information for these nonsteroidal anti-inflammatory drugs[8], and its usefulness is not proven in patients receiving oral corticosteroids.

Furthermore, although PPIs are considered

safe with few side effects, their use has been associated with an increased prevalence of certain diseases, and the risk of acute myocardial[12], nephritis or hypomagnesemia[14] intersticial[13]. In the case of bone metabolic disorder IBP consumption has been associated with the presence of fractures in young adults[15], behaving as an independent risk factor for the production of fractures, both as a different studies[3-6,16] metaanalysis[17] where an increased risk of vertebral, non-vertebral and hip fractures, especially in the elderly was observed. This has caused concern among health authorities who have published several notices on this topic[17-20]. Our results are indicative in this sense, not conclusive, because, although the prevalence of fragility fractures was much higher in the group that received PPI, methodological factors discussed below make us cautious when considering the real meaning of these findings.

The study has several limitations. We do not consider the co-morbidity of these patients. Therefore, we cannot establish with certainty which users of PPIs have a greater risk of fragility fractures due to these drugs as with patients taking the drugs there could probably be others receiving drugs whose gastric effects were protected (although incorrectly) that would produce increased bone fragility (as in the case of corticosteroids). Furthermore, taking these other drugs may in turn indicate the existence of conditions that damage the bone (for example, rheumatoid arthritis). Another limitation is the small sample size. On the other hand, this is only a preliminary study to confirm the suspected overuse of PPIs, with an indication that there is no clinical evidence of any kind and that carries a huge unjustified economic cost. We should keep in mind the possible side effects described in other studies discussed above.

Regarding the economic costs, in 2014, more than 3 million containers of PPI were sold in the Canary Islands, which generated an expense of 20 million euros[20], much of which, as we have just shown, without a correct indication. This relates to consumption financed by the Canary Island Health Service. Actual consumption may be much higher, because PPIs are dispensed without a prescription.

In conclusion, although these results are preliminary and include a small sample size, our study shows that more than half of the patients receiving PPIs and, of these, almost 40% take it with an indication which is not approved, which could, in addition to a significant unnecessary health spending, generate an increased risk of other diseases, including fragility fractures. Therefore, we recommend more in depth, broader studies in this direction.

Competing interests: The authors report that none has any conflict of interest.

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Table 1. Therapeutic indications of omeprazole as a model of the proton-pump inhibitor[8]

- Treatment of duodenal and benign gastric ulcer, including complicating treatment with nonsteroidal anti-inflammatory drugs (NSAIDs).
- Water Prophylactic duodenal ulcer, benign gastric ulcers and / or gastroduodenal erosions induced by nonsteroidal anti-inflammatory drugs (NSAIDs) in patients at risk (elderly and/or with a history of gastroduodenal erosions) requiring continuous NSAID treatment.
- Gastroesophageal reflux. Omeprazole is indicated for the treatment of reflux esophagitis, severe symptoms of reflux disease non-inflammatory, and mild symptoms that do not respond to conventional treatment.
- Zollinger-Ellison syndrome.
- Treatment of gastric and duodenal ulcers associated with dual therapy for helicobacter pylori (combination therapy with amoxicillin or clarithromycin) and triple therapy (combination therapy with two antimicrobials at once), the eradication rate is significantly higher with a shorter duration of treatment.

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Table 2. Characteristics of patients included in the study, depending on the no decision or PPI

		They take PPI	Do not take PPI	Value of p
Patients, n (%)		223 (54.1%)	188 (45.9%)	
Age (years), mean ± SD		63.3±13.7	50.9±17.5	0.001
Sex, n (%)	men	98 (53.6%)	85 (46.4%)	p=0.843
	women	125 (54.6%)	104 (45.4%)	

SD: standard deviation.

Table 3. Indication for receiving PPI*

Indication	Total	Men	Women	Value of p
Hiatal hernia	41 (10%)	15 (8.2%)	26 (11.4%)	0.288
Gastroesophageal reflux	32 (7.8%)	13 (7.1%)	19 (8.3%)	0.653
Peptic ulcer	19 (4.6%)	10 (5.5%)	9 (3.9%)	0.461
Helicobacter	5 (1.2%)	2 (1.1%)	3 (1.3%)	0.841
Gastric protector	164 (39.8%)	69 (37.7%)	95 (41.5%)	0.436

*: data calculated only in the subgroup taking the drug at the time of the survey.

Table 4. Presence of fragility fractures and current use of PPIs

Brittle fracture		They take PPI	Do not take PPI	Value of p	OR (IC 95%)
Yes		28 (12.6%)	5 (2.6%)	0.001	5.284 (1.998;13.976)
No		195 (87.4%)	184 (97.4%)		
Kind fracture	Vertebral	14 (6,3%)	4 (2.1%)	0.003	
	No vertebral	6 (2.7%)	1 (0.5%)		
	Hip	7 (3.1%)	0 (0%)		

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General and bone pain syndrome in a patient treated with tenofovir

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Summary

Tenofovir (TDF), is the only nucleotide analogue reverse transcriptase inhibitor for treating human immunodeficiency virus (HIV). Occasionally, it may cause acute renal failure and Fanconi syndrome.

We report the case of a 64-year-old male diagnosed with HIV infection 22 years previous and treated with tenofovir. In outpatient follow-up, the patient complained of progressive fatigue and diffuse aching bones. In several check-ups, increased alkaline phosphatase and parathyroid hormone (PTH) were observed. Over the past month, his condition worsened and he was admitted to hospital. Analytical data included marked glycosuria, hypophosphatemia, hyperphosphaturia and hypouricemia. All changes were resolved when TDF was discontinued.This illustrates the importance of clinical evaluations that include possible TDF-induced proximal tubulopathy in patients with general bone pain syndrome or mineral metabolism disturbances.

Key words: *general syndrome, tenofovir, osteomalacia, Fanconi syndrome.*

Introduction

Tenofovir (TDF), is the only inhibitor of nucleotide analogue reverse transcriptase for treatment of human immunodeficiency virus (HIV) infection. It is sometimes associated with renal impairment, including tubular dysfunction and Fanconi syndrome[1,2]. This syndrome involves a defect in the transport of amino acids, glucose, phosphate, uric acid, potassium, bicarbonate and protein at proximal tubule level[3].

Case report

We report the case of a 64-year-old man diagnosed with HIV infection 22 years previous, taking didanosine, tenofovir and lopinavir/ritonavir. In outpatient revisions over the past two years, the patient complained of diffuse bone pain and fatigue. Several analyses detected elevated total and bone alkaline phosphatase (AP) and the parathyroid hormone (PTH). The 25-hydroxyvitamin D (25-HCC), total and ionized calcium and other routine biochemical parameters in serum and elementary and sediment in urine were normal (Table 1). Plain radiography of the spinal column showed degenerative signs and CT scan was normal.

During the last month, his condition worsened, with increased fatigue, loss of strength, difficulty walking and a loss of 7 kg so he was admitted to hospital. Physical examination was unremarkable except for predominantly proximal muscle weakness. The analytical data highlighted mild hyperglycemia (154 mg/dl), marked glycosuria (4+), hypophosphatemia, unusally high phosphaturia, hypouricemia (2 mg/dl), mild metabolic acidosis (bicarbonate 19 mmol/l) and (945 mg/24h). Furthermore, there was moderate aminoaciduria with increased glycine (x2), valine (x2), serine (x4) and threonine (x4) values. FGF23 serum levels were 6 pg /ml. Other parameters shown in Table 1.

From these results, incomplete Fanconi syndrome was diagnosed with probable severe hypophosphatemia and osteomalacia linked to TDF. The drug was discontinued and treatment commenced with raltegravir and darunavir boosted with ritonavir. Furthermore phosphorus supplements, HCC-25 and 1.25-dihydroxyvitamin D (1.25 DHCC) were administered. His condition gradually improved with this regime. Six months after the withdrawal of TDF, bone pain and muscle weakness stopped, the patient had regained baseline weight and had normalized serum laboratory abnormalities, although phosphate reabsorption remained slightly low.

Discussion

The TDF is excreted by glomerular filtration and is actively transported by cells of the proximal tubule renal[1]. Although tubular disorders, including Fanconi syndrome, are a known complication of TDF treatment, acknowledged in its technical specifications and the subject of different publications[4,5], it is not often accompanied by clinical demonstrations[1,2]. Indeed, in a study of 422 patients with HIV infection, of which 381 received TDF, this drug was not found to be associated with globally altered levels of calcium, phosphorus, vitamin D or markers of bone remodeling, nor in bone mineral density (BMD)[6]. On the other hand, in a clinical trial involving 299 patients treated with TDF and followed up over 144 weeks, 10 cases of hypophosphatemia (similar to that found among stavudine frequency) were found, although none were necessary to remove the drug or developing Fanconi syndrome[7]. In that same study the authors found a slight decrease in spinal BMD, but not in the hip in those individuals treated with TDF. It has been suggested that concomitant administration of other drugs such as didanosine and lopinavir boosted with ritonavir, may increase the risk of tubulopathy[8,9].

Increased phosphate excretion with consequent hypophosphatemia was most relevant in this patient's evolution. The role of FGF23 in hypophosphatemia associated with PDT is controversial[10,11]. In our patient, FGF23 levels were decreased, which runs contrary to the implication of this phosphaturic factor and is consistent with a direct effect of the drug on the renal tubules. Although a bone biopsy was not carried out to confirm the accumulation of osteoid, clinical and laboratory manifestations, including increased PTH and FA are consistent with the existence of a hypophosphatemic osteomalacia[12,13]. The resolution after discontinuing TDF confirms the causal implication of this drug. However, this is a rare complication.

In a recent review of the literature, 53 cases of TFD-induced tubulopathy were found, of which 27 had bone changes consistent with osteomalacia. The median time from treatment initiation to the onset of renal impairment was 2.5 years[14]. The fact that these tubular alterations are not necessarily associated with a decreased glomerular filtration rate, next to that phosphatemia often not included in the biochemical parameters analyzed routinely, can lead to delays in diagnosis.

This case illustrates the importance of clinicians including the possibility of hypophosphatemia secondary to a proximal tubulopathy in the diagnosis of patients treated with vague TDF symptoms such as weakness or pain, which could otherwise be attributed to the underlying disease or other concomitant processes.

Competing interests: All authors declare no conflict of interest.

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Table 1. Analytical parameters observed in the patient 2 years and 6 months before admission, during hospitalization and 6 months afterward

	Normal values	24 months before	6 months before	To the admitted	6 months then
Cr, mg/dl	0.7-1.2	1.2	1.0	0.8	0.9
AP, U/l	40-129	231	200	180	132
PTH, pg/ml	10-45	56	51	25	-
P, mg/dl	2.5-4.5	1.9	-	1.1	2.6
Mg, mg/dl	1.6-2.5			2.4	2.3
Tubular reabsorption of phosphate, %	80-90			40	60

Cr: creatinine; AP: alkaline phosphatase; PTH: paratohormone.

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Review of the incidence of hip fracture in Spain

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Summary

The aging of the world population is an indicator of improving health worldwide. In developed countries, increased longevity has been accompanied by a so-called "compression of morbidity", that is, although people live longer, they do not spend more years suffering a poor health status. However, hip fracture itself may reduce life expectancy in almost two years and one in five patients will require permanent health care.

Epidemiological studies reporting the incidence of hip fracture in Spain are numerous. In most cases, they are retrospective and short-term studies, and their results have shown variations across regions. The overall incidence of hip fracture in our country, in subjects older than 65 years, have varied between 301 and $897/10^5$ inhabitants, lower figures than reported in other European countries or in the USA.

In this paper, we critically review the main published studies in Spain on the incidence of hip fracture.

Key words: *epidemiology, hip fracture, incidence, secular trend, osteoporosis.*

Introduction

The development of osteoporosis has been closely linked to the aging process. In this respect, the number of people aged 60 or more years worldwide has doubled since 1980, and is expected to reach 2,000 million by 2050[1]. The involution process associated with age implies a decrease in neuromuscular co-ordination, vision, hearing and autonomous warning systems. The co-existence of co-morbidities and diminished cognitive function decrease the responsiveness of the organism and imply a more fragile situation. Similarly, the increased consumption of drugs in the elderly, especially psychotropic medications further alters these capabilities, thus leading to falls with possible fracture. Due to its high mortality and morbidity, hip fracture is the most serious complication of osteoporosis. Indeed, hip fracture alone is capable of reducing life expectancy by almost two years and one in five patients who suffer this require permanent health care assistance[2].

Based on these data, the management of osteoporosis and hip fracture will be an issue of great importance in terms of care, hospital management and economic and social spending in the coming decades. Forecasting the evolution of this fracture is a crucial matter and that goes beyond the field of health care. Therefore. In order to interpret the incidence rates of hip fracture at present, we need to ascertain precisely the previous situation. In this paper, we review the most relevant articles published on the incidence of hip fracture in Spain over the past four decades.

Geographical variations in the incidence of hip fracture in Spain

There are many epidemiological studies describing the incidence of hip fracture in this country. In most cases, they are retrospective studies, local, covering short periods of time. Results have varied between different provinces. Thus, the overall incidence of hip fracture in Spain in people older than 65 years has been between 301 and 897/10^5 inhabitants[3], values below those of other countries in Europe or the US.

As in other geographical locations, Spain also presents a north-south gradient in hip fracture incidence rates. The highest numbers are reported in the north, in places such as Barcelona, and lesser in the southern regions, such as the Canary Islands.

Table 1 shows a summary of epidemiological studies on hip fracture incidence in our country.

Catalonia

Díez et al.[4] studied the incidence of hip fractures in Barcelona in 1984, using records of patients over 45 years admitted to acute care hospitals. A total of 1,163 patients, of whom 863 were women, were analyzed. The crude incidence of hip fractures in women was 252.2/10^5 and 115,6 inhabitants in men. The risk of fracture was 50% higher in women in any age group. In 1989, in the same city, Cucurull et al.[5] found the incidence was increased significantly in women, but was stable in men, in statistical terms.

Asturias

Altadill et al.[6] analyzed the epidemiology of hip fracture in Asturias. They reviewed the medical records of all patients over 45 years of age admitted during 1992 and residing in two health areas of Asturias, a representative group encompassing rural and urban areas. Pathological fractures were excluded. The authors identified 283 osteoporotic hip fractures, 225 of them women. The annual incidence was 219.6/10^5 inhabitants in individuals over 50 years. The incidence in women over 45 years was three times higher than that of men of the same age (271 vs 88/10^5, respectively). They also observed an exponential increase in incidence with age, finding that it doubled every five years from 75 years of age and in both sexes. The overall incidence of fractures was similar in both health areas: 76.5 and 83.5/10^5 inhabitants per year. The incidence of hip fractures in the rural habitat was 167.4/10^5-year residents over 45 years and 218.9/10^5 year in an urban setting, for the same age group. The incidence in individuals over 50 years was also higher in urban areas (266 cases/10^5 inhabitants/year) than in rural (185.7/10^5 inhabitants/year). No difference in the proportion of women and men from both population areas.

Castilla-Leon

Fernandez et al.[7] studied the incidence of hip fracture in Salamanca, in subjects over 50, from 1977 to 1988. They obtained an annual incidence of 195 cases/10^5 in women and 73/10^5 in men. During the 12 years analyzed, an increase in crude incidence of fracture of 143% was observed. Later, White et al.[8] conducted a retrospective study to determine the incidence of hip fracture in 1994-2002. They collected data on hospital discharges University Hospital of Salamanca patients over 65 diagnosed with hip fracture. A total of 2,726 cases were registered and was observed relative increase in hip fracture of 81% for women and 98% for males. The crude incidence fractures increased from 315 in 1994 to 496/10^5 inhabitants in 2002. This increase was not explained population aging, as during the study period, the number of subjects over 65 years increased 17% while the relative increase in hip fracture incidence was 57%.

The Canary Islands

In the Canary Islands, Sosa and colleagues analyzed the epidemiological and demographic characteristics of hip fracture over two defined periods. In the first[9], they studied the incidence of hip fractures from January 1, 1989 to December 31, 1993, in people age over 49 years in Gran Canaria, using records culled from the island's public and private hospitals. The total number of fractures was 1,175, of which 848 were in women. The adjusted incidence showed an increase in fractures from 127.8/10^5 in 1989 to 170.1 in 1993, an increase that, although observed in both sexes, was more pronounced in men. A higher incidence was recorded in autumn and winter.

Table 1. Epidemiological studies on the incidence of hip fracture age of 10^5 persons/year in females (F) and males (V) in Spain

Author (year)	Period	Location	Population study	Cup of incidence (10^5/year) (F/V)
Ferrández L (1992)	1977-1988	Salamanca	>50 years	195/73
Díez A (1989)	1984	Barcelona	>45 years	252/115
Olmos JM (1992)	1988	Cantabria	>49 years	277/100
Sosa M (1993)	1989-1993	Canary Islands	>49 years	221/170
Altadill A (1995)	1992	Asturias	>45 years	271/88
Arboleya LR (1997)	1994-1995	Palencia	>49 years	337/121
Serra L (2002)	1996-1999	Spain	>65 years	695/270
Herrera A (2006)	2002	Spain	>60 years	913/417
Blanco J (2006)	1994-2002	Salamanca	>65 years	699/225
Hernández JL (2006)	1988-2002	Cantabria	>50 years	389/101
Álvarez-Nebreda ML (2007)	2000-2002	Spain	>65 years	678/262
Sosa M (2013)	2007-2011	Canary Islands	>50 años	205/89
Azagra R (2014)	1997-2010	Spain	>65 years	766/325
Sosa M (2015)	1989-1993	Canary Islands	>49 years	204.5/91.4
	2007-2011			246/108.1
Etxebarria-Foronda I (2015)	200-2012	Spain	>65 years	153.24

In the second study, carried out during 2007 and 2011[10], a total of 2,222 cases were collected of hip fractures in patients over 50, of whom 1,593 (71.7%) were women. The number of fractures increased with age until the early 90s. The female/male ratio was 2.53. The overall annual incidence was 150 cases/10^5 inhabitants, and 205.4 in women and 89.1 in men. During the study period, the incidence of hip fracture in Gran Canaria remained broadly stable, with the highest overall incidence in 2010 and the lowest in 2007, with a difference between both of 34.1 cases/10^5 inhabitants/year. 29.7% of all fractures occurred in winter.

The most recent work of this group, published in early 2015[11], has aimed to compare changes in the incidence of hip fracture between the two periods mentioned. The age-adjusted incidence of hip fracture increased by 7.3% annually between 1989 and 1993. These findings suggest a trend towards stabilization of the incidence of hip fractures in the Canary Islands, mainly benefiting men. In women, however, the incidence has continued to increase.

Cantabria

In the north central area of Cantabria, two studies have been published so far into the epidemiology of hip fracture. The first, made by Olmos et al.[12], the incidence of hip fracture in men and women older than 49 years was analyzed during 1988, stratifying the data by place of residence (rural or urban) and on the time of year when the fracture occurred. The overall annual incidence rate was 198/10^5 inhabitants. For women, the figures were 277/10^5 and, in males, 100 cases/10^5 inhabitants. No significant differences in the incidence of hip fractures in rural and urban areas were found nor any evidence of seasonality.

Later, Hernandez et al.[13] analyzed the incidence of hip fractures in both sexes in 2002. The data obtained were compared with those of 1988, establishing for the first time the long-term trend of hip fracture in Cantabria. On this occasion, the authors observed increased incidence of hip fracture of about 50%, especially among women with respect to the values obtained 14 years earlier. However, adjusting the crude values depending on the age, no significant changes were observed. The recorded increase was attributed to factors related to the aging population. An interesting finding of this study was a greater increase in the number of cervical hip fractures (72.5%) than for trochanteric fractures (41%). As this type of fracture is considered the typical osteoporotic disease, given the aging of the population mentioned previously, it would seem logical to find an increase in the number of trochanteric fractures and not the cervical. The authors related this fact with the possible effect of anti-osteoporotic drugs and with the increase in size and physical exercise, factors that

favor an increase in cervical fractures. A seasonal pattern in the incidence of hip fracture in males in 2002 was also observed, not present in 1988, which consisted of an increased fracture rate in the summer and winter in connection with the spring and autumn. In this case the cause was more likely to be increased based on time out of the home during summer as residents tend to spend more time practicing outdoor sports activities, especially in the case of men. In winter months, with unfavorable weather conditions, there is an increased probability of falls and possible fractures. This work highlighted, from the results commented, that the relationship between the incidence of hip fracture in Spain compared to northern European countries remained constant.

National studies

Only five studies published to date have analyzed the incidence of hip fracture across Spain. In the first, Serra et al.[3], analyzed data from the National Register of the Minimum Basic Data Set (MBDS) at the Ministry of Health regarding hip fracture (identified by codes 820.0 to 820.9 of ICD-9) from 1996 to 1999. In this period a total of 130,414 cases of hip fracture in Spain in subjects older than 65 years were recorded. The overall crude incidence was 517 cases/10^5 inhabitants/year (270 cases in men and 695 in women). The results in the various regions showed large differences in incidence rates, the lowest in the Canary Islands and the highest in Catalonia (221 and 658 hip fractures per 10^5 inhabitants/year, respectively). The incidence by age group increased exponentially, from 107 cases/10^5 inhabitants/year in the age group 65 to 69 years, reaching 3,992 cases/10^5 inhabitants/year in individuals over 94 years. The incidence of hip fractures in women was almost double that of men in all age groups up to age 94.

Herrera et al.[14] conducted a multi-center study in 77 Spanish hospitals, recording osteoporotic hip fractures in patients over 60. The retrospective study evaluated fractures which occurred in 2002, and a prospective phase evaluated hip fractures in May 2003. In the retrospective phase 13,195 hip fractures were recorded, 74% of them in women. The overall average incidence was 6.94/10^3 inhabitants, 4.17/10^3 inhabitants/year in the case of men and 9.13/10^3 inhabitant/year in women. Extrapolation of these data allowed the authors to calculate an average of 61,173±3,878 osteoporotic hip fractures in patients older than 60 years in Spain in 2002. In the prospective phase, a total of 1,399 hip fractures were recorded. The annual incidence in males was 0.36/10^3 and 0.80/10^3 women. With these data, the authors estimated a prevalence of osteoporotic hip fractures 7.20 per 10^3 inhabitants/year in 2003 in subjects over 60 years in Spain.

Alvarez-Nebreda et al.[15] conducted a retrospective study of patients over 65, treated for hip fractures in the 19 Spanish Regional Communities from 2000 to 2002, data from the National Registry of the Ministry of Health (MBDS). They reported 107 718 cases, of which 74% were women. The adjusted annual incidence of hip fracture was 503 cases/10^5 inhabitants/year, 262 in men and 678 in women. The authors found differences between different communities, the Canary Islands showing the lowest incidence of hip fracture and the city of Melilla, the highest (312 and 679/10^5 inhabitants, respectively). The annual incidence rates increased exponentially with age (97 cases per 10^5 inhabitants/year among patients 65 to 69 years by 1898 and 10^5 inhabitants/year over 85 years). The incidence rate in women was twice that of men in all age groups up to 85 years.

Azagra et al.[16] analyzed the incidence rates of hip fracture in Spain in subjects older than 65 during two time periods: 1997-2000 and 2007-2010. As the MBDS source from the Ministry of Health and the codes used were analyzed from 820.0 to 820.9 of ICD-9. A total of 119,857 hip fractures in men and 415,421 women were counted. Incidence rates by sex were 259.24/10^5 inhabitants/year in men and 664.79/10^5 inhabitants/year in women in 1997 and 325.30/10^5 inhabitants/year and 766.37/10^5 inhabitants/year 2010, respectively.

In this work, a downward trend was observed in the incidence of hip fracture in women 65 to 80 years old accompanied by a significant increase after 85 years in both sexes. The authors pointed out that changes in population structure in Spain could be responsible for increased hip fracture rates in the population aged 85 or more. Furthermore, the widespread prescription of antiresorptive drugs, especially bisphosphonates, in women and men under 70 could be responsible for the decline in the crude rate of hip fracture found in the youngest of the sample population.

Finally, Etxebarria-Foronda et al.[17], based on data collected in the same national MBDS analyzed the trend in the incidence of hip fracture by age, among women of different Spanish autonomous regions, from 2000 to 2012. In 2000, the incidence of hip fracture in women was 131.26/10^5 inhabitants/year, which in 2012 amounted to 153.24/10^5 inhabitants/year. In this study, a continuous increase in absolute numbers was noted in the rate of hip fracture. However, after adjusting these rates by age, a decreasing trend was observed in certain sectors of the population. Accordingly, the authors demonstrated the presence of statistically significant changes in the trend of incidence rates in all age groups in women over 65 years. In the group of women between 65 and 74 years, the annual reduction was 2.2% and was slightly lower in the group of 75-84 years. The incidence rate in women over 85 increased by 0.58% on a yearly basis. The authors suggested that given the absence of major changes in the population structure, drugs for osteoporosis could be the main factor involved in the observed changes.

Discussion

As we have seen, most of the studies in Spain are cross-sectional, ie. quantify the incidence of hip fracture in a specific city or region over a given

time period of time. Often the analyzed population varies between the studies and others, as well as the duration of the time period studied. Furthermore, the design of the work, the analysis methods used, results and conclusions are not quite reach homogeneous.

Studies published since the second half of the 90s, and especially those made during the first decade of the century, have begun to enhance the analysis of the incidence of this type of fracture, carrying out comparisons between the rates found in different geographical areas examined and considered in the interpretation of the results, the possible influence of factors such as climate, habitat and the effect of lifestyle changes and drug treatment for osteoporosis.

This situation should make us reflect as to what extent the international agencies responsible for planning strategies for the prevention and treatment of osteoporosis and hip fracture are based on data that properly reflect developments in the incidence of hip fracture in Spain. In 2011, Cooper et al.[18], published an extensive report made from the analysis of 40 studies on the incidence of hip fractures in 40 countries from 4 of the 5 continents (Africa was not included). In this report, the question of the evolution of the incidence of hip fracture from the point of view of analyzing long-term trends identified, through the use of models of age-period-cohort, the age-specific rates of hip fracture in the final decades of the twentieth century. In this report, Spain was represented only by the study by Hernandez et al. in 2006, cited previously.

A year later, another report by Kanis et al. was published, by the Working Group for Epidemiology and Quality of Life of the International Osteoporosis Foundation (IOF)[19] based on the analysis of 72 studies from 63 countries. Unlike the previous study, this did not refer to secular trends in the populations studied, but noted that there are geographic differences in the incidence of hip fracture, approximately 10 times, depending on the area considered. It also found a decreasing north-south and urban-rural gradient. The precise reasons for these variations were not well defined, but related to genetic, environmental and demographic factors. In this report, Spain was represented using data from four studies, some of which collected data over more than three decades[4,9,20,21]. However, the methodological quality was deemed "good" or "appropriate."

In this scenario, it is not unreasonable to posit that most of the data used as reference for the incidence of hip fracture in Spain, are not entirely faithful nor do they reflect the situation current of the same in our country. Along with this lack of precision, we must consider the projections made on aging in Spain. The National Statistics Institute (INE) indicated that in our country, the number of people over 65 has doubled over the past 30 years. This process has been exacerbated by the low birth rate that has been recorded for several decades. Current data show that the Spanish population over 65 years represents about 17% of the total population, with more than 7 million people, of which approximately 25% are octogenarians. The same organization states that, in 2050, people over 65 will constitute more than 30% of the population, ie. almost 13 million people, of which octogenarians will number more than 4 million. Similarly, a United Nations report[22] predicts Spain will be the oldest country in the world in 2050, the year when 40% of our population will be over 60 years.

In addition to this bleak panorama, we would highlight the limited follow-up control in these patients after hip fractures occur. In this sense, it would be desirable to carry out a multidisciplinary treatment of these patients in different units, a fact that would result in better control in the acute phase, especially when complications develop during the postoperative period and the exacerbation of pre-existing conditions. This situation is particularly important in the case of older patients, usually presenting poly-pharmacy and a high frequency of co-morbidities. which makes them an especially complex group. In addition, it is worth noting that, despite improvements in anesthesia and control of infections after surgery, the mortality rate for this type of fracture has barely changed in recent decades[24,25]. Furthermore, the use of anti-osteoporotic drugs after hip fracture remains low and may even be decreasing[23].

In the past decade, advances in the understanding of the patho-physiology of osteoporosis and the development of anti-resorptive and osteoforming drugs make it essential that these patients be evaluated and monitored by teams of specialists with specific training in this field, which has been designated in recent studies[26] cost-effectiveness.

In conclusion, further work to update data on the incidence of hip fracture in Spain, especially its secular trend, and to unify criteria, both in the design and methods of analysis of the results should be obtained. This update may be of particular interest in defining the intervention thresholds based on absolute fracture risk, implemented at national and international level. Specific training should be provided to health professionals involved in health care after hip fracture, prevent the occurrence of new fractures and to treat chronic age-related diseases that worsen the prognosis and quality of life after the fracture. The development of multidisciplinary fracture units could provide a valuable option to improve the care of these patients and the social and health management of osteoporotic fracture.

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Secular trend in the incidence of hip fractures in the world

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Summary
Osteoporosis is the most common metabolic bone disease and the main effector of the development of fractures in people over 50 years. When analyzing the evolution of the incidence of hip fracture it is important to consider the effects of the implementation of the strategies undertaken to prevent and treat early forms of disease and falls.
In most chronic diseases with an environmental etiological component, identified or not, an interval of several decades occurs between initial exposure to the main causative agent and the clinical onset of the disease. The systematic study of the secular trend of a disease shows different phenomena that help to understand its pathogenesis. At the same time, it constitutes an activity of surveillance that allows warn about its future relevance.
The evolution of the incidence rate of hip fractures has not been uniform over time in different countries. It is a matter of great interest to identify whether the observed temporal changes in rates are associated with an aging population or the result of a large number of circumstances of the same population over time.
In this paper we review the main studies published around the world that explore, in greater or lesser extent, in the analysis of the secular trend in the incidence of hip fracture in order to bring this concept to the reader and offer an overview on the evolution of the incidence of hip fracture and the causes of this evolution.

Key words: *epidemiology, hip fracture incidence, secular trend, osteoporosis.*

Introduction

Osteoporosis is the most common metabolic bone disease and the main cause of fracture in people over 50 years of age[1]. Health promotion programs carried out by public institutions in most Western countries over the past 50 years have led to enhanced health care[1], access to medicines and rehabilitation programs, accessible to most of the population in industrialized countries. This fact, which has improved the health of many and has conditioned an increase in life expectancy, is also responsible for a change in the specific causes of mortality, an overall increase in morbidity and in the expression of different diseases, including osteoporosis.

When analyzing the evolution of the incidence of hip fracture it is important to consider the effects of the implementation of strategies undertaken to prevent and treat early forms of the disease and osteoporosis-related falls. Numerous reports detect variations in hip fracture incidence, including but not limited to geographical factors. Most of these studies concur that a crude increase in the number of hip fractures in both male and female patients over 50 in the second half of the last century. However, as a result of the "demographic transition" described by Omran[2], more people are living longer, and therefore more at risk of suffering a hip fracture, specific age-adjusted rates are required for analytical purposes. Thus, it would be possible to ascertain the real evolution of the incidence of this type of fracture and the factors responsible for this evolution beyond the mere aging population.

Secular trend of hip fracture

The term "secular trend" implies systemic change in the age-specific rates depending on the time described. It represents an intricate set of social, epidemiological and demographic factors present in a population over time. In most chronic diseases with an environmental etiological component, whether identified or not, an interval of several decades occurs between initial exposure to the main causative agent and the disease's clinical onset. Thus, changes in the observed secular trends correspond to variations in the exhibitions produced years previous when the individuals under study were young. Therefore, the analysis of the data, with particular attention to the specific incidence rates by age, is very useful in interpreting secular trends and helps explain the phenomena observed in a given period. The models of age-period-cohort commonly used in descriptive epidemiology studies to analyze the trend in the incidence and mortality from different diseases, but, in general, they are applied to any situation to assess the effect the temporal occurrence of an event.

Thus, the systematic study of the secular trend of a disease shows different phenomena that help to understand its pathogenesis. At the same time, it constitutes a surveillance function that warns of its future relevance. Secular trends may provide information on the effects of programs such as early detection, prevention strategies or the impact of new drug therapies. Correct interpretation also contributes to decision making regarding the distribution of resources, a fact that becomes particularly relevant in the current historical moment.

In assessing the change in disease frequency over a period of time and in the same population, one may observe certain factors that basically depend on three effects: the effect of age, the period effect and the effect of the date of birth[3].

1) Effect of age

This refers to the process of aging. It is observed when there is a change with age in the frequency of a particular disease. Many diseases have a tendency to increase in prevalence with age, a fact that reflects the aging, defined as the combination of biological, social and psychological changes, influences the susceptibility to present a specific disease process.

2) Effect of period

This implies a change that uniformly affects all age groups and all population cohorts. The diagnostic measures determine the identification of a specific disease in a given period of time and are applicable to all age groups, as in the case of diagnostic and therapeutic improvements, such as bone densitometry and the spread of anti-osteoporotic drugs in the case of osteoporosis. Furthermore, changes in the criteria of the International Classification of Diseases (ICD), are listed as changes in trends associated with the timing of the event.

3) Effect of date of birth (cohort effect)

People born at a certain time, carry with them throughout their entire life a greater chance of developing a disease at some point. This effect is seen in the case of subjects exposed to natural disasters, war, radiation or toxic drugs (as in the case of thalidomide). However, it may also reflect its effect of individuals' the type of feeding during infancy and even the diseases that have been submitted during the first years of life. This cohort effect implies an unexpected change that would result in the distribution of cases depending on the age group.

Epidemiological significance

In developing countries, it is expected that the global population and life expectancy will double over the next 25 years. Regarding Western countries, although population is not expected to increase significantly in the coming years, forecasts indicate that the percentage of the elderly population in Europe will increase by 33% in the next three decades. It has been estimated that in 2050 the number of hip fractures will be 6.26 million, of which approximately 50% will occur in Asia[4]. The clinical and care relevance of hip fracture, therefore, implies one of the greatest challenges facing health authorities in the next four decades.

For these reasons, there is considerable interest in identifying whether the observed temporal changes in rates are associated with an effect of

age, a period effect or a birth cohort effect, an objective which is not always possible, given accurate linear relationships between these factors.

Table 1 summarizes the main epidemiological characteristics and the results of published studies worldwide that, to a greater or lesser extent, delve more deeply in the analysis of the secular trend in the incidence of hip fracture.

Incidence of hip fracture in North America

There are numerous published studies in the United States (US) and Canada that suggest the possibility of an effect of age, period and birth cohort effect[5] in the evolution of the incidence of hip fracture.

USA

Melton et a.l[6,7] found a 5-fold increase in hip fracture incidence in the period from 1928 to 1942 and 1973 to 1982, which encompassed 135.8 to 675.8 per 10^5 inhabitants. This change was due almost entirely to the increase in the incidence of hip fracture in women until 1950, and in men until 1980, observed in all age groups. The decline in incidence rates thereafter led to a 9% drop in the rate of hip fractures in the period between 1973 and 1982 and between 1983 and 1992, reaching 612.7 per 10^5 inhabitants. They also noted an increase of 13.7 years in the age at which the first hip fracture appeared, a fact explained by the aging of the population.

Bacon[8] observed a linear increase in the incidence of hip fracture in males ≥80 years. However, rates did not change significantly in women or the group of young men.

Brauer et al.[9] obtained information about drugs consumption through a survey conducted between 1992 and 2005 and, with these data, extrapolated the trend of the use of bisphosphonates, estrogen and estrogen receptor modulators. During the study period, the average annual hip fractures in women was 957.3 per 10^5 inhabitants and 414.4 per 10^5 inhabitants in men. The age-adjusted incidence increased between 1986 and 1995 and then declined steadily between 1995 and 2005. In both men and women, the increase in the incidence of hip fracture between 1986 and 1995 was more pronounced in subjects over 75 years.

The Framingham study, a cohort study of population base carried out between 1948 and 1996, confirmed a gradually increasing incidence rate of hip fracture during the second half of the past century[10]. This study suggests the presence of a birth cohort effect on the risk of hip fracture rates by 20% and 40% higher among women born between 1901 and 1910 and between 1911 and 1921, respectively, compared with those born in the previous decade. Samelson et al.[11], based on the premise that bone strength in old age is a function of accumulated bone mass in the first two decades of life, as well as a loss of bone mass from middle age, they determined the rates of hip fracture from the age specific Framingham Study, and studied the relationship between birth cohort and hip fracture risk. For each birth cohort they found an exponential relationship between age and the risk of hip fracture, both among women and men. Compared with women born in 1900, the incidence was 1.2 and 1.4 times higher among women born between 1901 and 1910 and between 1911 and 1921, respectively. In males, compared with the oldest birth cohort (1887-1900), fracture risk was 50% higher in men born between 1901 and 1910 and twice as high for those born in the last study period (1911-1921).

Leslie et al.[12] identified 570,872 patients hospitalized with a principal diagnosis of hip fracture between 1985 and 2005. During the 21 years analyzed, rates of hip fracture decreased in both sexes and all age groups with a decline in women from 118.6 to 80.9 fractures per 10^5 person-years in men and from 68.2 to 51.1 fractures per 10^5 person-years. The sharpest absolute decline occurred in the group of subjects over 85 years, in both women and men. Regression analysis identified a change in slope in about 1996.

Jean et al.[13] analyzed whether the pattern observed by Leslie's group could be explained by a period effect, a birth cohort effect, or both. They appreciated significant period effects both in men and women. Compared with incidence rates of hip fracture between 1985 and 1989, the observed rates between 2000 and 2004 were reduced by 21% and 32% in males and females, respectively. Birth cohort effects were also observed in both sexes. Thus, the cohorts born before 1950 had a higher risk of hip fracture, while those born after 1954 had a lower risk.

Incidence of hip fractures in Europe

Scandinavia

The countries of Scandinavia (Finland, Sweden, Norway and Denmark) show the highest incidence of hip fracture in the world. There are many studies that have analyzed the secular trend in the incidence of hip fractures in the different countries of this northern Europe region. Overall, the incidence of hip fractures in the Scandinavian countries increased between 1950 and 1990. Over the past two decades there seems to have been a decline, which was most evident among both women and men.

Sweden

In Sweden, Zain et al.[14] analyzed the incidence of hip fracture during the years 1965, 1970, 1975 and 1980. In each five-year period, the crude number of hip fractures increased between 21% and 25%. The incidence of hip fractures in the population increased from 430 per 10^5 inhabitants in 1965 to 650 per 10^5 inhabitants in 1980. The age-specific incidence increased, especially in individuals over 85 years. A subsequent study in Malmo Sernbo et al.[15] also showed an increase in hip fracture incidence between 1950 and 1985, among both men and women.

Table 1. Studies on the incidence of hip fractures in the world

Country	Author (appointment)	Period	Study population	Code ICD	Incidence rate (10^5/year)		Yearly % change
USA							
	Melton et al.[6]	1928-1982	≥65 years	ICD 9:820-9	Δ +540		+2
	Melton et al.[7]	1983-1992	≥65 years	ICD 9:820-9	Δ -63		-0.8
	Bacon[8]	1965-1993	≥50 years	NA	NA		Not change
	Brauer et al.[9]	1985-1995	≥65 years	ICD 9:820-9	Δ F +87	Δ M +64	+0.9
		1995-2005			Δ F -256	Δ M -64	
	Framingham[10]	1948-1996	≥50 years	NS	NA		+1
Canada							
	Leslie et al.[12]	1985-2005	No age restriction	ICD 9:820-9 ICD 10-CA:S72.0-.2	Δ F -39		-1.6
					Δ M -17		
Sweden							
	Zain et al.[14]	1965-1980	≥55 years	ICD 9:820-9	Δ +220		+2.2
	Sernbo et al.[15]	1950-1985	≥55 years	NS	Δ F +530		NA
					Δ V +240		
	Rogmark et al.[54]	1992-1995	≥50 years	NS	F 850		-0.5
					M 360		
	Rosengren et al.[16]	1987-2002	≥50 years	ICD 9:820-9 ICD 10-CA:S72.0-:2 ICD 9 841.82x or - ICD 10 NFB, NFJ	Δ F -90	1987-1996 -0.11	
						1996-2002 -1.37	
					Not change	1987-1996 +0.35	
						1996-2002 -0.66	
Norway							
	Omsland et al.[17]	1999-2008	≥50 years	ICD 9:820-9 ICD 10-CA:S72.0-2	Δ F -110		NA
					Δ M -30		
Finland							
	Kannus et al.[18]	1970-1997	≥50 years	ICD 8:820.X ICD 9:820-9 ICD 10:S72.0-2	Δ F +175		+2.2
					Δ M +121		
	Kannus et al.[19]	1997-2004	≥50 years	ICD 8:820X ICD 9:820-9 ICD 10:S72.0-2	Δ F -82		-2.4
					Δ M -15		

Table 1. Studies on the incidence of hip fractures in the world *(cont.)*

Country	Author (appointment)	Period	Study population	Code ICD	Incidence rate (10^5/year)	Yearly % change
Denmark						
	Giversen[55]	1987-1997	≥50 years	ICD 8:82000-3 ICD 10:S72.0-2	Δ F +18 Δ M +8	+4.1
	Abrahamsen et al.[20]	1997-2006	≥60 years	ICD 10:S72.0-2	F -22% M -20%	+1
United Kingdom						
	Spector et al.[21]	1968-1995	Without age restrictions	ICD 9:820	1968-1978 F +61% 1968-1979 M +73%	+2
	Evans et al.[22]	1968-1986	≥65 years	ICD N820, N821	Δ F +120 Δ M +20	+6 (until 1978)
	Wu et al.[23]	1988-2008	≥45 years	ICD 10:S72.0-2	Not change	NA
Netherlands						
	Boereboom et al.[24]	1986-1993	≥65 years	NA	Δ F +120 Δ M +110	+1.3
	Goettsch et al.[25]	1993-2002	NA	ICD 9:820-9	Δ -10	-0.5
Austria						
	Mann et al.[26]	1994-2006	≥50 years	ICD 9:820-9 ICD 10:S72.0-2	Δ F +121 Δ M +86,5	+0.8
Germany						
	Icks et al.[27]	1995-2004	No age restriction	ICD 9:820-9 ICD 10:S72.0-2	Δ +20	+0.5
Italy						
	Agnusdei et al.[28]	1980-1991	≥50 years	NA	Not change Δ M +3.62	NA
	Rossini et al.[29]	1999-2002	≥45 years	ICD 9:820.9;821.1	+9%	NA
	Piscitelli et al.[31]	2003-2005	≥45 years	ICD 9:820.9;821.1	Δ F +38 Δ M +30	NA
	Piscitelli et al.[32]	2000-2009	≥65 years	ICD 9:820.9;821.1	+29.8%	NA

Table 1. Studies on the incidence of hip fractures in the world *(cont.)*

Country	Author (appointment)	Period	Study population	Code ICD	Incidence rate (10^5/year)	Yearly % change
China						
	Chalmers et al.[39]	1965-1967	Without age restrictions	NA	F 262 M 176	NA
	Lau et al.[42]	1966-1995	≥50 years	NA	F x 2.5 M x 1.7	+7.5
	Koh et al.[43]	1991-1998	≥50 years	ICD 9: 820.0;820.2;820.8	F 402 M 152	F +1.2 M +0.7
India						
	Dhanwal et al.[45]	2009	≥50 years	ICD 10:S72.0-2	F 159	NA
Japan						
	Orimo et al.[44]	1987-2007	≥39 years	NS	Δ F 89 Δ M 21	NA
Australia						
	Crisp et al.[37]	1997-2007	≥50 years	ICD 9:820-9 ICD-10 S720-2, ICD-10-AM W00, W01, W03-W08, W18, W19, W22, W50, W51, W548	Δ F -75 Δ M -26	NA
New Zealand						
	Rockwood et al.[33]	1950-1987	≥65 years	NA	NA	+2
	Fielden et al.[34]	1988-1999	≥65 years	ICD 9:820	NA	-1.2
	Stephenson et al.[35]		≥65 years	821, 827, 828, 804, 820	NS	NS
Cameroon						
	Zebaze et al.[47]	1996-1998	≥65 years	NS	F 24,4 M 20,7	NA
Morocco						
	El-Maghraoui et al.[46]	2006-2009	Without age restrictions	NS	F 86 M 73	-0.4 +3.1

ICD: international classification of diseases; F: females; M: males; NA: not available; NS: not specified.

Recently, Rosengren et al.[16] analyzed the possible existence of a period-cohort effect. The adjusted incidence rate decreased with age in women, from 680 in 1987 to 590 per 10^5 person-years in 2002. However, the figures for men remained stable. Regression analysis identified a trend toward change in 1996. The existence of a period effect and a cohort effect was more pronounced among women than among men, showing a significant reduction in the incidence of hip fractures in post birth cohorts.

Norway

The NOREPOS[17] study analyzed the annual incidence of hip fracture in Norway and its secular trend between 1999 and 2008. The adjusted rate hip fracture by 10^5 person-years in women was 910 in 1999 and 800 in 2008. In men, the rates were 410 and 380, respectively. These figures correspond to a decrease in the incidence of hip fracture age-adjusted 13.4% in women and 4.8 in men. Among women a statistically significant decrease in all age groups from 70 years was observed. In groups of men 75 to 84 years, there was also a decline in the incidence of fracture, whereas in the other age groups the incidence rates were stable. Despite this decline in the age-adjusted incidence, the absolute number of hip fractures in women was stable and increased in males. The most likely explanation for this was that the number of women and men over 50 increased by 11% and 17%, respectively, over the 10-year period analyzed.

Finland and Denmark

In Finland, Kannus et al. observed that hip fracture rates adjusted for age in subjects over 50 years of age, 20% of women and 6% for men between 1997 and decreased 2004[18,19]. Meanwhile, in Denmark, Abrahamsen reported that in patients over 60 years the incidence of hip fracture between 1997 and 2006 was reduced by 20% in both sexos[20].

United Kingdom

Since the early 1950s there have been numerous studies that have described an increasing trend in the incidence of hip fractures in the United Kingdom. Spector et al.[21] analyzed data from hospital discharge for hip fracture in England and Wales in the 1968-1985 period. They observed an increase in age-adjusted and sex steadily over the first 10 years of the study, followed by a subsequent stabilization, which the authors attributed to the decline in physical activity in the elderly population. Evans et al.[22] observed a similar pattern after analyzing the data of hospital admissions for hip fracture between 1979 and 1985, collected in the Oxford Record Linkage Study. They also found a clear effect of birth cohort, with differences in rates in births between 1883 and 1917, similar to the results of the Framingham cohort.

In a more recent study, Wu et al.[23] found that hip fracture rates have continued to increase in England until 2009. Both the number of hip fractures as well as crude fracture rates increased over a 11-year study period, but there were few changes in the age-adjusted rates. In women, the largest percentage increase in rates of hip fracture was observed in the 55-to-64 age group, and the largest absolute increase in women over 85 years. In males, the largest percentage increase was observed in the 45-54 age group, while the largest absolute increase occurred in men over 85 years.

Netherlands, Austria, Germany and Hungary

In the Netherlands, the incidence of hip fracture, adjusted for age, increased linearly between 1972 and 1987[24], but a subsequent study suggests a stabilization between 1993 and the end of the century[25]. Similarly there has been a stabilization of hip fracture incidence in Austria[26] and Germany[27] between 1990 and 2000. In these two countries, there have also been significant reductions in fracture rates adjusted for age from 2000 to 2005.

Therefore, although initial research Central European countries reported an increase in the incidence of hip fractures adjusted for age in both sexes, recent studies report a stabilization and, more recently, indicate a decline in the incidence of this type of fracture.

Southern Europe

Apart from Spain, only in Italy were studies carried out to assess the secular trend of hip fracture. Agnusdei et al.[28] studied the incidence of hip fracture in the province of Siena, between 1980 and 1991, using records of all cases of hip fracture contained in orthopedics wards of several area hospitals. The secular trend among males increased linearly, from 57.5/10^5 person-years in 1980 to 108.9/10^5 person-years in 1991, which represented an annual increase of 3.62 cases per 10^5 person-years. No significant trend was observed in women. The overall incidence rate during this period was 157/10^5 person-years, much lower than in the countries of northern or central Europe.

Subsequently, Rossini et al.[29] analyzed the incidence of hip fracture. They found an increase of 9% in 2002 in compared with 1999 data.

Piscitelli et al.[30,31] carried out an extension study using data on hospital admissions for hip fracture in subjects over 65 throughout the country, during the years 2003 and 2005. They recorded nearly 90,000 cases, of which 78% occurred in women, 84.3% of whom were 75 or more. Hospitalization for hip fracture in both sexes showed an increasing trend throughout the period under review. Hospital costs increased to € 467 million in 2005, and rehabilitation costs rose to € 531 million in the same year.

The most recent work published in Italia[32] analyzes the epidemiology of hip fracture through registered hospitalizations from 2000 to 2009, incidence rates stratified by sex and age in patients ≥65 years. This work includes a sub-analysis during the 2007-2009 triennium analyzing the inci-

dence of hip fractures in the five-year-older population. From 2000 to 2009, a total of 839,008 hospitalizations were recorded for femoral neck fractures, a figure which corresponded to an overall increase of nearly 30% in the ten years covered by the study period. The incidence per 10,000 population increased markedly in people ≥75, from 158.5 to 166.8 (+5.2%) in women and from 72.6 to 77.5 (+6.8%) in men. In the five-year age analysis conducted during the last 3 years of the study it was observed that patients older than 84 presented a gradual increase in hip fracture incidence, from 35,472 in 2007 to 37,899 in 2008 and 39,244 in 2009. The incidence of hip fractures in women under 75 years of age increased from 2000 to 2004 by 5.9%, but then decreased by 7.9% between 2004 and 2009.

Incidence of hip fracture in Oceania

The evolution of the incidence of hip fracture in New Zealand and Australia has followed a similar pattern to those observed in North America and Europe.

New Zealand

Rockwood et al.[33] analyzed the hip fracture rates adjusted for age in New Zealand between 1950 and 1987. These authors observed a disproportionate increase in the number of fractures in the population group over 75 years, particularly among women over 85, which could not be explained solely by the increase in population in this age group. They performed a regression analysis according to predictions of population growth, and estimated that in 2011 the incidence of hip fracture would double.

Fielden et al.[34] conducted a follow-up study between 1988 and 1999 to compare hip fracture incidence rates in New Zealand during that period of time with those predicted in 1990 by Rockwood. In both men and women, the number of hip fractures between 1988 and 1993 was similar to that expected number, but since 1995 the number was significantly lower than expected.

Stephenson et al.[35] were reconsidered why the results observed by Fielden did not fit the predictions of Rockwood's group. They noted that Fielden's study provided no details regarding case selection. So these authors analyzed the incidence of hip fractures again with special attention to the inclusion criteria.

The design of Stephenson's study had three differences in the inclusion criteria compared with those used by Fielden's team: a) the inclusion of diagnostic codes 821 (fracture of other unspecified parts of femur), 827 (other fractures multiple poorly defined lower extremity), 828 (multiple fractures with involvement of both lower limbs, fractures of the lower limbs and upper limbs, and fractures of the lower extremities with costal involvement and / or sternum) and 804 (multiple fractures involving skull or face with other bones), plus the usual array 820 (femoral neck fracture), used in most published works; b) the inclusion of cases of re-diagnosed hip fracture, and c) the exclusion of patients treated on an out-patient or early readmission basis. Thus, estimates of trends in specific rates of hip fracture by age, especially among women, showed that the decline was much less important than that observed by Fielden et al.

Australia

In Australia, the population estimates based on secular trends and population growth projections suggest that the number of hip fractures will increase by between 4 and 5 times in the year 2051[36]. Despite this, several studies suggest a decreased incidence in standardized hip fracture by age in this country. Crisp et al.[37] noted that crude numbers of hip fracture increased by 11% in both sexes between 1997 and 2007. This increase was due to an increase in the number of cases in older age groups. The incidence of hip fracture among subjects aged 50 to 79 years either remained stable or declined slightly in both sexes.

Incidence of hip fracture in Asia

It is estimated that about 30% of hip fractures that occur in the world take place in different Asian populations, especially in China[38]. Globally, most studies in Asian populations noted an increasing trend in the incidence rates of hip fracture adjusted for age, in both sexes, until the mid-90s and then decreased the same. More recent studies agree that the upward trend in the incidence of hip fracture is not over yet.

Studies by Chalmers et al.[39] and Lau et al. Hong Kong[40-42] revealed large increases in the incidence of hip fracture adjusted by age, in both men and women, between 1966 and 1985 (1.7 times in men and 2.5 times in women) and were followed by a period of stabilization between 1985 and 1995. In women, there was an increase in the incidence of fractures up to 1996, the year from which a decrease was observed. Hip fracture incidence in men began to stabilize after 1985 and decreased from 2000, when the incidence rates nearly matched those recorded in the UK in the same period.

The incidence of hip fractures in Singapore is among the highest in Asia, and is similar to that seen in Hong Kong. Koh et al.[43] estimated an annual increase of around 1% between 1991 and 1998, compared with the data obtained in a previous study in 1965. In Japan, Orimo et al.[44] observed that the incidence of fracture hip had increased in both sexes between 1992 and 2007. In terms of age groups, however, incidence rates in males 60 to 69 years and women 60 to 79 years were the lowest, but remained much higher in older age groups.

Studies of Hong Kong and Singapore suggest that secular trends may have reached a plateau, but research in Japan indicated that the incidence of hip fractures continues to increase. Recently, Dhanwal et al.[45] have conducted a small retrospective study in Rohtak, a district of northern India, which found rates of hip fracture midway between Western countries and Africa.

Incidence of hip fracture in Africa

No data are available on the secular trend of hip fracture in most African countries. Osteoporosis and fragility fractures are generally considered rare in Africa but in fact, there are no policies or preventive screening programs or specific treatment in most sub-Saharan countries.

Research shows an incidence[46,47] one or two times lower than that found in Western and Asian countries. However, there is no evidence that Africans and present higher BMD levels. This population presents risk factors for osteoporosis, such as low calcium intake, high parity and prolonged breastfeeding[48-50]. The values summarized in Table 1 are most likely the result of the shorter life expectancy in these countries and coding errors, so that the data on Africa are not reliable.

Discussion

As we have seen, the evolution of the incidence rate of hip fractures has not been uniform over time in different countries analyzed. The differences in the patterns of the trend of hip fracture can be related to the changing population demographics, changes in exposure to different risk factors for the occurrence of this type of fracture, with changing life styles and the result of the various measures taken to minimize the effect of such factors. On the other hand, its origin may also be placed under the conditions present at birth and during the first years of life of the people analyzed.

In the research into countries which presented a growing trend of hip fracture, the authors' attempts to justify this trend may be summarized in the presence of an effect of age, a period effect or an birth cohort effect present in the populations studied. Bacon's study[8] indicated the high prevalence of smoking among older men (period effect) as the main culprit for increased incidence of hip fracture in that population sector. Sernbo et al.[15] attributed this trend to the aging of the population (age effect) and the tendency toward a more sedentary life (period effect) increasing the incidence of hip fracture registered in the city of Malmo.

The work of Spector et al.[21], Evans et al.[22] and the Framingham[10] suggest that the effect of environmental factors during the early stages of life affect the acquisition of bone mass, therefore increasing the risk of osteoporosis and fractures present in adulthood (birth cohort effect). As we know, 40-80% of an individual's BMD is determined by genetic factors[51,52]. Some studies suggest that peak bone mass may be the most important factor involved in the development of osteoporosis. Similarly, limited intrauterine growth during the first years of life has been associated with the presence of thinner bones and an increased risk of fractures in adulthood[53].

The studies that report decreasing hip fracture incidence note the coincidence in time of the start of this decline with aminobisphosphonate marketing (period effect), but it is never exclusively attributed to the effect of drug therapies. There is broad consensus in the literature when considering the effect of other measures such as changes in lifestyle, supplementation with calcium and vitamin D, decreased smoking, moderation in alcohol consumption, preventing falls and increased physical activity as agents that could justify the decrease in the incidence of hip fracture (period effect). The lack of reduction of hip fracture in older subjects may reflect less access or willingness to prescribe or take medicine for osteoporosis in these individuals[37,44].

The beneficial effect of bisphosphonates in the evolution of hip fracture incidence, although it may seem obvious, is, at least, controversial. Rosengren[16] indicated that it was unlikely that antiresorptive drugs led to the decrease in the incidence of hip fracture in Sweden, as its use was not widespread until the late 1990s. Similarly, Leslie[12] detected a decreased incidence of fracture before the generalized measuring of bone mineral density and the use of current hip antiosteoporotic drugs. In this sense, Brauer[9] pointed out that the use of these drugs justified only a 9% reduction in the incidence of hip fracture in women in his study (compared to 23% found), and, given their limited use in men could not explain the reported decline among the male population.

In the presence of a birth cohort effect, the studies analyzed in Hong Kong[39-42], Sweden[16] and the US[10] (Framingham) do not rule out their influence on the results. This would mean that patients over 50 during the 1990s, having had unfavorable social and economic situation in their childhood, generally characterized by poor nutrition and poor access to health care, may be predisposed to less healthy bones. During pregnancy, maternal stress affects the development of the fetal skeleton and bone mass acquired during childhood. Similarly, the history of lower intrauterine growth during the first years of life has been associated with the presence of thinner bones and an increased risk of fractures in adulthood[53]. These data suggest that environmental factors influence the acquisition of bone mass during the early stages of life, and therefore the risk of osteoporosis and fractures present in adulthood.

One aspect worth emphasizing, which could influence the disparity in the results that have been reported, is the difference in the design of the different studies. As noted in Table 1, both the criteria used to define what is considered hip fracture as well as the age of the people included have not been homogeneous. For example, the ICD codes have not always been the same. Although in most of the research, the study analyzed population comprised individuals 50 or older, the range was very wide, between 39 and 65. Therefore, the different age ranges could distort the comparison of the results provided by the different authors. Furthermore, the use of different classifications could lead to biases as in the previous case lead to errors in interpreting the data. In this regard, only one of the studies reported in

this paper refers to a possible bias of classification of hip fractures in the interpretation of results obtained[35].

Conclusions

The secular trend in the incidence of hip fractures in the world has not been uniform over the past decades. In most developed countries the incidence increased in the second half of the twentieth century up to the last decade, which seems to present a stabilization or even a decline in the incidence. However, in parts of Asia and Africa the trend is still growing.

The detailed analysis of current trends suggests that changes in consumption patterns associated with aging occur slowly over time, and are more closely related to income than to the demographic structure of the population. This complex reality makes it difficult to predict future trends in consumption, since any growth in revenue among the elderly is quite uncertain in the coming decades.

Furthermore, it would be desirable to achieve greater uniformity in the design and methodology of the studies to assess the incidence of hip fracture more accurately. It would also be desirable to carry out more studies to develop a better understanding of the models of age-period-cohort, and to attempt to analyze the incidence of this type of fracture without using a purely numerical interpretation based on the comparison of magnitudes. This would enable us to understand this entity globally and face the challenge of hip fracture in the twenty first century.

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Summary Annual Congress of American Society for Bone and Mineral Research 2015. A subjective overview

Introduction

This past October 2015, the annual congress of the American Society for Bone and Mineral Research (ASBMR) was held in Seattle, USA.

Those in attendance observed a constant through all the conference sessions: research aimed at finding new interrelationships in bone mineral metabolism beyond the bone itself or to better understand the patho-physiology or obtain new therapeutic resources.

SEIOMM and the Journal of Osteoporosis and Mineral Metabolism consider it interesting to provide our readers with a personal overview of the proceedings with a summary of the issues that seem most relevant and representative of current research trends in bone metabolism, as I explain below.

Keynote Speakers

Bruce Spiegelman: Bone, Fat and Energy Regulation

In this lecture, the author emphasized the various types of adipocytes: white, brown and beige adipocytes, focusing mainly on their different functions, which are often opposed. So whereas the white adipocyte stores energy and is a "pro-obesity" cell, the brown eliminates energy and would be an "anti-obesity" cell. After a detailed review of the pathophysiology, he concluded by venturing a hypothesis about how we could mobilize fat from white to brown adipocyte, and how it could be handled in the dietary sense. Finally, he posited if this might be possible in humans.

Forum Discussions

ASBMR/ECTS Symposium: skeletal consequences of diabetes and obesity

Serge Ferrari began reviewing the pathophysiology of type 2 diabetes and its relation to bone fragility, highlighting the recognized factors of lower bone turnover and decreased PTH along with microstructural alterations that lead diabetic patients have more bone mineral density but also more fractures, especially those related to the cortical bone. He also recognized that urinary pentosidine seems to increase in those diabetic patients at increased risk of fracture, as well as levels of sclerostin, which have been linked to the risk of vertebral fracture. Thus, metformin and sulfonylurea use appears to decrease the risk of fracture, while treatment with rosiglitazone and other thiazolidinediones increased this risk. Some studies have even reported that insulin treatment increases the risk of non-vertebral fracture, which needs to be confirmed.

Juliet Compston then discussed obesity and bone health, indicating that weight gain decreased risk of fracture, but if this risk is adjusted by BMI itself, the morbidly obese and extremely obese are more likely to suffer fractures. She said that 80% of obese women with fractures have normal bone mineral density and that the obese with non-vertebral fractures have less bone mineral density at three key sites: the spine, hip and forearm.

THE CONSTANT THROUGHOUT THE CONFERENCE WAS RESEARCH AIMED AT FINDING NEW INTERRELATIONSHIPS IN BONE MINERAL METABOLISM BEYOND THE BONE ITSELF OR TO BETTER UNDERSTAND OF THE PATHO-PHYSIOLOGY OR OBTAIN NEW THERAPEUTIC RESOURCES

Finally, William Leslie spoke about diabetes, obesity and the risk of fracture, commenting the contradictory data and the progress made in recent years. He indicated that fractures in the obese are significant, that BMI in the fractures has a site-specific effect and that obesity and diabetes have a detrimental effect on bone tissue as well as that of adipose and muscle. He concluded that there is a need to develop joint strategies for treating these problems.

Proceedings

Clinical debate. Should the diagnosis of osteoporosis be altered to include patients at high risk of fracture rather than relying on the T-score?

In favor of the motion: Nelson Watts. Against: John Kanis

This was one of the most interesting topics of the congress and had raised great expectations, which, at least in my opinion, were not fulfilled (due, in large measure, to many technical difficulties).

Watts defended the need to change the definition of osteoporosis to include fracture risk. He proposed defining osteoporosis as "a disease with a high risk of fracture due in part to increased bone fragility". For this, it relied on the position paper for the clinical diagnosis of osteoporosis "The clinical diagnosis of osteoporosis: a position statement from the National Bone Health Alliance Working Group" signed by Ethel Siris and other authors, including Watts himself.

Kanis defended the position of the T-score below -2.5, arguing that, as Voltaire put it, "the best was the enemy of the good," indicating that diagnosis of osteoporosis based on this criterion was adequate, completing it with other criteria such as a fragility fracture or T-score combined with fracture risk values to 10 years over 20% for any fracture or 3% for hip fracture calculated by FRAX scale ®.

In my opinion, Watts won the debate "on points". His arguments were more convincing clinically and perhaps favored by the fact that he was "playing on home turf". Regrettably, all the technological uncertainties marred the discussion.

Meetings with the expert

Michael Lewicki: Communication of benefits and risks of osteoporosis treatment

Lewicki presented the potential risks associated with treating osteoporosis using bisphosphonates. The short-term side effects such as (frequently by parenteral route) acute gastrointestinal upset, hypocalcemia, "fever" reaction, and long term effects, such as osteonecrosis of the jaw and shaft fractures. He also reviewed the questionable side effects, such as atrial fibrillation, esophageal cancer or alteration in the repair of fractures, to finish listing the possible beneficial effects such as reducing the risk of cancer (breast, colorectal, gastric) of stroke, diabetes mellitus and reduced mortality. Later he coordinated a debate about the possible appropriateness of "therapeutic holidays".

Robert Josse: Therapeutic Holidays. When and how?

At this meeting the author courageously expressed his dissenting view concerning therapeutic holidays. He believes that osteoporosis is a chronic disease whose treatment does not cure and treatment is stopped when usually the beneficial effect is lost, more or less quickly. Regarding medication holidays but not real discontinuation, he wondered if the "retention of bisphosphonates" that occur in the bone is sufficient to ensure a reduction of fracture risk. He also questioned whether it is appropriate to lose the beneficial side effects, such as reducing mortality, interrupting a treatment that is not harmful.

He said that the incidence of side effects such as diaphyseal humeral fracture is very low, on the order of 1.7 cases per 100,000 treatments per year in the first two years, and 113 cases per 100,000 treatments / year from the 8- 9 years of treatment. He concluded that osteoporosis was the only disease in which treatment was discontinued before the complication appears.

Oral communications and posters

Of the numerous clinical submissions, these ten were selected, as I considered them the most interesting:

1. Reference MO1142. *Eighteen months of treatment followed by abaloparatide with six months of treatment with alendronate in postmenopausal women with osteoporosis – Results of the ACTIVExtend Trial. Felicia Cosman, et al.*

In this work, known as ACTIVE by its authors, the results of phase 3 double-blind randomized study presented abaloparatide compared with teriparatide, in which 2,463 patients (all women with postmenopausal osteoporosis) received 18 months 80 abaloparatide ug sc, or teriparatide 20 mcg sc, or placebo. All the women received calcium and vitamin D. In the branch extension, treatment lasted 24 months, with continuous alendronate. The two treated groups showed abaloparatide and teriparatide increased BMD and reduced fracture risk compared with the control group, but in the (L2-L4) spine obtained with abaloparatide, increase was higher than teriparatide.

2. Reference 1092. *The effects of a longer-term, low-protein diet on calcium absorption*

WATTS DEFENDED THE NEED TO CHANGE THE DEFINITION OF OSTEOPOROSIS TO INCLUDE FRACTURE RISK. HE PROPOSED DEFINING OSTEOPOROSIS AS "A DISEASE WITH A HIGH RISK OF FRACTURE DUE IN PART TO INCREASED BONE FRAGILITY"

and kinetic Measures of bone turnover in young women. Jessica Bihuniak et al.
This study was conducted in 11 premenopausal women who were prescribed a diet low in protein (<0.7 g/kg/day) for 6.5 weeks, carrying out a study of calcium absorption (radioactive) together with the bone turnover markers and PTH. The authors conclude that low protein intake, defined as less than 0.8 g / kg, produces gastric malabsorption, increased PTH and greater loss of urinary excretion of calcium in young women.

3. Reference 1153. *Reduced mortality and subsequent fracture risk with oral bisphosphonate treatment in secondary fracture prevention: an 8-year observational follow-up study. Tineke van Geel, et al.*
The authors analyzed the effect of oral bisphosphonates on the risk of new fragility fractures and mortality after 8 years of follow-up. 9,439 patients of both sexes aged over 50 years who had suffered at least one fracture were reviewed, and after 8 years of follow mortality decreased from 15% to 9% and also observed a reduced risk of new fractures after making adjustments.

4. Reference 1115. *Predicts sarcopenia fracture risk in 65-year old healthy community dwellers. Trombetti Andrea, et al.*
In this paper, the authors studied 930 patients of both sexes in a prospective study with a duration of 3.4 years on average. They found an association between the presence of sarcopenia and risk of fragility fracture in patients over 65 years and independently calculated risk by FRAX®.

5. Reference 1144. *Efficacy of odanacatib in Women with postmenopausal osteoporosis: subgroup analyzes of data from the phase 3 Long-term Odanacatib Fracture Trial (LOFT). Kenneth G. Saag, et al.*
The authors presented the results of a study of the phase 3 odanacatib, called LOFT study, which included 16,713 women over 65 years of age without vertebral fracture and a T-score between -2.5 and -4 , 0, or a vertebral fracture and a T-score between -1.5 and -4.0. They were randomized and classified into 2 groups, one treated with odanacatib (50 mg / week) and another with those receiving placebo. All the women were given calcium and vitamin D. Patients receiving odanacatib showed a reduction of vertebral, non-vertebral morphometric vertebral and hip fractures. Among the results, the authors suggested that studying all cardiovascular events was a higher number of strokes in patients receiving odanacatib.

6. Reference SA 0328. *Longitudinal cohort study of once weekly in glucocorticoid- induced osteoporosis teriparatide in Japanese patients. Ikuko Tanaka, et al.*
In this paper the authors present the results obtained using teriparatide administered weekly to patients with steroid osteoporosis. 87% of patients treated with bisphosphonates and teriparatide 13% weekly and were monitored for 1 year. a statistically significant risk reduction of new vertebral fractures, which was 16% in patients receiving bisphosphonates and 10% in those with teriparatide weekly decline was observed. What was not clear from the presentation and I could not ask the author not agree with him on the poster, is whether teriparatide was the same as is commonly used or if it is a different gelenic formulation.

7. Reference SA 337. *Bone mineral density with response rates teriparatide, denosumab, or both: to respond DATA analysis of the Study. Paul Wallace, et al.*
In my opinion, one of the most interesting papers of the congress. The authors studied 94 women at high risk of fracture, randomizing them in 3 treatment groups: only teriparatide, only denosumab or both drugs combined. They followed up for 24 months. The results showed that patients receiving the combination therapy had a statistically significant increase in bone mineral density than the other 2 groups in all anatomical locations in which it was determined: Total lumbar spine, femoral neck and hip. The sample size did not yield results on reducing the risk of fracture, but still worth continuing with this type of studies that looks very promising for severe of patients with severe osteoporosis.

8. Reference 1143. *Romosozumab improves strength at the lumbar spine and hip in postmenopausal women with low bone mass compared with teriparatide. Keaveny TM, et al.*
Work done on a small number of patients, but with very interesting results. In one group (n=28) received teriparatide 20 mcg/day, in another (n=24) romosozumab, 210 mg/month (both subcutaneous), and the third group (n=27) received placebo. Efficacy on bone strength in the spine and hip was evaluated used a finite element analysis performed on scans obtained. The observed increase in resistance with rosomozumab column was 27.3% per year, whereas teriparatide the increase was 18.5%; in the placebo group a decrease of 3.9% was observed. In the femur, romosozumab only showed an increase, which was 3.6%. By increasing the resistance in the trabecular and cortical compartments, the overall strength of the bone increased significantly, and this augurs romosozumab excellent results in reducing long-term risk of fracture.

ABOUT THE THERAPEUTIC VACATION ROBERT JOSSE BELIEVES THAT OSTEOPOROSIS IS A CHRONIC DISEASE WHOSE TREATMENT DOES NOT CURE AND TREATMENT IS STOPPED WHEN USUALLY THE BENEFICIAL EFFECT IS LOST, MORE OR LESS QUICKLY

9. Reference 1067. *Vertebral fracture risk in elderly diabetic*

men: The MrOS Study. Nicola Napoli, et al.
One result over MrOS study conducted in men in recent years. In it, the authors studied the possible association between type 2 diabetes mellitus and the incidence of vertebral fracture in a population of 5,994 men over 65 years, of which 875 were diabetic and 80 used insulin for treatment. BMD DXA and QCT was determined. After an average of 4.6 years, a side column radiography control was carried out.

No increased incidence of vertebral fractures among diabetics, or incident or prevalent was found. Conversely, they obtained an association with patients who had lower bone mineral density.

10. Reference 1073. *Change in fracture risk after bariatric surgery from a pattern Associated with obesity to a typical pattern of osteoporosis: A study using healthcare administrative databases. Catherine Rousseau, et al.*
The authors studied a total of 10,662 patients who received bariatric surgery between 2001 and 2012 and compared the results with other 2 groups: one formed by 31,986 obese who were not operated on and another, a group of 31,986 age-matched non-obese. The risk of fracture comparing the 3 groups with a mean of 4.2 years were analyzed. After surgery, the risk of fracture in the lower extremities decreased by 33% in the group which underwent surgery, but the risk of upper limb fracture increased by two and by three the risk of this fracture would occur in pelvis or hip. In the other 2 groups (obese and non-obese intervention) the risk remained stable. The authors conclude that after bariatric surgery pattern changes fracture risk in these patients, from the typical pattern of postmenopausal women.

Others

There was a very limited number of papers on odanacatib in this conference as opposed to ASBMR last year in Houston, where many were presented. This makes me suspect that they will abandon the research and sale of this drug. The number of strokes described the previous year must have caused alarm and a reconsideration of the strategy. There is no official information or any other data. This is an assumption.

Moreover, I noted that an engineered PTH molecule, abaloparatide, cropped up in several aforementioned oral communications and posters. It presents exceptionally good results in both increased bone mineral density and reduction of fracture risk, which suggests that the next drugs in the therapeutic panorama of osteoporosis will be rosomozumab and abaloparatide.

I NOTED THAT AN ENGINEERED PTH MOLECULE, ABALOPARATIDE, CROPPED UP IN SEVERAL AFOREMENTIONED ORAL COMMUNICATIONS AND POSTERS. IT PRESENTS EXCEPTIONALLY GOOD RESULTS IN BOTH INCREASED BONE MINERAL DENSITY AND REDUCTION OF FRACTURE RISK, WHICH SUGGESTS THAT THE NEXT DRUGS IN THE THERAPEUTIC PANORAMA OF OSTEOPOROSIS WILL BE ROSOMOZUMAB AND ABALOPARATIDE.

Many tables, meetings with the expert and various communications were related to metabolic issues including obesity, diabetes and nutrition, in an effort perhaps, as I indicated at the outset, to open new physio-pathogenic bone-related lines and also to find new potential therapeutic targets.