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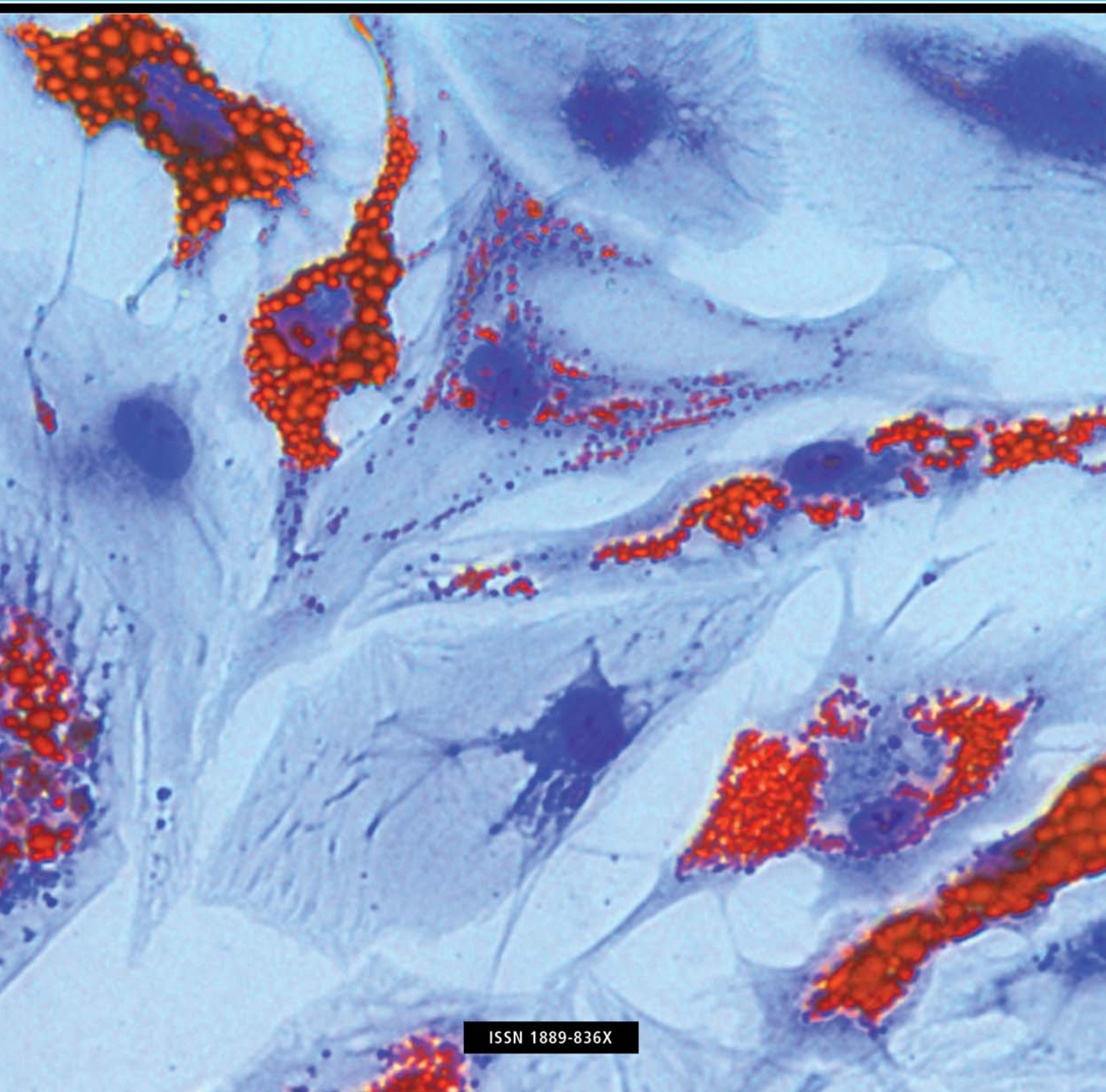

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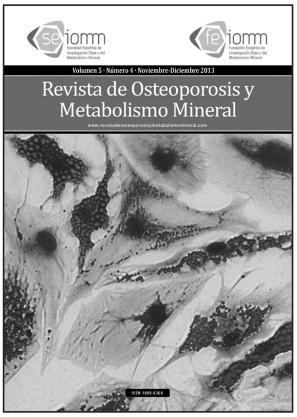

Our cover
Fat vesicles stained with oil red resulting in cultured adipocytes differentiation mesenchymal stromal cells

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Osteoporosis and steroid antagonists of the Wnt way

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The association between an excess of glucocorticoids and osteoporosis was indicated more than 80 years ago by Harvey Cushing when describing the disease which takes his name. Subsequently, after the introduction of the glucocorticoids as anti-inflammatory drugs, it was confirmed that exogenous hypercortisolism was also detrimental to the skeleton, in such a way that steroidal osteoporosis is now considered to be the most common form of secondary osteoporosis in our ambit[1]. Osteopenia induced by corticoids affects predominantly trabecular bone and is most intense during the first months of treatment, when more than 10% of bone mass may be lost[2]. In addition to inducing the loss of bone, the glucocorticoids alter its quality, which would explain the notable increase in fractures (nearly 75%) during the first three months of treatment, even before bone mineral density falls.

The mechanisms which are involved in the reduction in the quantity and quality of bone tissue are various. The glucocorticoids act directly on the osteoblasts, inhibiting their replication, differentiation and functional activity, and favouring both their apoptosis and that of the osteocytes[3,4]. They also act on the osteoclasts, reducing their proliferation but prolonging their survival[3,5]. On the other hand, the glucocorticoids exert an indirect effect on the generation of osteoblast cells by suppressing the expression of bone morphogenetic proteins (BMP), and of the Runx2 transcription factor, which is required to induce osteoblast differentiation from the mesenchymal mother cells[6]. In addition, these drugs increase the expression of PPARγ, which favours the differentiation mother cells into adipocytes and slows their differentiation into osteoblasts, contributing to an increase in fat in the bone marrow at the expense of the osteoblasts and of trabecular bone[7].

Finally, the corticoids may also intervene in the Wnt (wingless) pathway, which acts to modulate the differentiation and activity of bone cells. This complex signalling pathway is made up of a number of components, including ligands, membrane receptors, intracellular and antagonist effectors[8]. The best known mechanisms for transmission of the signal of the Wnt ligands are those included in what is called the canonical pathway, in which β-catenin plays a central role, although there are other alternative or non-canonical pathways which use different mediators[9]. The Wnt ligands are glucoproteins capable of bonding with their receptor and initiating the activity of the pathway. The membrane receptors are made up of frizzled proteins and proteins related to the receptor of low density lipoproteins type 5 and 6 (LRP5 and LRP6). Finally, various types of molecules have been described with an inhibitory action on the Wnt pathway. In some cases these are molecules which act as decoys which bond with to the Wnt ligands and thus compete with its bonding to the receptor. This is the case for some soluble proteins of the frizzled type which are secreted into the extracellular environment. Another inhibitor molecule is sclerostin, a glucoprotein of 190 amino-acids coded by the SOST gene which is expressed in the osteocytes and which bonds to LRP5/6, impeding the formation of the LRP 5/6-frizzled-Wnt complex. The sclerostin is released into the blood circulation, making it possible to determine its blood concentration[10]. There is a strong correlation between the content of sclerostin in the bone and its level in the blood, which would indicate that the production of this protein occurs in the bone and that its measurement in the blood may reflect its activity in the tissue[11].

Other molecules capable of antagonising the Wnt signals by bonding themselves to the LRP 5/6 and Kremen co-receptors are those of the dickkopf family. There are at least four members of this family of which the type 1 (Dkk-1) is especially significant in the bone[9,12,13]. Similarly to sclerostin, Dkk-1 may also be determined in the blood, with a higher concentration of this antagonist having been described in postmenopausal women or those with low bone mass. It has also been suggested that the reduction in the effect of teriparatide may be related to an increase in the concentrations of this antagonist[14].

In this edition of the Review of Osteoporosis and Mineral Metabolism Grifé et al.[15] analysed the values of blood sclerostin and Dkk-1 in patients who has started treatment with glucocorticoids, confirming that, contrary to what occurs in experimental studies, steroid treatment is associated with a reduction in Dkk-1, while no changes are observed in concentrations of sclerostin. As the authors note, there are various reasons which may justify these findings. Sex, age, renal function, estrogenic status, the existence of associated diseases or the quantity of bone mass are determining factors in levels of sclerostin and probably also of Dkk-1[14,16,17]. On the other hand, there is also the possibility that the blood values of both antagonists do not adequately reflect their expression in tissue. Moreover, contrary to what might be expected *a priori*, there was no relationship between the blood concentrations of the antagonists of the Wnt pathway and those of the markers for bone remodelling. However, it should be noted that the data published to date have been contradictory, with in some cases an inverse relationship between levels of sclerostin and some markers for formation having been described[17], while in other studies this relationship has not been able to be confirmed[16,18,19]. In any case, the results of this excellent work invite further studies which analyse the effect of glucocorticoids on the Wnt pathway antagonists, as well as their relationship with bone mass and markers for remodelling, which would without doubt help to clarify the role the Wnt pathway antagonists play in the development of steroidal osteoporosis.

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Blood sclerostin and Dkk-1 in patients who start treatment with glucocorticoids. Preliminary results

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Summary

Background and objectives: The Wnt pathway and its inhibitors (sclerostin and Dkk-1) have a primary role in the regulation of bone mass and osteoblastogenesis. The objective of this study was to analyse the effect of treatment with glucocorticoids (GCC) on the inhibitors of the Wnt pathway and their relationship with bone mass and the parameters for bone turnover.
Methods: A transverse study including 15 patients (9 women and 6 men) with an mean age of 51±21 years at the start of treatment with GCC (≥7.5 mg/day, ≤6 months). Levels of sclerostin, blood Dkk-1 and blood markers for bone turnover (procollagen 1 N-terminal propeptide [P1NP], osteocalcin [OC], and carboxy-terminal telopeptide of collagen type 1 [CTX]) were determined, and bone densitometry (DXA) in the lumbar spine was carried out, in all patients. The results were compared with a control group.
Results: The mean dose of glucocorticoids was 58±21 mg/day, in the majority of patients (73%) indicated by idiopathic thrombocytopenic purpura. The patients treated with glucocorticoids had a reduction in the parameters for bone formation compared with a control group (OC: 7.4±2.8 vs 24.4±6.2 ng/ml, $p<0.01$) and a reduction in blood Dkk-1 (29.6±23.6 vs 48.3±15.6 pmol/L, $p=0.02$). No significant differences were observed in values for blood sclerostin, although this correlated positively with the dose of GCC received and lumbar bone mineral density.
Conclusion: Contrary to what is seen in experimental studies, the start of treatment with glucocorticoids is associated with a reduction in blood levels of Dkk-1. These results indicate the necessity of analysing these inhibitors and their relationship with remodelling and bone mass during this process over the long term.

Key words: *sclerostin, Dkk-1, glucocorticoids.*

Introduction

Treatment with glucocorticoids (GCC) is associated with a marked loss of bone mass and the development of fractures in the initial phases of treatment, as well as being one of the most common causes of secondary osteoporosis[1]. The GCCs act especially on the osteoblasts and osteocytes, reducing the replication, differentiation and function of the osteoblasts and inducing apoptosis in the osteoblasts and osteocytes. These changes lead over time to a reduction in the formation and quality of bone, which is the finding most characteristic of secondary osteoporosis induced by GCC[2-4].

The Wnt pathway, a cell signalling pathway, has a fundamental role in the modulation of osteoblast activity. This pathway is integrated through various components which include ligands, membrane receptors, intracellular effectors and antagonists. The Wnt pathway antagonists, notable among which are sclerostin and Dkk-1, bond with the membrane receptors (essentially LRP-5 and -6) and inhibit the activity of this pathway, and consequently osteoblast activity.

Recent experimental studies, both *in vitro* and *in vivo*, indicate that treatment with GCC reduces the differentiation of the osteoblasts through the Wnt pathway by means of an increase in its inhibitors, sclerostin and Dkk-1[5-7]. However, currently there are hardly any clinical data on the effect of treatment with such inhibitors in GCC. Thus the objective of this study has been to analyse the blood levels of sclerostin and Dkk-1 in patients who have recently begun treatment with GCC, and evaluate its relationship with the markers for bone remodelling and bone mineral density (BMD).

Patients and methods

Study population

A transverse study which included patients who had initiated (<3 months) treatment with doses equal to, or greater than, 7.5 mg/day of prednisone or equivalent. The patients were proposed by the haematology service of the Hospital Clinic Barcelona (August 2010 to 2012) and recruited consecutively.

All the patients complied with the following inclusion criteria: aged over 18 and with normal values of creatinine, liver function, calcium and phosphorus. Excluded were patients who had followed treatment with GCC for more than 6 months, those with diseases or processes which affected bone metabolism (Paget's disease, rheumatoid arthritis, hyperparathyroidism, hypercortisolism, malabsorption syndrome, malignant tumours, transplant, pregnancy or recent breastfeeding) and/or those who followed treatment with drugs which interfered with bone metabolism (bisphosphonates, strontium ranelate, selective estrogen receptor modulators, calcitonin, estrogen therapy, denosumab, osteoformers thiazides or anti-convulsives).

In all the patients the risk factors for osteoporosis were evaluated, including: family history of femoral fracture, personal history of fractures, tobacco and alcohol consumption, age at menopause, dietary intake of calcium (mg/day) and history of renal lithiasis. Recorded in addition was the cause of treatment with GCC, the dose and treatment regime (accumulated dose [mg] and duration [days]).

The results were compared with a healthy control group of similar age and sex.

The study was carried out with the approval of the ethics committee of the hospital and conformed with the directives pertinent to research in humans. All the patients signed their informed consent to their inclusion.

Analytical tests

Blood was taken from all patients at between 8 and 10 am after a night of fasting. A biochemical profile was carried out which included calcium, phosphorus, creatinine, and total alkaline phosphatase, determined by standard techniques.

The following biochemical markers for formation were measured: osteocalcin (OC, radioimmunoassay, Elsa-Osteo-Cis, Gif-sur-Yvette, France) and amino-terminal propeptide of procollagen type I (PINP, automated method Cobas e411, Roche); and of bone resorption: carboxy-terminal telopeptide of collagen type I (CTX, automated method Cobas e411, Roche).

Blood levels of sclerostin and Dkk-1 were measured using ELISA (Biomedica, Austria), with a coefficient of intravariation of 4-6% and 7-8%, and a coefficient of intervariation of 5-7% and 9-12%, respectively.

Bone mineral density

The BMD in the lumbar spine and femur were determined in all patients using dual X-ray absorptiometry (DXA; Lunar Prodigy, Radiation Corporation Madison, WI, U.S.). The densitometric risk categories (normal BMD, osteopenia and/or osteoporosis) were defined according to WHO criteria[8].

Statistical analysis

The results have been expressed as the mean ± standard deviation from the mean (SD). The differences between the means of the continuous variables were analysed using the Mann-Whitney non-parametric U test, and the differences between proportions by means of the Fisher test. To evaluate the association between variables the Pearson coefficient of correlation was used. Values $p<0.05$ were considered statistically significant. The statistical analysis of the data was carried out using the SPSS software programme (version 18.0, Chicago, U.S.).

Results

The clinical characteristics of the patients included in the study are shown in Table 1.

15 patients were included (9 women [4 postmenopausal] and 6 men), with an average age of 51±21 years. The average dose of GCC used was 58±21 mg/day (range 20-100 mg/day) and the average duration of treatment was 42±24 days (range 4-90 days). 73% of the patients had received treatment for idiopathic thrombocytopenic purpura, 20% for haemolytic anaemia and 3% for both causes (Evans

syndrome). 27% had family history of fracture of the femur and 20% had densitometric osteoporosis. Dietary intake of calcium was 593±305 mg/day and only one patient was an active smoker. The patients who had followed treatment with GCC showed a significant reduction in markers for formation with respect to the control group (PINP: 18±9 vs 47±9 ng/ml, p<0.01; OC: 7.4±2.8 vs 24.4±6.2 ng/ml, p<0.01). No significant differences were reported in values of markers for resorption with respect to the control group (CTX: 0.60±0.27 vs 0.45±0.16 ng/ml, p=0.21).

Those patients treated with GCC had a reduction in blood levels of Dkk-1 compared with the control group (29.6±23.6 vs 48.3±15.6 pmol/L, p=0.02), while concentrations of sclerostin were similar in both groups (39.6±23.3 vs 33.8±20.3, p=0.6) (Figure 1).

In the group of patients treated with GCC, the values of sclerostin were positively correlated with the accumulated dose of GCC (r=0.573 p=0.026) and lumbar BMD (r=0.550, p=0.034) (Figure 2). The values of Dkk-1 were not related with any of the parameters analysed. No relationship was observed between the values for markers for bone remodelling and blood values of sclerostin and/or Dkk-1. In the control group, the values of sclerostin were positively correlated with age (r=0.661, p=0.02) (Table 2).

Table 1. Clinical characteristics of patients treated with GCC

	Patients with GCC (n=15)
Age (years)	51±21
Sex (female/male)	9/6
Risk factors for osteoporosis:	
BMI (kg/m^2)	25±5
Dietary calcium intake (mg/day)	593±305
History of kidney stones (%)	13
Active smoking (%)	7
Alcohol consumption habitual (%)	13
Family history:	
Femoral fracture (%)	27
Treatment regimen with GCC:	
Daily dose of GCC (mg/day)	58±21
Duration of treatment with GCC (days)	42±24
Cumulative dose of GCC (g)	2.5±1.3
BMD (g/cm^2):	
Lumbar	1.122±0.156
Femoral neck	0.927±0.113
Total femur	0.958±0.109

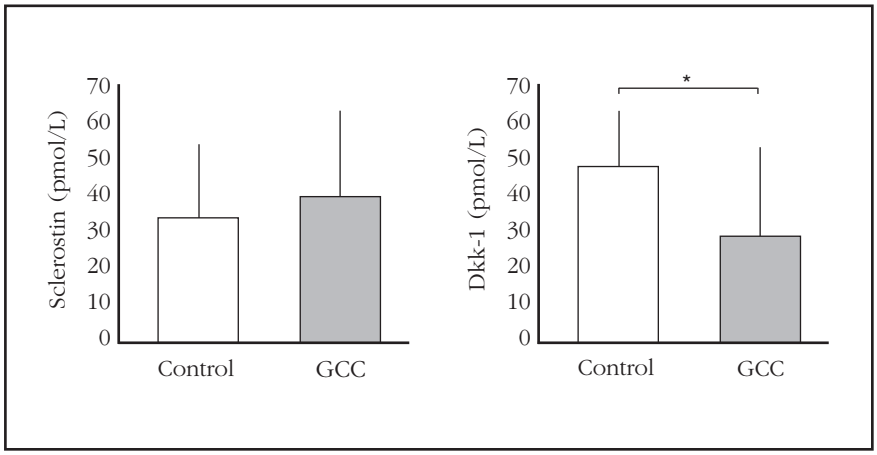
GCC: glucocorticoids; BMI: body mass index; BMD: bone mineral density.

Discussion

Contrary to what occurs in experimental studies, the initiation of treatment with GCC in patients with haematological processes is associated with a reduction in blood levels of Dkk-1. This effect, however, differs as a function of the antagonist analysed, since no significant changes were observed in concentrations of sclerostin at the time of the evaluation.

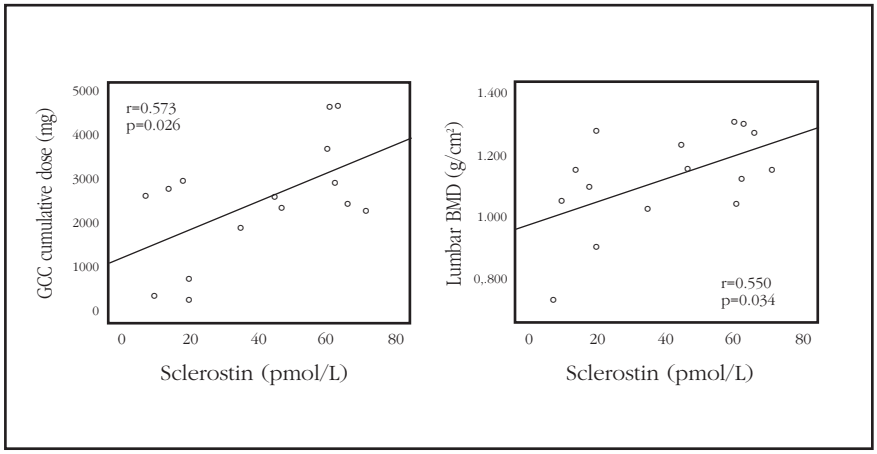
The patients included in this study had low blood levels of Dkk-1 after initiating treatment with GCC at medium-to-high doses. These findings contrasted with the results of earlier experimental studies. Thus, in cultures of osteoblasts and osteocytes (MLO-Y4 cells), treatment with dexamethasone resulted in an increase in Dkk-1[9], which is associated with the dose and period over which treatment is received[7]. There are similar results in animal experimentation models, in which have been observed an increase in the expression of Dkk-1 in the bone tissue after initiating treatment with GCC[7] and an attenuation of the deleterious effect of GCC in the bone by blocking the effect of Dkk-1 in mice[5]. Even though the causes of these differences are not clear, the method of treatment with GCC, including the dose and period of treatment, may explain, in part, these results[6]. Neither can we discount a counter-regulatory effect of this Wnt pathway antagonist in situations of prolonged exposure to GCCs, or that these blood levels may indicate not only the cell function of the osteoblasts and osteocytes but also the number of cells, which, as is well known, reduces (due to an increase in apoptosis) with treatment with corticoids.

Figure 1 . Blood levels of sclerostin and Dkk-1 in patients in treatment with GCC (grey) in comparison with healthy controls (white)



* p=0.02

Figure 2. Correlation between values of sclerostin, the accumulated dose of GCC (mg) and lumbar bone mass (lumbar BMD)



However, it is worth commenting that there have recently been preliminary indications of a reduction in blood values of Dkk-1 at three months from the start of treatment with GCC[10], and similarly, recent studies have described a paradoxical response of Dkk-1 similar to that observed in our study, in other clinical situations. Thus, contrary to expectations, reduced values of Dkk-1 have been observed in immobilised patients[11], and an increase in blood values of Dkk-1 in patients with primary hyperparathyroidism[12]. Also it has been described a paradoxical response of Dkk-1 after treatment with teriparatide[13] and denosumab[14]. These authors have suggested that there is a relationship between bone remodelling and values of Dkk-1. In any case, it is important to remember that there may be factors which influence blood values of Dkk-1, such as an underlying disease and concomitant treatment, among others, which should be taken into account when analysing the concentrations of this antagonist. In addition, the relationship between blood levels and expression in tissue is controversial[7].

In our study, blood values of sclerostin after the initiation of treatment with GCC were similar to those in the control group. However, a positive correlation was observed between values of sclerostin and the accumulated dose of GCC, suggesting a GCC-dependent effect on this Wnt pathway antagonist. Studies in mice treated with GCC have reported an increase in the expression of sclerostin after treatment[3]. However, in patients who started treatment with GCC (in the first 96 hours) a reduction in values of sclerostin has been reported[15], a finding which has not been observed in postmenopausal women treated with GCC[10], nor in patients with hypercortisolism due to Cushing's syndrome[16], in whom there has been observed an increase in values of sclerostin.

The circulating levels of sclerostin in the general population has been associated with age, sex, estrogenic *status* (postmenopause) and the total quantity of bone mass[17-19], which means that these factors need to be taken into account when its concentration is analysed. So, in the healthy subjects included in our study we observed a positive correlation of levels of sclerostin with age, a finding which was not observed in the group treated with GCC, possibly due to the direct effect of the GCC on the Wnt pathway. In this group of patients it was observed, however, that there was a positive correlation between blood levels of sclerostin and lumbar BMD, a relationship which has also been observed in other studies and which has been attributed to a higher production of sclerostin by the osteocytes, due to the greater quantity of bone[18,20].

The relationship between the markers for bone remodelling and the Wnt pathway antagonists is uncertain and varies in different clinical situations. Thus, García-Martin et al.[21,22] in a group of patients with diabetes mellitus type 2, described an inverse correlation between values of sclerostin and markers for bone formation (bone AP) and bone resorption (sCTX and TRAP 5b). Similar data have been reported in the general population[18] and in

patients immobilised after a vascular-cerebral accident[23], although in other situations, such as in patients with chronic renal insufficiency, no significant correlations have been observed[20,24,25]. In our study, although significant reductions in markers for bone formation (P1NP ad OC) were observed after treatment with GCC, these were related with neither Dkk-1 nor sclerostin.

The main limitations of this study were the reduced number of patients included, and the lack of follow up of these patients.

In conclusion, the effect of treatment with GCC on blood values of the Wnt pathway antagonists differs as a function of the antagonist being evaluated. While levels of Dkk-1 diminished at the start of treatment, the values of sclerostin showed no significant changes. All this suggests the necessity of carrying out prospective studies including a greater number of patients with better follow up to analyse the effect of the GCCs on the Wnt pathway antagonists.

Conflict of interest: The authors declare that there is no conflict of interest.

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Table 2. Correlation between values of sclerostin and values of Dkk-1, markers for bone remodelling and the other parameters analysed (Pearson r with and without adjusting for age[§])

	GCC (n=15)	Controls (n=20)
Age (years)	0.03/-	0.661*/-
BMI (kg/m^2)	0.254/0.267[§]	0.320/-0.036[§]
Markers of bone remodeling:		
PINP (ng/ml)	-0.295/-0.375[§]	-0.12/0.131[§]
OC (ng/ml)	-0.204/-0.212[§]	-0.593/0.134[§]
CTX (ng/ml)	-0.249/-0.360[§]	0.098/0.296[§]
Wnt pathway antagonists:		
Dkk-1	0.01/0.01[§]	-0.205/0.309[§]
BMD (g/cm^2):		
Lumbar	0.537*/0.651[§]*	-
Femoral neck	0.171/0.30[§]	-
Total femur	0.187/0.258[§]	-

GCC: glucocorticoids; BMI: body mass index; PINP: amino-terminal propeptide of pro-collagen type I; OC: osteocalcin; CTX: carboxy-terminal telopeptide of collagen type I; BMD: bone mineral density.
* $p<0.05$

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Response of osteoblasts to compounds of strontium or calcium: proliferation, differentiation, mineralisation and whole genome response

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Summary

Background: The mechanisms which trigger osteogenesis are not yet clear. The objective of this study was to evaluate the role of strontium and calcium, provided in different molecular forms, as inductors of different mechanisms of osteoblast stimulus, including proliferation, differentiation and mineralisation of preosteoblast cells. The whole genomic response was also investigated using the microarray technique.
Methods: An experimental study was designed with murine preosteoblast cells MC3T3-E1, which were stimulated for 3 hours and 7 days. Biochemical and genome gene expression studies of mouse (Affymetrix) were carried out.
Results: Strontium bonded with ranelate (SrRn) was the most powerful inductor of the capacity of mineralisation in comparison with the other compounds used (2.55 times that of the control). The studies of whole gene expression showed that after 3 hours 2030 genes change, of which 1644 are specific to this phase. On the other hand, after 7 days of treatment only 329 genes change, of which 147 are specific. The biological processes most enriched after 3 hours are those involved in the regulation of transcription (147 genes), metabolic processes (140 genes) and protein phosphorylation (44 genes) among others, while at 7 days these are changes relating to the cell cycle (18 genes) and carbohydrate metabolism in general (12 genes).
Conclusion: Strontium bonded with the ranelate anion performed as the most powerful inductor of osteogenesis compared with other anions such as chloride or the hydroxides. The stimulation for 3 hours showed greater changes in gene expression in comparison with 7 days. The biological processes affected may be useful in speculating on the signalling cascades involved in the activation of the osteoblast, and on new molecular targets for therapeutic purposes.

Key words: *osteoporosis, osteogenesis, strontium, calcium, gene microarray, gene expression.*

Introduction

The skeleton provides support and mineral equilibrium to the organism. Skeletal tissue is formed during growth and is maintained through adult life by the continuous renewal of the bone matrix, through a process known as bone remodelling. In this process an important role is played by two types of cell, osteoclasts and osteoblasts. During growth bone formation exceeds resorption, resulting in a net gain. Conversely, during aging a disequilibrium occurs which results in a negative bone balance[1]. Extrinsic mechanisms, such as changes in levels of hormones and growth factors, and intrinsic mechanisms associated with cell senescence, may cause the dysfunction of the osteoblasts[2,3]. This causes an increase in the number of bone remodelling units, which encourages trabecular perforation and a reduction in endocortical bone which in turn cause reduced bone resistance[4,5].

With the aim of limiting the excess resorption which follows the menopause, a series of antiresorptive drugs has been developed, such as bisphosphonates, monoclonal antibodies against cytokines involved in osteoclastic differentiation, cathepsin K inhibitors, etc[6,7]. Their long-term effects are, however, not known[7]. A significant challenge in the treatment of osteoporosis is the identification of strategies capable of reversing the deterioration of bone formation linked to age. To date, the number of anabolic agents which promote osteoblastogenesis is limited. The availability of parathyroid hormone (PTH) was a significant advance in the treatment of osteoporosis[8]. The intermittent provision of PTH increases bone formation in patients with osteoporosis, which results in an increase in trabecular bone mass and cortical thickness[8-10]. However, this anabolic treatment has some limitations linked to the low half-life and high cost of this molecule.

Strontium (Sr), a cation close to calcium in the periodic table has been shown to act pharmacologically on bone metabolism[11,12]. In certain experimental models it appears to develop an anabolic action, which has generated interest in associated pathways capable of promoting bone formation. In ovariectomised rats (OVX), an animal model for postmenopausal osteoporosis, it was observed that, both short- and long-term, treatment with Sr impedes the loss of trabecular bone induced by deficiency in estrogens[13,14]. Controlled clinical trials in postmenopausal osteoporotic women showed that treatment with Sr reduced the relative risk of vertebral fracture in comparison with the placebo group[15], as well as the risk of all non-vertebral fractures and hip fracture, as was analysed in a subgroup of patients of advanced age[16,17].

Various mechanisms may explain the reduction in risk of fracture induced by Sr in osteoporosis. One of the possible mechanisms is the increase in bone mineral density (BMD)[18]. Another would involve the effect of Sr on bone resorption and formation[19]. In support of this finding, it was found that 12 months of treatment with Sr causes an increase in the number of osteoblasts, formation of the matrix and a reduction in the number of osteoclasts in patients with osteoporosis[20,21]. In addition to the effects on bone cells and bone microarchitecture, Sr can increase bone resistance by means of changes in the properties of the bone matrix. A small fraction (less than 10%) of strontium is incorporated in the bone[22]. Given this, the beneficial effect of Sr on bone resistance cannot solely result from its pharmacological effects on the bone cells, bone remodelling and the microarchitecture, but may also arise from its effects on the properties of the bone matrix. Therefore, more basic and clinical analyses are required to clarify these effects at a molecular level.

In this work, we have studied the effects of different compounds of Sr and a calcium salt on different parameters related to osteogenesis using preosteoblast cultures as a model. In addition, we carried out a whole genome study by means of microarray with the aim of obtaining information on the genes and signalling pathways involved in osteoformation. For this, we used a microarray of mouse GeneChip Mouse Gene 1.0 ST, which contains more than 750,000 probes which represent 28,000 genes. The genes were analysed at different times, short (3 hours, acute phase) or long (7 days, chronic phase). The changes in gene expression were grouped according to the most notable metabolic processes and those related to the proliferation, differentiation and activity of the osteoblasts.

Materials and methods

Cell cultures

The murine preosteoblast cell line MC3T3-E1 (Sigma-Aldrich, St. Louis, US) was used. The cells were kept in a culture medium composed of α-MEM (Invitrogen, California, US) supplemented with 10% foetal bovine serum (FBS, Invitrogen, California, US), 2 mM L-glutamine, 100 u/ml penicillin and 100 µg streptomycin (Invitrogen, California, US) in an incubation cabinet at 37 ° C in a humid atmosphere with 5% CO_2. In order to carry out the tests they were left to grow until 60-70% confluence.

Before any stimulation the cells were kept for 24 hours in a medium with 2.5% FBS. After this period the cells were treated with strontium ranelate (SrRn, AK Scientific Inc., US), strontium chloride ($SrCl_2$), calcium chloride ($CaCl_2$) and strontium hydroxide ($[Sr(OH)_2]$ (Sigma-Aldrich) at a concentration of 2 mM for the periods specified in each experiment . The treatments were carried out in a medium of α-MEM supplemented with 2.5% FBS, 2 mM L-glutamine, 100 u/ml penicillin and 100 µg/ml streptomycin. For the prolonged treatments, the cultivation medium was refreshed by 50% and the corresponding treatment added every 3 days.

Cell proliferation test (XTT)

The cells were seeded in a plate of 96 wells (density $4x10^3$ cells/well). They were incubated for 48

hours with the aforementioned stimuli. The cell proliferation was measured with the Cell Proliferation ELISA II test (XTT-assay, Roche Applied Science, Mannheim, Germany) following the manufacturer's instructions. After the incubation period, a measurement of absorbance at 450 and 650 nm at 37° was taken in a Victor™X3 2030 Multilabel Reader (Perkin Elmer, Massachusetts, US.) plate reader.

Measurement of alkaline phosphatase (ALP) activity
The MC3T3-E1 cells were seeded in plates of 96 wells at a confluence of 60% and subjected to the aforementioned stimuli for 24 hours, 3 days and 7 days. After the treatments the cells were washed with PBS, harvested with Triton 100 0.1% PBS and sonicated at 4°C. After centrifuging at 4°C at 20,000 g for 5 minutes, the supernatants were harvested, from which the alkaline phosphatase activity was determined using the ALP Reagent test (Thermo scientific, Massachusetts, U.S.). The absorbance at 405 and 660 nm (reference absorbance) was determined at 37°C each minute for 10 minutes in a Victor™X3 2030 Multilabel Reader (Perkin Elmer, U.S.) plate reader.

The concentration of protein was determined using the Bradford colorimetric method (BioRad, Hemel Hempstead, United Kingdom). The ALP activity was expressed as µg of product formed per minute of reaction over the quantity of protein present in the supernatant (U/mg).

Mineralisation tests
To quantify the mineralisation alizarin red was used (ARS, Sigma-Aldritch, St. Lois, Missouri, U.S.) an organic colourant capable of bonding with the deposits of calcium in the cells. It is used therefore as a marker for the capacity to form a calcified matrix. For the experiments, the MC3T3-E1 cells were seeded in plates and incubated with the different treatments SrRn, $SrCl_2$, $CaCL_2$ and $Sr(OH)_2$ for 24 hours , 3 days and 7 days. Then the cells were washed with PBS and fixed with formaldehyde 10% for 15 min. After fixing, they were treated with a solution of ARS (40 mM, pH4.2) for 20 min at room temperature. To detect their capacity for mineralisation the cells marked with ARS were incubated with acetic acid 10% for 30 min at room temperature. Subsequently, the cell suspension was harvested and incubated at 85°C for 10 min followed by 5 mins on ice, to harvest the supernatants after centrifuging at 20,000 g for 15 min. Potassium hydroxide (KOH) 10% was added to each supernatant, and finally, the intensity of fixing with ARS was determined by measuring absorbance at 405 nm in a plate reader Victor™X3 2030 Multilabel Reader (Perkin Elmer, U.S.).

Extraction of total RNA
The cells were cultivated in triplicate at a concentration of 106 cells per sample. After this, they were divided into an equal number of samples with or without treatment with 2 mM of SrRn for 3 hours and for 7 days. After these periods of incubation the culture medium was eliminated and the extraction of total RNA was carried using the trizol method according to the maker's (Invitrogen, California, U.S.) specifications. The quantity and purity obtained was confirmed by determining absorbance at 260 and 280 nm using Nanodrop ND-1000 (Thermo Fisher Scientific, Wilmington, Delaware, U.S.).

Microarrays of gene expression
The analysis of the expression of whole genome was performed using the GeneChip Mouse Gene 1.0 ST Array (Affymetrix, Santa Clara, California, U.S.) microarray which has more than 750,000 probes, with a longitude of 25 oligonucleotides which represent 28,000 mouse genes. Biological triplicates were obtained for each condition: controls, 3 hours and 7 days of treatment. We started from 300 ng of total RNA, having previously analysed its integrity ((Bioanalizador, 2100 Agilent), in order to synthesise the single-stranded cDNA. Its later fragmentation and marking was carried out following the manufacturer's (Affymetrix, Santa Clara, California, U.S.) indications. The hybridisation took place over 17 hours at 45°C with rotation at 60 rpm in the oven recommended by Affymetrix: "GeneChip Hybridization Oven 640" (Affymetrix, Santa Clara, California, U.S.). After this period, the microarrays were passed through a wash process and, finally, the marking was carried out with streptavidin-phycoerythrin using the "GeneChip Fluidics Station 450" (Affymetrix, Santa Clara, California, U.S.). Subsequently, the microarrays were scanned with "GeneChip Scanner 3000 7G" (Affymetrix, Santa Clara, California, U.S.) and images of each of the samples obtained. The quality controls of these images were performed using the programme "Affymetrix Genechip Command Console".

Statistical analysis of the results
The normalised .CEL files of the images of the samples from all the experiments were analysed with the software Partek Genomics Suite version 6.6 (Partek Inc., St. Louis, Missouri, U.S.). The microarrays were normalised using the RMA algorithm. The differential gene expression was identified using ANOVA by means of a highly restrictive analysis using False Discovery Rate <0.05 (FDR<0.05).

The expression of the baseline genes which changed over time between the control at 3 hours compared with the control at 7 days were discounted from the analysis to enable the more specific observation of those genes which changed after the stimulation with SrRn.

The cell processes included the significant genes, both for the acute and the chronic phases, were analysed using the software Pathway Studio version 9.0, database ResNet9.0;2012Q3 (Mammal) (Ariadne Genomics, Rockville, Maryland, U.S.).

The biochemical and cell proliferation data obtained were analysed with the programme GraphPad Prism version 4 (GraphPad Software,

Inc., California U.S.). The comparative statistical study was carried out using single factor analysis of variance (ANOVA) and the Bonferroni test for the multiple comparatives. The level of statistical significance was established at values $p<0.05$, for all variables analysed.

Results

The effect of different salts of Sr in MC3T3-E1 pre-osteoblast cultures

Cell proliferation

Figure 1A presents the effect of different salts studied on cell proliferation determined by the XTT method. All the stimuli induced cell proliferation negative with respect to the control after 48 hours of treatment. $CaCl_2$ induced the highest degree of proliferation, with an increase of 2.13 times, which was highly significant ($p<0.001$). All the salts of strontium produced a slight increase in cellular proliferation ($p<0.05$) in the period studied with respect to the controls. There were no significant differences between the salts studied.

Cell differentiation (alkaline phosphatase and mineralisation)

ALP is a marker for early osteoblast differentiation and an increase in its activity is considered to be an indicator of an increase in the activity of the mature osteoblasts. Figure 1B shows the activity of ALP with the different salts over 7 days. Measurements were also taken at shorter time periods (24 hours and 3 days) but no increase in ALP activity was observed with any of the stimuli (data not shown), therefore, during this period of time the cells still remained undifferentiated. However, the evaluation at 7 days produced an increase in ALP activity, such that the strontium as a chloride or hydroxide salt achieved an increase of 4.45 which was highly statistically significant ($p<0.001$). The salts $CaCL_2$ or SrRn showed a lower increase in the activity of ALP, of approximately 2 times with respect to the control, of statistical significance ($p<0.01$).

Since it is known that Sr is incorporated into the bone matrix, the impact of the salts of Sr and of $CaCl_2$ on the capacity of the mineralisation of the pre-osteoblasts MC3T3-E1 was evaluated. Similar to what was observed in the ALP tests no mineralisation was observed at 24 hours or 3 days (data not shown), but the test was positive at 7 days after the stimulus (Figure 1C). The SrRn achieved an increase of 2.55 times in mineralisation measured by ARS with respect to the control and 2.2 times if we compare it with other compounds of strontium ($p<0.05$), which also appear to induce mineralisation slightly when compared to the control. The cells stimulated with SrRn showed a greater capacity for mineralisation even than the $CaCL_2$ salt studied, with a strong statistical significance ($p=0.06$).

In conclusion, the Sr bonded with ranelate induced slight cell proliferation, and an increase in ALP activity sufficient to achieve the maximum mineralising capacity of all the salts investigated. With this knowledge, the investigation of the gene expression mediated by this salt was begun.

Analysis of gene expression using microarray

The effect of SrRn on the gene profile of MC3T3-E1 cells was also investigated over 3 and 7 days of stimulus. These periods of study were selected in earlier studies by our group, where we detected the most intense changes in gene expression related to signalling cascades Wnt and NFAT, both important routes in the process of osteogenesis[23]. The results obtained by microarray were made in triplicate and are represented in a principal components analysis (PCA) map (Figure 2). It is observed that the samples from the same condition resemble each other and therefore the ellipses are small. The fact that the ellipses do not overlap confirms to us that there are genetic differences with the other conditions. The samples included within the 3 hour stimulus were very different, especially in comparison with those not treated (control). After 7 days, the differences narrowed, which suggests that the impact on gene expression reduces in relation to the acute phase.

A comparative statistical analysis was carried out using single factor analysis of variance (ANOVA) with an FDR ≤ 0.05. We observed that in the acute phase there are 2,030 genes which modify their expression, of which specific to this phase are 1,644 genes which change significantly when the samples are treated with SrRn. On the other hand, after 7 days of stimulus, 329 genes changes in a statistically significant way, with only 147 of those specific to the chronic phase. The remaining genes were common to the two phases. In this study those genes which changed their expression among the controls at 3 hours and 7 days were discounted and we focussed on the genes which changed specifically in each period of time. Represented in Figures 3A and 3B are the biological processes in which those genes with differential expression may be integrated. The representation is made according to the number of genes which change, from higher to lower, although it should be underlined that all the changes presented are statistically significant. The cellular processes differed significantly according to the period of treatment, 3 hours or 7 days, with SrRn. In the acute phase the cellular processes associated with those genes whose expression changed corresponded to the regulation of transcription (147 genes), to general metabolic processes (140 genes), to transport (89 genes, of which 52 are related to transport of proteins) and 44 genes related to the phosphorylation of proteins. On the other hand, processes such as cell death, DNA repair or response to DNA damage were less enhanced. In contrast, at 7 days of stimulation, chronic phase (Figure 3B), the changes were different and the biological processes most enhanced were those metabolic processes which are involved above all in the metabolism of glucose (6 genes) and of carbohydrates in general (6 genes), cell cycle and division (10 genes), cell prolifera-

tion (6 genes) and, to a lesser extent, those related to hypoxia and lipopolysaccharide metabolism.

Discussion

In this work, we studied the effect of three compounds of strontium on murine MC3T3-E1 pre-osteoblasts. We observed that Sr promotes cell proliferation and differentiation, as well as mineralisation, but its potential changes according to the anion to which it is bonded. If in the culture medium there is a source of organic phosphate, we observe discrete zones of mineral deposits which contain hydroxyapatite. Among the strontium compounds studied, the Sr bonded with ranelate was the most powerful inductor of mineralisation in comparison with chloride or hydroxide (Figure 1C). This process was accompanied by a small increase in the activity of ALP associated with the osteoblast phenotype. Conversely, the highest alkaline phosphatase activity was observed with Sr bonded with both chloride or hydroxide, demonstrating the versatility of the action of the cation according to the molecular framework which accompanies it. The cells used in this study have been very well described and are therefore an excellent model for studies of mineralisation, however they have a low expression of ALP. Other subclones of the same cell line have been characterised which, in contrast, have high ALP activity and do not have mineralisation capability[24]. It is known that little ALP (0.05 U/mg) activity is required in order for inorganic phosphorus to be obtained *in vitro*[25]. In mesenchymal cells from bone medulla low ALP activity has also been observed, as well as their being capable of mineralisation[26]. Therefore, the activity of ALP in the samples stimulated with SrRn is necessary and sufficient to produce a mineralisation matrix.

The sequence of the induction of the formation of mineralised foci progressed in a regular order over the time periods studied of 0-1-3-7 days. Experiments carried out with ascorbic acid have shown that this process is prolonged to 2 to 3 weeks[27], showing the efficacy of the action of SrRn. Presumably, successive waves of change in gene expression occur over the period of 0-7 days required for the differentiation of the pre-osteoblasts to a mineralisation phenotype. We carried out studies of up to 21 days of treatment with the stimuli, observing that the most intense changes in the cell, such as gene expression and activation of signalling cascades related to osteoformation processes, occur very shortly after the stimulus[28].

Given the high degree of mineralisation observed, and the fact that this process can only be executed by mature osteoblasts, strontium bonded with ranelate (SrRn) is for

Figure 1. The effect of different compounds of Sr in MC3T3-E pre-osteoblast cultures. Cell cultures without stimulation and treated with $CaCL_2$ were used as controls. (A) cell proliferation during 48 hours of stimulation; (B and C) alkaline phosphatase activity; ALP and the capability of mineralisation determined by the alizarin red method after 7 days of treatment, respectively. The results are represented as the mean ± standard deviation of triplicates, *p<0.05, **p<0.01, ***p<0.001, comparisons made with respect to the control (0)

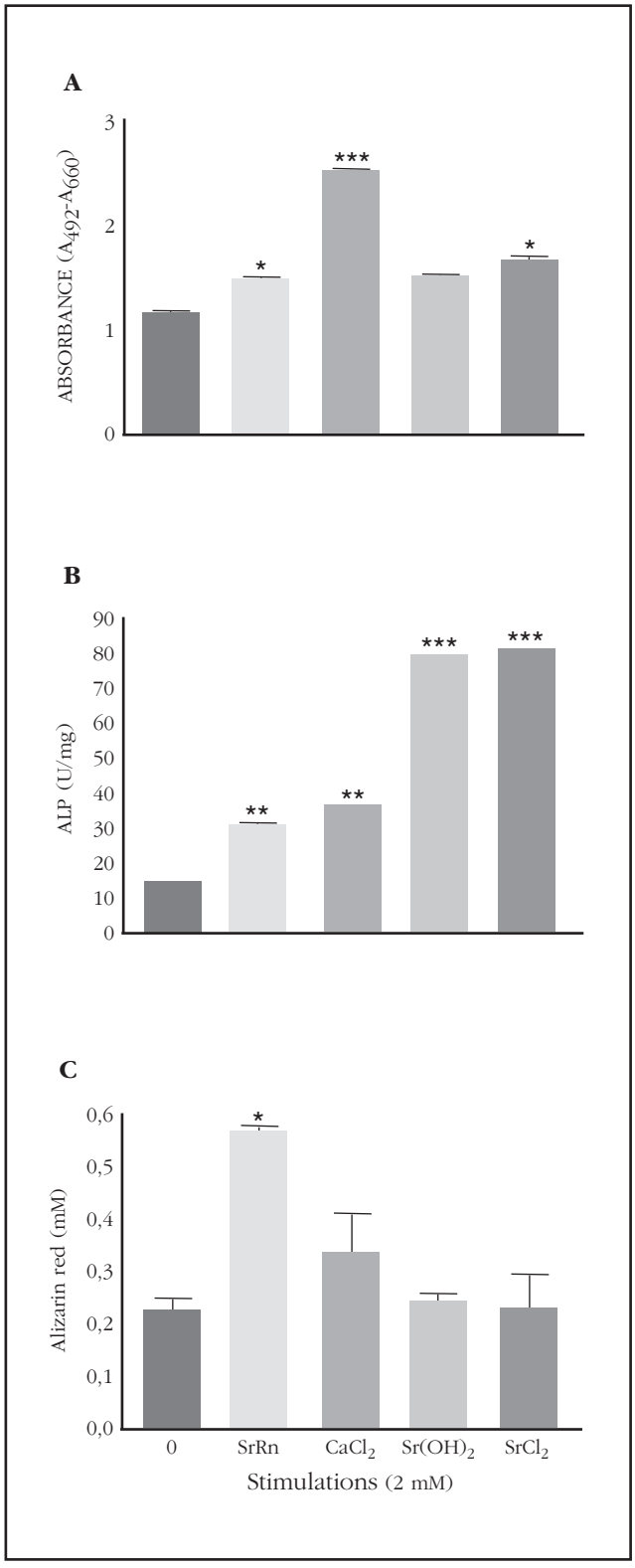
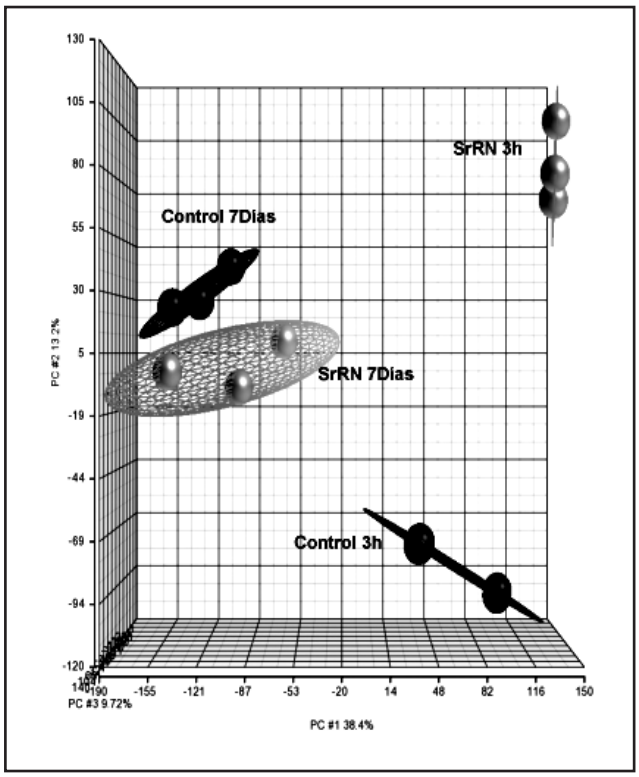


Figure 2. Representation of principal components analysis (PCA) map. All the samples are represented in 3D through a non-supervised analysis of principal components. The ellipses are drawn to include 61.3% of the genes in each group



us an excellent stimulus for the study of the cell processes which occur when osteogenesis or bone formation take place in these cells. We have used an expression matrix from the mouse which contains 28,000 genes. Earlier studies only used 588 or 8,700 probes[29,30]. Our matrix allows the investigation of the genes of numerous growth factors, cytokines, interleukins and their receptors, as well as key genes involved in different stages of embryo development. MC3T3-E1 cells are a well-established osteogenic clonal cell line, which provide an excellent model for the study of patterns of gene expression in osteoblast differentiation. In our study, the SrRn induced in these cells a process of maturation which can be divided into two phases, which result in the formation of a mineralised matrix (Figure 1C). Each of these phases requires strictly regulated expression of genes and transcription factors.

The first phase of this process of maturation (3 hours after stimulation, acute phase) is triggered on contact with the stimulus, SrRn. During this phase the MC3T3-E1 cells are not yet differentiated, and are not capable of producing a mineralisation matrix. However, in these cells the expression of many genes is activated, up to a total of 1,644, in which the 10 most enriched cell processes from our global gene expression study are shown in Figure 3A. In this stage many genes change which are related to the regulation of transcription, metabolic processes in general, molecular transport, etc. It is notable that among those general metabolic process enriched in this short period of time is protein phosphorylation-dephosphorylation, a process which participates in a large number of cell routes of great interest. Some of these have been shown to be involved in the maturation of the osteoblasts, such as the Wnt and NFAT signalling routes[31,32]. However, we currently have insufficient information regarding the cell pathways which are activated during the differentiation, proliferation and maturation of osteoblasts, in which doubtless the processes of protein phosphorylation-dephosphorylation are crucial.

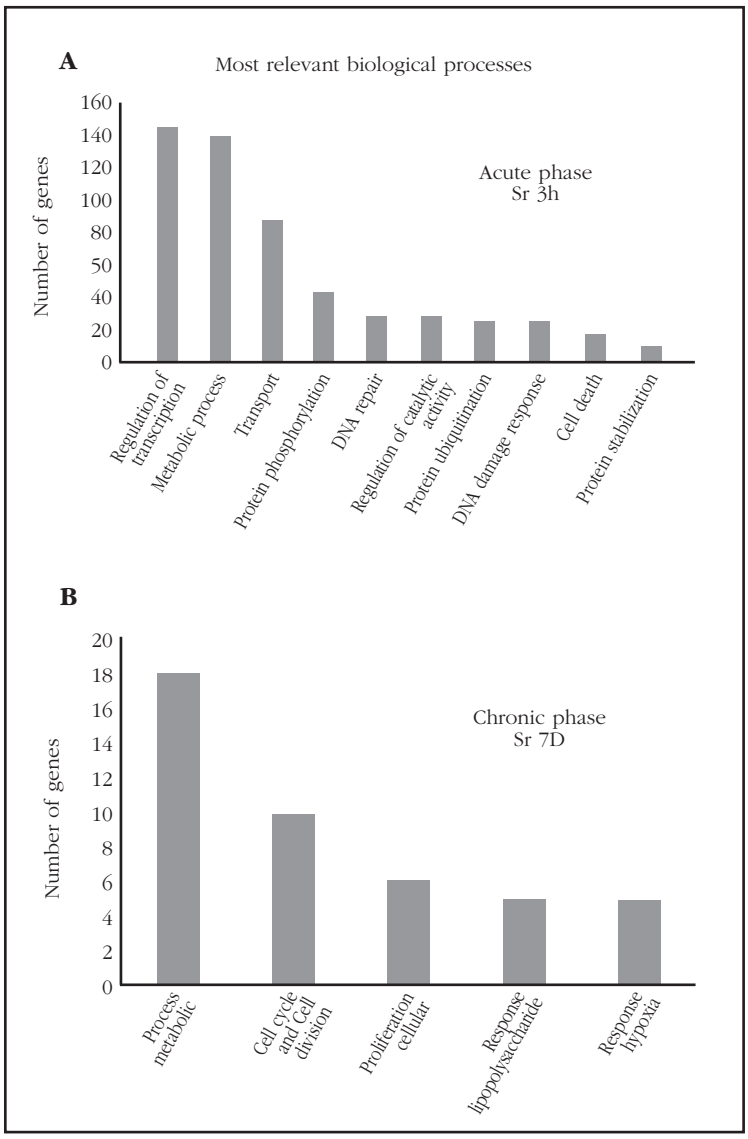
In accord with this pattern, in an earlier work we confirmed that the Wnt/β-catenin and NFAT pathways, both powerful osteoprogenitors, are activated within only 15 minutes of stimulus with SrRn, which rapidly induces changes in gene expression. In contrast, 21 day cultures do not show activation of these pathways (data not shown).

Once confluence is achieved, and coexistent with an increase in ALP activity and mineral matrix deposit (Figures 1B and 1C), the cells enter what is known as the differentiation phase (7 days). This is characterised by an increase in the formation of the bone matrix, and is associated with the change in expression of 147 genes. The cell processes which are increased in this phase are those related to the energetic state of the cells: carbohydrate metabolism in general, metabolic process of glucose and other carbohydrates, response to lipopolysaccharides or gluconeogenesis. This makes this cell line an excellent model for the study of the molecular events involved in the process of osteogenesis. The microarrays allow the simultaneous monitoring of a great number of genes associated with the metabolism of bone, which enables the detection of potential targets for therapeutic or diagnostic purposes. The use of microarrays is becoming ever more accessible and the data generated from this type of approach are largely descriptive. However, the general information which is obtained is a very useful baseline for more analytical studies of functional routes which come into play during cell differentiation, an event which is mediated by receptors, transcription factors, proteins, enzymes, etc., which are in turn regulated by changes in gene expression.

The analysis here presented offers a dynamic vision of these events during the differentiation of osteoblasts. The results of the expression matrix which is presented here complement earlier studies[29,30]. However, many earlier gene studies in osteoblasts were analysed under different conditions, with different cell lines, or under induction by different agents. The ability to compare data collected from hundreds of genes under a combination of conditions with data from other systems strengthens our general understanding of the

molecular basis of osteogenesis. These data are valuable not only for a better understanding of osteogenesis but also for comparison with other types of tissues. This information should help in the development of more efficacious treatment for bone disorders and in the prediction of secondary effects on bone metabolism of drugs which are aimed at the same factors for intervention in other diseases.

Figure 3. Representation of the most significant biological processes of each phase in which are integrated those genes modulated by the treatment. The representation is shown from higher to lower according to the number of genes which change. All are statistically significant (p<0.001)



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The reality of osteoporosis in patients hospitalized in Internal Medicine

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Summary

Purpose: a) to know the prevalence of previous osteoporosis and vertebral fractures in patients admitted to an Internal Medicine department from a third-level hospital; b) to determinate the proportion of patients discharged with a diagnosis of osteoporosis, and the percentage of those receiving treatment; c) to quantify the risk of fracture by applying the FRAX calculation tool; and d) to know the serum levels of 25OHD in these patients.

Patients and methods: Retrospective study, based on the review of clinical charts of all the patients admitted to the Internal Medicine department of Marqués de Valdecilla University Hospital, during April 2012. The information was gathered by a standardized protocol, including demographic, clinical, radiological and laboratory variables.

Results: Three hundred patients were studied (mean age, 80 years). Thirty-four (11.3%) had a previous diagnosis of osteoporosis and 14 (4.8%) of them were, or had been, on treatment. A diagnosis of osteoporosis, in the hospital discharge report, was noted in 14 patients. No treatment was prescribed in one of them. According to the FRAX calculation tool, mean risk for major osteoporotic fracture was 10.5%, and mean risk for hip fracture was 5.4%. Mean serum 25OHD level was 16 ng/ml, and more than 80% of patients had values below 20 ng/ml.

Conclusion: Osteoporosis is an underdiagnosed and undertreated disease, in patients admitted to an Internal Medicine Department, whatever the reason. Moreover, we have observed a high prevalence of 25OHD deficiency among these patients. Hospitalization can represent an excellent opportunity for the internists and other clinicians, to pay attention to the presence of osteoporosis and its related complications.

Key words: *vertebral fracture, osteoporosis, diagnosis, chest-X-ray, 25OHD, Internal Medicine.*

Introduction

Osteoporosis is a disease characterised by a reduction in bone mass and by alterations in the microarchitecture of bone tissue, which leads to an increase in its fragility and, consequently, a higher risk of suffering fractures[1]. The prevalence of osteoporosis increases with age, from 15% in women between 50 and 59 years of age to more than 80% in those over 80[2].

The principal osteoporotic fractures are vertebral, hip, wrist, humerus and pelvis. These fractures bear significant health and social implications. Furthermore, in a high percentage of patients they lead to significant morbidity, such as loss of mobility, loss of ability to carry out independently basic activities of daily living, chronic pain and even depression[3,4]. Similarly, osteoporotic fractures, above all hip fractures, are associated with a significant increase in mortality[5], this fact is especially important, since it has been estimated that the annual incidence worldwide of hip fractures in women will have increased by 3.5 times between 1990 and 2050[6].

In the last few decades, in hospitals in general, and in general internal medicine services in particular, an increase has been seen in the average age of patients being hospitalised who, in addition, usually have multiple comorbidities and are polymedicated. In fact, the prevalence osteoporosis, as a disease linked to aging, is increased in these individuals[7]. Admission to hospital represents on many occasions an opportunity to diagnose, initiate treatment and programme appropriate monitoring in those patients with osteoporosis, with the aim of attempting to prevent the complications of the disease, mainly fractures. We have carried out this work with the aims of: a) ascertaining the prevalence of previous osteoporosis and of vertebral fractures in patients admitted to the internal medicine service of a tertiary hospital; b) determining the proportion of patients discharged with a diagnosis of osteoporosis and the percentage of those who are treated; c) quantifying the risk of fracture by applying the FRAX® tool, and; d) finding out the blood levels of 25-hydroxyvitamin D (25OHD) in these patients.

Material and methods

A retrospective descriptive study was designed, based on a review of the clinical histories of all patients admitted to the internal medicine service of the Marqués de Valdecilla University Hospital during the month of April 2012. This centre serves as a health referral centre for a population of 350,000 inhabitants of Cantabria. The internal medicine service has approximately 130 hospital beds.

The variable data for the study were gathered using a standardised protocol in a computerised database management system. These variables were: risk factors for osteoporosis, comorbidities, drugs with an influence on bone metabolism, results of X-rays of thorax and/or dorso-lumbar spine, blood levels of 25OHD and prescribed treatments, differentiating between treatments at time of hospital admission and at discharge. Also recorded was the presence or not of family history of hip fracture and personal history of fractures.

Menopause was defined as being early if it had occurred before the age of 45. It was considered that a patient had clinical osteoporosis if they had suffered a typical osteoporotic fracture (hip, vertebral, humerus, forearm) earlier or had a vertebral fracture in the radiological study requested on admission, in the absence of other causes which may have accounted for it (high impact trauma, bone metastasis, myeloma or other bone diseases).

The comorbidities analysed were: chronic obstructive pulmonary disease –COPD– by means of a diagnosis based on tests of compatible respiratory function); ischemic cardiopathy or cardiac insufficiency, cerebrovascular accident, dementia, Parkinson's disease; active neoplasia, or in remission; venous thromboembolism; cataracts; rheumatoid arthritis; diabetes *mellitus* type 1 or 2 (HbA_{1c} ≥6.5% or two tests for glycemia in fasting ≥126 mg/dl or a casual glycemia of 200 mg/dl or more than one patient with cardinal symptoms); arterial hypertension (through measurement and confirmation of levels of TAS > 140 mmHg and/or TAD >90 mmHg persistently); dyslipemia; hyperthyroidism and hypothyroidism; hyperparathyroidism; hypogonadism; intestinal malabsorption syndrome; malnutrition, urolithiasis; chronic hepatopathy; chronic renal insufficiency (defined as a glomerular filtrate <60 ml/min/m^2 according to the four variable MDRD equation, maintained for at least 3 months). The drugs evaluated were: corticoids, beta-blockers, anticonvulsives, statins, proton pump inhibitors (PPIs), serotonin re-uptake inhibitors, oral anticoagulants, opiates, hypnotics and benzodiazepines. Treatments for osteoporosis recorded in the discharge reports included: denosumab, selective estrogen receptor modulators (SERMs), strontium ranelate, teriparatide, PTH 1-84, calcitonin or bisphosphonates.

Blood levels of 25OHD were determined using electrochemiluminescence (Elecsys 2010, Roche Diagnostics, GMBH, Mannheim, Germany).

A vertebral fracture was defined as a reduction in height of the vertebral body greater than or equal to 20%, assessed through a review of lateral X-rays of the thorax and/or thoracic-abdominal spine. The X-rays were reviewed independently by the two authors and disagreements (<5%) were resolved by consensus. All the patients diagnosed with osteoporosis had had at least one laboratory study which included biochemistry, haemogram and VG, thyroid hormone, proteinogram and 24 hour calciuria.

Results

300 patients were included in the study of whom 157 (52.3%) were women and 143 were men. The average age was 80 years. The average body mass index (BMI) was 29.3 kg/m^2.

In terms of the risk factors related to osteoporosis or fragility fracture, the most frequent was the presence of cataracts (76 patients), followed by malnutrition (33), hypothyroidism (26), malabsorption syndrome (24) and chronic hepatopathy (24). Seven patients had history of hypogonadism and hyperparathyroidism, and it was only possible to confirm 4 women with history of early menopause.

In tables 1 and 2, respectively, are shown the comorbidities of the patients included in the study and their consumption of drugs.

A total of 34 subjects (11.3%) had had a previous diagnosis of osteoporosis (5 men and 29 women), although only 14 of these (4.8%) were receiving or had received treatment for the disease (zoledronic acid in one patient, other bisphosphonates in eight patients, three patients were receiving denosumab and teriparatide and strontium ranelate had been subscribed in one case each). Of the 34 cases of osteoporosis, in 6 cases the patients were receiving oral corticoids, in one case for rheumatoid arthritis, and in five cases with chronic obstructive pulmonary disease (it was not possible to quantify the exact doses of the glucocorticoids administered). In one case there was history of early menopause, and in another long-term hyperthyroidism. Treatment had been prescribed in only two of these case.

On the other hand, of the 34 patients with diagnosis of osteoporosis, in 16 of them it was confirmed that they had suffered at least one vertebral fracture.

All the patients had an X-ray of the thorax taken during their admission and in 31 there was also an X-ray taken of the thoracic-lumbar spine. 50 patients were identified as having vertebral fractures. Taking into account the total of all the patients studied and given that only 16 had history of radiological vertebral fractures, in 12% of all patients the presence of vertebral fractures had passed unnoticed by the clinician responsible. On the other hand, 68% of all fractures had passed unnoticed. Of the 50 patients with vertebral fractures, in only one case was this attributed to multiple myeloma.

Overall, and in accord with earlier data, 74 cases of clinical osteoporosis (defined by vertebral, hip humerus or wrist fracture) were identified. Therefore, 40 subjects had not had a diagnosis of osteoporosis. At the time of discharge, in only 14 patients was the diagnosis of osteoporosis confirmed in the clinical record, and of these, in one case no treatment was programmed.

On the other hand, the average risk of major osteoporotic fracture (vertebral, hip, humerus or wrist) measured by application of the FRAX® scale was 10.5% (SD: 8.7) and the risk of hip fracture 5.4% (SD: 5.5). According to the FRAX® tool developed for Spain, there were 27 patients with a risk higher than 20% of major osteoporotic fracture, and 118 patients had a risk higher than 3% for fracture of the hip. The risk of fracture using the FRAX® tool could only be calculated in 174 patients, due to a lack of information in the clinical histories of the other subjects of the study, while those treated for osteoporosis were excluded.

The average blood value of 25OHD was 16 ng/ml, although this was only obtained from 45 patients, this being a test which was not included in the protocol of requests for laboratory tests on admission. More than 80% of the patients had values below 20 ng/ml, and more than 50% (26 patients) had serious hypovitaminosis (≤10 ng/ml). Only two patients with hypovitaminosis D did not receive oral supplements at discharge.

Discussion

Osteoporosis is a process which generally develops asymptomatically over a long period of time, a fracture being the first sign in the majority of cases. A predisposition to the development of fractures is, from a clinical point of view, the central phenomenon of the disease. Among these fractures, of greatest significance is fracture of the hip, which preferentially affects older people, is frequent in women and which varies greatly in its incidence, its mortality and in length of stay in hospital[8].

Vertebral fractures are another complication characteristic of osteoporosis, although more than 50% of them pass unnoticed[9,10]. It is important to remember that the presence of an osteoporotic fracture, independently of the densitometry value, increases even more the risk of subsequent fractures, which means that the diagnosis is as important as the monitoring of the disease. In a study published by Sosa et al.[11], it was concluded that the presence of vertebral fractures increased the risk of new fractures. Furthermore, the authors observed this type of fracture in 62.6% of patients hospitalised or treated for fracture of the hip. Although there is no universally accepted definition of vertebral fracture, the majority of authors are in agreement in considering that there must be a loss of height of the vertebral body of at least 20%[12]. We know that lateral X-rays of the thorax are a very useful tool in enabling the identification of vertebral fractures[9]. In our work 50 patients were identified with fractures of this type, mostly unnoticed by the clinician (in our service, thoracic X-rays are not analysed by a radiologist unless specifically requested) and as a consequence, are not reflected in the discharge notes.

On the other hand, according to the definition of clinical osteoporosis which we have used, 74 cases were identified, which shows that almost 50% were not recorded in the clinical record (there being 34 patients with a previous diagnosis of osteoporosis). Furthermore, only 13 patients in whom their discharge notes reflected a diagnosis of osteoporosis received treatment, which indicates once more that, although osteoporosis is a highly prevalent chronic disease, it continues to be underdiagnosed and undertreated.

In respect of the results obtained using the FRAX® tool, it is known that the Spanish version of

Table 1. Comorbidities in the patients in the study

Disease	N	%
Hypertension	200	67
Dementia	112	37
Heart failure	111	37
Dyslipidemia	93	31
Diabetes *mellitus*	91	30
COPD	78	26
Ictus	72	24
Ischemic heart disease	68	23
Neoplasia	67	22
Chronic renal failure	60	20
Alcoholism	35	12
Thromboembolic disease	34	11
Smoking	28	9
Parkinson's disease	17	6

COPD: chronic obstructive pulmonary disease

Table 2. Consumption of drugs in hospitalized patients

Drug	N	%
PPIs	154	51
Hypnotics	126	42
Statins	90	30
Anticoagulants	56	19
Beta-blockers	36	12
Opiates	33	11
Thyroid hormone	23	8
Corticosteroids	18	6
Anticonvulsants	9	3

PPIs: proton pump inhibitors

FRAX® underestimates by 50% the number of major fractures[13]. So, we could take into account the recommendations of NOF, which is to treat patients if their risk of major fracture is ≥20% and of hip fracture ≥3%. Thus in our work, more than 30% of patients (for major fracture) and more than 80% (for hip fracture) had indications to receive treatment, although it should be borne in mind that this guide recommends starting treatment when there is a diagnosis of osteoporosis, whatever the FRAX® indicates. This makes us think that patients admitted to the internal medicine service usually have a high risk of osteoporotic fracture.

In terms of the determination of the level of 25OHD in the blood, it seems that, while this is not normally requested routinely during hospital admission, the staff do usually prescribe treatment when there is a deficit.

When dealing with a multifactorial disease in which are involved, among others, genetic and environmental factors, one of the keys to diagnosis is to identify its risk factors[2]. Hospitalisation may be an opportunity to diagnose osteoporosis and vertebral fracture. However, in line with the data provided in this work the risk factors for fracture are not generally evaluated during admission and osteoporosis continues to be forgotten by the clinician despite its known epidemiological, health and social significance. Furthermore, it is a highly prevalent disease in older patients, in whom, with increasing frequency, are associated malnutrition, comorbidities and polymedication (a notable finding in this work is the significant consumption of hypnotics) which themselves pose additional risk of falls and, therefore, of fracture[2].

However, there are highly efficacious and easy methods of treatment for osteoporosis, especially in polymedicated patients, which ensure therapeutic compliance, and which may be considered, when indicated, during admission to hospital[14].

Our study has various limitations. Firstly, those inherent in any retrospective study. In addition, not all the patients had X-rays of the lumbar spine, which means that it was not possible to determine the true prevalence of vertebral fractures in this section of the spine. Finally, the determination of 25OHD in the blood was carried out in a small sub-group of hospitalised patients, which means that we cannot generalise the results to all the patients studied. However, our work group has studied levels of 25OHD in a broad sample of patients hospitalised in our internal medicine service (approximately 400 individuals) and the results were similar (data not published).

In conclusion, osteoporosis is a disease rarely considered by the clinician in patients hospitalised due to other causes, in spite of its having a prevalence similar to other diseases such as diabetes mellitus, dyslipemia or dementia. As a consequence, it continues to be a condition which is underdiagnosed and secondarily undertreated, in spite of there being many therapeutic options. In the light of the findings of our work we suggest that the internist, and in general, any clinician, should

maintain a high level of suspicion of this disease in hospitalised patients, in order to try to reduce associated complications, especially fractures.

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Multiple skeletal-related events in a patient with breast cancer

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Summary
Bone metastases are common in advanced cancer, occurring up to 75% of patients with advanced breast cancer. Complications of bone metastases include bone pain, hypercalcemia and skeletal-related events (SERs), such as fracture, need for radiation or surgery to bone, or spinal cord compression.
A 50 year-old patient with advanced breast cancer, who has multiple skeletal-related events and poorer overall quality of life.

Key words: *bone metastases, skeletal-related events, cancer, breast.*

Figure 1. (a) X-rays of left humerus, in which are observed a fracture in the middle third and the fixation with an intramedullary nail. (b) X-rays of left femur with lytic metastasis and intramedullary nail

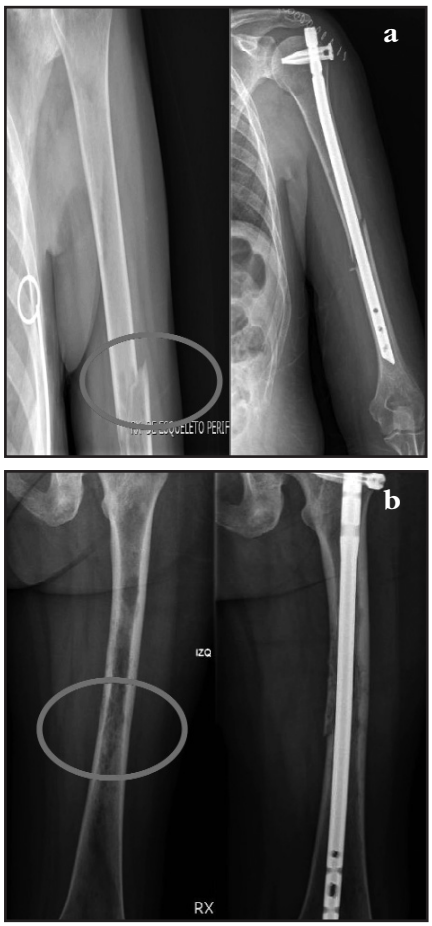
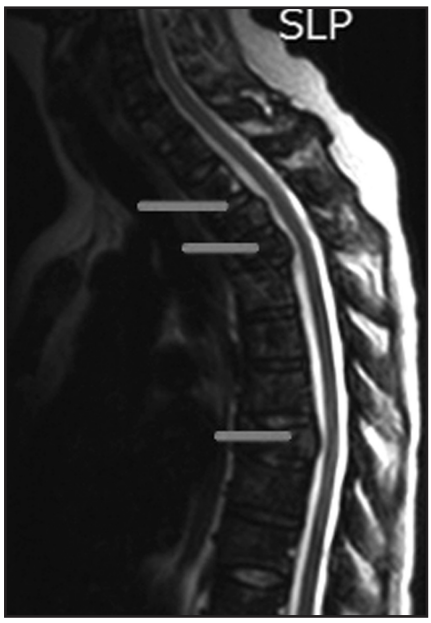


Figure 2. Magnetic resonance of the spine, which evidences medullary compression at the second, third and seventh dorsal vertebrae



Introduction

One of the most aggressive characteristics of cancer in general is its capacity to produce metastases, with the skeleton being one of the most common locations for their development. The activity of osteoclasts produces a local destruction of the bone and, as a consequence, skeletal-related events (SREs) such as pathological fractures and medullary compressions which may require treatment with radiotherapy or surgery, with consequent hospitalisation[1].

Presented here is the case of a patient with advanced breast cancer with multiple SREs with affectation of her quality of life, and which required an efficacious treatment based on a multidisciplinary approach.

Clinical case

A patient of 50 years of age diagnosed in December 2002 with infiltrating ductal carcinoma of the left breast, hormonal receptor-positive with the clinical state T3 N0 M0, treated with induction chemotherapy with 4 cycles of fluorouracil-epirubicin-cyclophosphamide, modified radical mastectomy, 4 cycles of docetaxel and adjuvant radiotherapy in the costal wall, left supraclavicular fossa and internal mammary nodes up to 50 Gy. This was followed by hormone therapy with aromatase inhibitors and periodic checks.

In April 2005 the patient reported mechanical lumbalgia which did not recede with WHO first or second level analgesia. A magnetic resonance (MR) scan was carried out in the spine, which found a single metastasis in L4, which was confirmed histologically. An intervention was performed with a fixation followed by radiotherapy on L3-L5 receiving 30 Gy, and chemotherapy initiated with vinorelbine associated with zoledronic acid 4 mg intravenous every 28 days over 2 years (24 doses in total) followed by periodic checks.

In February 2013 the patient complained of pain of a mechanical nature in the upper left limb which developed over a number of hours. A simple radiography of the left humerus was performed which showed a fracture of the inferior third of the diaphysis, which was fixed with an intramedullary nail, along with radiotherapy (Figure 1a). The biopsy was compatible with metastasis originating from the breast. In the axial thoracic-abdominal-pelvic computerised tomography (CT) scan there was evidence of progression in the bone, confirmed by bone tracking (BT).

During admission the patient reported pain in the glenohumeral joint, and after the carrying out of an X-ray a fracture of the right acromion was observed which required fixing.

In April 2013 the patient presented with difficulty walking and acute mechanical pain in the proximal part of the left leg that was not eased with WHO level 3 analgesia. In the X-ray a fracture of the diaphysis of the left femur was found, on which an intramedullary fixation was carried out (Figure 1b).

During admission she presented with pain in the upper dorsal zone with a positive spinal apophysis percussion test, due to which a CT scan was requested, which showed a bulge in the posterior wall of D3 and D7, which was confirmed through MR (Figure 2). After evaluation for radiotherapy it was decided to administer 20 Gy to D2-D3 and D7, and 20 Gy to the left femur. At the time of producing this work (June 2013) the patient was in treatment with capecitabine, despite presenting a score of 3 on the Performance Status scale, secondary to the multiple skeletal-related events which resulted in dependency for the activities of daily living.

Discussion

Physiologically, there is a balance between bone formation and resorption. The osteoblast line cells are involved in oste-

oblast function and differentiation by means of the RANK factor (Ligand Receptor of the Activator of Nuclear Kappa-B factor) present in their membrane. The bonding of RANK to its ligand (RANKL) stimulates differentiation, the survival of the osteoclast precursor cells and the activity of the mature osteoclasts, thus increasing the expansion of the osteoclast mass and bone resorption[2].

On the other hand, osteoprotegin (OPG) has been identified as a protein which inhibits the development of osteoclasts. When there is sufficient OPG in the environment this protein bonds with the RANKL of the osteoclasts, impeding their interaction with the osteoclast precursors, which slows the process of bone resorption. Changes in the RANKL/OPG quotient are decisive in the pathogeny of bone loss, from osteoporosis to bone metastasis.

Bone is a target tissue for metastasis in breast cancer. This is due, in part, to the irrigation of the bone itself, and to growth factors IGF-1, FGF and PDGF, which exert an attraction on cancerous cells and are a suitable medium for cell growth[3].

The bone balance is altered by the arrival of the tumorous cells. It is necessary that the neoplastic cells are retained in the sinusoids of the bone medulla, that they migrate, that they pass through the vascular wall and adhere to the extracellular matrix of the bone surface of the periosteum in order to be able to stimulate the osteoblasts and osteoclasts. In breast cancer there may be an increase in bone resorption over formation, favouring the formation of osteolytic bone metastases. This imbalance is related to an increase in markers for bone resorption such as urinary N-terminal telopeptide of collagen type 1 (uNTX), C-terminal telopeptide of collagen type 1 (CTX), the alkaline phosphatases, (AFs), the amino-terminal propeptide of procollagen type 1 (PINP) or tartrate-resistant acid phosphatase (TRAP-5b)[4].

Clinical trials have shown that the bisphosphonates inhibit the resorption of bone mediated by the osteoclasts, which means that it could be a therapeutic option for the prevention of bone loss induced by oncological treatment secondary to hormonal deprivation, especially if the patient has a low bone mineral density or has risk factors for the development of fractures from minimal traumas. In the initial stages of non-metastatic breast cancer, zoledronic acid administered every 6 weeks, oral ibandronate monthly and weekly risedronate have been shown to prevent bone loss associated with the use of aromatase inhibitors in breast cancer in postmenopausal women. On the other hand, SREs occur in 64% of patients with breast cancer who are not treated with bisphosphonates, which is why the American Society for Clinical Oncology (ASCO) recommends treatment with intravenous bisphosphonates in patients with pain or destruction of bone evidenced by radiography[5-7].

Other, more recent treatments are those monoclonal antibodies such as denosumab, which have a great affinity with RANKL, impeding the RANKL/RANK interaction on the surface of the osteoclasts, thus diminishing bone resorption. In a phase II clinical trial carried out in patients with breast cancer and bone metastasis in which were compared 120 mg subcutaneous denosumab, plus an intravenous placebo, and 4 mg intravenous zoledronic acid, adjusted for renal function, plus a subcutaneous placebo every 4 weeks, it was shown that denosumab reduced the time until the first bone event by up to 23% (HR 0.82; CI 95%, 0.71-0.95; p=0.1 superiority) and the risk of multiple events by up to 18% (HR 0.77; CI 95%, 0.66-0.89; p=0.001), compared with the zoledronic acid [8]. These data translate into a greater quality of life for the patient[9,10]. In addition, unlike zoledronic acid, denosumab is not nephrotoxic, which means that there is no requirement to adjust according to creatinine clearance, it is administered subcutaneously and has lower toxicity.

The better understanding of bone metabolism, advances in molecular biology and a better characterisation of the signaling systems of the RANK/RANKL/OPG pathways represent an advance in the treatment of bone metastases, as well as in the prevention of states of osteopenia and osteoporosis secondary to oncology treatment, given that these negatively influence the morbimortality of our patients.

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The history of SEIOMM (1987-2013)

During the 1980s, the medical professionals associated with diseases of the locomotor apparatus began to pay attention to metabolic bone diseases, and among these, a pathology very common in advanced age, osteoporosis, which, until this time had passed almost unnoticed due to the absence of precise methods of diagnosis. Bone fractures, to a great extent brought on by osteoporosis, were frequent and provoked disorders and disabilities, above all in women after the menopause. The appearance of highly precise methods for the diagnosis of osteoporosis and of efficacious drugs for its treatment led to an increase in interest in this pathology on the part of medical specialists directly or indirectly associated with the locomotor apparatus such as internists, rheumatologists, endocrinologists, gynaecologists and nephrologists. In Spain this situation resulted in the formation of the Spanish Society for Bone and Mineral Metabolism Research (SEIOMM) and led to a pharmaceutical company offering DXA densitometry at 14 Spanish hospitals, facilitating the diagnosis and study of osteoporosis.

A management board presided over by Horacio Rico Lenza, and formed of Luisa Traba (Vice President), Sergio Serrano (Secretary) and Emma Rosa Hernández (Treasurer) established the basis of the constitution of this multidisciplinary organisation and organised the first symposium in Barcelona on the 6th December 1987, which saw a large number of participants. This symposium was the opportunity to hold the Assembly at which was appointed SEIOMM's first board of directors.

Earlier, in April 1987, the European Foundation for Osteoporosis and Bone Diseases had been formed, with the main objective of promoting the understanding of the physiopathology, diagnosis, treatment and prevention of osteoporosis and other bone diseases. To achieve this it was considered necessary to support biomedical, clinical, nutritional and epidemiological research into these diseases and to promote their understanding, establishing education and training for doctors and other health professionals in this field. The same activities were taken on by SEIOMM and have been carried out, as far as possible, since the Society's foundation. SEIOMM is now a benchmark at an international level.

From December 1987 to December 1991

During this period the board of directors was made up of Daniel Roig Escofet (President), Ramon Peréz Cano (Vice President), María Luisa Mariñoso Barba (Secretary) and María Teresa Gonzaléz Álvarez (Treasurer). In addition, board members were appointed, each representing an Autonomous Community, whose role was to act as a link between the members in their respective Communities and the board of directors.

One of the first activities of the board of directors was to appoint a committee which would study the previous articles of association of the Society and propose modifications which they considered necessary. In accordance with this, new articles were drafted which were submitted for approval by the Extraordinary Assembly held in Seville on 3rd May 1989.

These articles had 35 sections, from which some specifics may be highlighted. The main objective of SEIOMM was the promotion of basic and clinical research into osteoporosis and other bone dise-

A MANAGEMENT BOARD PRESIDED OVER BY HORACIO RICO LENZA, AND FORMED OF LUISA TRABA (VICE PRESIDENT), SERGIO SERRANO (SECRETARY) AND EMMA ROSA HERNÁNDEZ (TREASURER) ESTABLISHED THE BASIS OF THE CONSTITUTION OF THIS MULTIDISCIPLINARY ORGANISATION AND ORGANISED THE FIRST SYMPOSIUM IN BARCELONA ON THE 6TH DECEMBER 1987, WHICH SAW A LARGE NUMBER OF PARTICIPANTS. THIS SYMPOSIUM WAS THE OPPORTUNITY TO HOLD THE ASSEMBLY AT WHICH WAS APPOINTED SEIOMM'S FIRST BOARD OF DIRECTORS

ases. The national symposium of the Society would be held biennially, and a Monograph Meeting would be held on a current theme in the inter-symposium years. To each of these activities would be added a meeting of the General Assembly. Extraordinary Assemblies could be called for the modification of the articles of association or to deal with matters of vital importance and whose resolution could not wait for the next ordinary General Assembly. The board of directors would be made up of a President, Vice President, Secretary, Treasurer and a representative of each Autonomous Community; but a permanent executive board was also established with four representatives from the Autonomous Communities. This would have decision-making powers. The board of directors would produce the agenda for the General Assembly of SEIOMM, which would coincide with the national symposium or the monograph meeting. The scientific programme of the symposia would be produced by the organising committee and would need to be approved by the board of directors of the Society. It was also determined that the President would be able to hold office for a maximum of 4 years. These articles were approved by an absolute majority of the Assembly.

It was considered that SEIOMM should establish and maintain contacts with other institutions or societies with similar objectives, both nationally and abroad. The first international contact was made with the European Foundation for Osteoporosis and Bone Disease. This occurred at a meeting held in Davos (Switzerland) in April 1988, which was attended by representatives of different national societies in Europe with similar objectives. The President of SEIOMM Daniel Roig Escofet attended as the Society's representative. Another Spanish representative, Aurelio Rapado Errazti, President of another Spanish association, the Spanish Association for Osteoporosis and Metabolic Bone Diseases (AHOEMO) also attended. This association had been established in 1988 as a federation of a number of Spanish scientific societies. Its objective was to inform the general public on the prevalence and serious effects of osteoporosis, as well as to give information to the press and other communications media, and sponsor research into all aspects of the disease through grants, prizes and large population studies. Its activities were social in character, whereas those of SEIOMM were exclusively scientific. Subsequently a number of activities were carried out jointly.

The relationship with the European Foundation resulted in SEIOMM organising an "International Symposium on bone metabolic diseases" in the Platja d'Aro (Girona) in October 1990, with the aim of making the Society better known externally. Attendance was high and specialists in these diseases attended from various European countries, as well as from South America, since SEIOMM had also established relationships with the Iberoamerican Society of Osteology and Mineral Metabolism (SIBOMM), of which Dr Díaz Curiel was secretary and in which SEIOMM had a representative.

At the Assembly, Professors Horacio Rico Lenza and Miguel Garrido Peralta were made honorary members.

During this period SEIOMM organised, in addition to the aforementioned international congress, a symposium in Seville (1989) and another in Oviedo (1991).

From November 1991 to October 1995

At the III Symposium held in Oviedo in November 1991 a new board of directors was appointed, now being formed by Jorge Cannata Andía (President), Concepción de la Piedra Gordo (Vice President), José Bernadino Díaz López (Secretary) and Javier del Pino Montes (Treasurer).

One of the details from this period is the participation of SEIOMM in the development of the Spanish Review of Bone Metabolic Diseases as the organ of the Society, along with AHOEMO and the Osteoporosis Working Group of the Spanish Society of Internal Medicine, and the appointment of Jorge Cannata Andía as joint editor representing SEIOMM. SEIOMM continued to maintain its external relationships, and in May 1992 an International Symposium on Osteoporosis was held in Barcelona attended by representatives of various international societies. Dr Concepción de la Piedra Gordo was appointed as a member of the SIBOMM board, representing SEIOMM.

The introduction of densitometry in a number of Spanish hospitals facilitated the development of work relating to osteoporosis. Two types of research were requested of those receiving of the densitometers. One of these was a free choice. The other was common to all the groups and consisted of the measurement of the bone density of groups of people of different ages, with the aim of getting an idea of the normal values for bone mass in our country. This latter work, originally conceived of as having a transverse design, continued in a second phase with a longitudinal design in which the development of bone mass monitored over three years was studied.

In 1992, as a result of this work the "Study of bone density in the Spanish population. A multicentric osteoporosis research project" was published, with the participation of the following hospitals: the La Paz Hospital (Madrid), the San Carlos University Hospital (Madrid), the Jiménez Díaz Foundation (Madrid), the Clinical University Hospital (Salamanca),the Insular Hospital (Las Palmas de Gran Canaria), the Prínceps d'Espanya Hospital (Hospitalet), the Santa Creu i Sant Pau Hospital (Barcelona), the Esperanca Hospital (Barcelona), the La Fe Hospital (Valencia), the Virgen Macarena University Hospital (Seville), the University Hospital (Granada), the General Hospital of Asturias (Oviedo), the Miguel Servet Hospital (Zaragoza) and the University Clinic (Pamplona). Also involved, as well as SEIOMM, were the Osteoporosis Working Group of the Spanish Society for Internal Medicine (GTO),

AHOEMO and the medical department of the pharmaceutical company Rhóne Poulenc Rorer.

The results of the study were published in various reviews and in the Spanish Review of Metabolic Bone Diseases. Notable among these was the article "Normal values for bone mineral density in the Spanish adult population". And in 1996, another work on this subject: "New frontiers in the study of bone density in the Spanish population" was published.

In 1993 SEIOMM awarded prizes to works on "Vitamin D metabolites and osteoporosis". The first prize was shared between two studies: "Osteoporotic hip fractures in old people. A social problem of physiopathology and prevention" by J.M. Quesada and J. Alonso, and "Levels of 25(OH)D in postmenopausal women and old people: its relationship with bone density" by Maria E. Martínez, M.T. del Campo, M.J Sanchéz-Cabezudo, J.A. García, J. Coya, M.T. Sánchez-Calvín, A. Torijos and L. Munuera. In addition, three second prizes were awarded.

During this presidency the AHOEMO was transformed. In 1993 it changed its name to the Hispanic Foundation for Osteoporosis and Metabolic Bone Diseases (Fundación Hispana de Osteoporosis y Enfermedades Metabólicas Óseas [FHOEMO]), with a scientific committee formed by four members of SEIOMM (Jorge Cannata Andía, Bernadino Díaz López, Jesús González Macías and Daniel Roig Escofet) and four other members of different scientific societies previously associated with AHOEMO. The desirability of developing a protocol or document defining the relationship between SEIOMM and FHOEMO in order to facilitate bilateral collaboration was raised

At the executive board meeting of 19th May 1993 it was decided to name Drs Aurelio Rapado Errazti and Daniel Roig Escofet as honorary members for their outstanding work and effort in activities related to bone and mineral metabolism.

In this period SEIOMM symposia were held in Córdoba (1993) and Alicante (1995) and two monograph meetings, which the articles of association had established, were organised, one on "Glucocorticoids and bone" in Zaragoza (1994) and the other on "Paget's Disease" in Salamanca.

From October 1995 to October 1999

A new board of directors, whose appointment coincided with the Alicante symposium, was formed by Jesús González Macías (President), Adolfo Díez Pérez (Vice President), Manuel Sosa Henríquez (Secretary) and Manuel Díaz Curiel (Treasurer).

In this period the normal activities of the Society continued, predominantly the dissemination of new developments in bone diseases through courses, conferences and written materials, relationships with other organisations, and the promotion of research. A letter was sent to all hospital directors general to explain the need to highlight the significance of bone densitometry. Notable among other activities was a course on densitometry organised by the SEIOMM and led by Luís del Río Barquero, in Barcelona in November 1997. Also, a monograph meeting organised jointly by the IOF (International Osteoporosis Foundation), FHOEMO and SEIOMM held in Madrid and presided over by Dr Antonio Torrijos Eslava on "the Menopause".

Relationships with overseas societies related with osteoporosis continued. As a minimum, a representative of SEIOMM went to Paris every six months to attend meetings with the European Foundation for Osteoporosis and Bone Disease (EFFO), which transformed during this period into the International Osteoporosis Foundation (IOF), although there was no Spanish member appointed to its scientific committee. At the III Iberoamerican Congress on Osteology and Mineral Metabolism organised in Mexico in 1996 by SIBOMM, Dr Manuel Díaz Curiel was appointed as the SEIOMM delegate to that Society. In addition, Dr Adolfo Díez Pérez became the representative of SEIOMM to the European Calcified Tissue Society, and was chosen as a permanent member of the council of that society. There were also conversations with Professor Gennari, President of the International Federation of Societies on Skeletal Diseases (IFSSD) and with the World Federation on Osteoporosis (WFO) to try to integrate SEIOMM with these societies on condition that the Society would be able to play an active part, and be represented on the boards of directors or the executive councils of these societies. These conversations were not fruitful, but in the end these societies were integrated into EFFO, subsequently called the IOF, and disappeared.

At the General Assembly of SEIOMM held in Madrid on 16th October 1998, a modification of the statues was approved which created working groups with the aim of channelling the scientific activities of the members into one or other line of research according to their interests. At the end of this period there were already four functioning working groups, dedicated to "bone densitometry", " quality of life", "management of clinical history" and "research into ultrasound and bone metabolism", coordinated respectively by Luis del

THE INTRODUCTION OF DENSITOMETRY IN A NUMBER OF SPANISH HOSPITALS FACILITATED THE DEVELOPMENT OF WORK RELATING TO OSTEOPOROSIS. TWO TYPES OF RESEARCH WERE REQUESTED OF THOSE RECEIVING OF THE DENSITOMETERS. ONE OF THESE WAS A FREE CHOICE. THE OTHER WAS COMMON TO ALL THE GROUPS AND CONSISTED OF THE MEASUREMENT OF THE BONE DENSITY OF GROUPS OF PEOPLE OF DIFFERENT AGES, WITH THE AIM OF GETTING AN IDEA OF THE NORMAL VALUES FOR BONE MASS IN OUR COUNTRY

Río Barquero, Adolfo Díez Pérez and the last two by Manuel Sosa Henríquez. Over time other groups were added such as one on "risk factors" coordinated by Carmen Valdés Llorca.

The board of directors carried out an internal audit aimed at understanding the state of the Society for the administration. A detailed study was then proceeded with whose results were discussed at the meeting of the permanent executive board which took place in Santander on 16th February 1998. The following actions were carried out:

a) Regularisation of SEIOMM through its registration with the Register of Associations and compliance with the corresponding employment laws.

b) Regularisation of the situation of the secretary, proceeding with their contract and registering with the Social Security department.

c) Contracting with consultancies specialising in human resources, finance and accountancy so that monthly accounts could be managed, and annual accounts and a budget for the following year prepared.

In this period another honorary member was appointed: Dr Horacio Rico Lenza. SEIOMM was also modernised: an e-mail was set up for the services of members to enable communication with the board of directors and an internet web page was established for SEIOMM, developed by Xavier Nogués.

And there was a change: the biennial scientific meetings until then called symposia became congresses. The congresses were held in Granada (1997) and Sitges (1999) and the two monographic meetings in Madrid, on on "Biochemical markers for bone remodelling" and the other on "Densitometry". The latter was timed to coincide with a joint meeting with FHOEMO and EFFO.

From October 1999 to November 2003

A new board of directors was chosen: Adolfo Díez Pérez (President), José Manuel Quesada Gómez (Vice President), Nuria Guañabens Gay (Treasurer) and Xavier Nogués Solán (Secretary). In a motion to the General Assembly of SEIOMM held on 5th October 2002, the Society was notified that the President, Adolfo Díez Pérez had resigned due to his new position in a multinational company based in the U.S, and the presidency passed to the Vice President, José Manuel Quesada Gómez.

This period saw the death of two significant personalities in the field of bone diseases, and honorary members of SEIOMM: Dr Horacio Rico Lenzo, President of the first management board of SEIOMM, and Dr Aurelio Rapado Errazti, President of FHOEMO. The latter position passed to Dr Manuel Díaz Curiel.

Relations with national and overseas bodies were maintained, notably, an accord with the International Bone and Mineral Society (IBMS) by which for three years from January 2001 members of SEIOMM also had full rights to the IBMS, receiving the review Bone free. In addition, Dr Manuel Díaz Curiel was appointed as President of SIBOMM. SEIOMM participated in a congress of the Spanish Society of Internal Medicine and in another of the Spanish Society of Family and Community Medicine. The relationship with FHOEMO was maintained, with each Society reflecting its own chatacter: scientific exclusivity for SEIOMM and social exclusivity for FHOEMO. SEIOMM as generator of conferences, scientific meetings and other events aimed at professionals, and FHOEMO spreading knowledge of the diseases in the population through social and cultural activities.

The working groups already established were sustained. In 1999 a SEIOMM working group on protocols and clinical history was created, coordinated by Manuel Sosa Henríquez. The result of this work was SEIOMM's Clinical History, whose first version was presented at the congress held in Menorca in 2001 and subsequently modified at the monographic meeting in Toledo in 2002. This Clinical History was presented on a CD, with the collaboration of the Italfármaco laboratories.

A work entitled "Guide to Clinical Practice" produced by the working group on "Management of Clinical History" was published in the Spanish Review of Bone Metabolic Diseases and the Spanish Clinical Review. The content of the guide was developed over a period of two years. A draft was presented at the congress held in Menorca in 2001 and, and subsequently a debate was organised in a forum open to all SEIOMM members at the monographic meeting in Toledo. The introduction explained that the progressive increase in the incidence of osteoporosis, parallel to the demographic of aging in Spain, its morbidity and mortality, as well as its health and economic impact had led SEIOMM to develop this guide as a first step aimed at the population group most affected. It offered an indicative framework, in which the interested professional groups could develop action protocols adapted for each healthcare environment.

The Guide to Clinical Practice was developed by a group of experts from different specialities (internists, rheumatologists, endocrinologists, gynaecologists, nephrologists and specialists in family and community medicine), coordinated by an expert in evidence-based medicine. The group was formed by J. Calaf (gynaecologist), J. Cannata (nephrologist), B. Díaz (internist) A. Díez Pérez (internist), J. González Macías (internist), N. Guañabens (rheumatologist), F. Hawkins (endocrinologist), A. Morales (rheumatologist), M. Muñoz Torres (endocrinologist), X. Nogués (internist), J. M. Nolla (rheumatologist), P. Orozco (family doctor), R. Pérez Cano (internist), J. del Pino (internist), J. M. Quesada (endocrinologist) and M. Sosa (internist).

The Society, alone or in collaboration with the pharmaceutical industry, stimulated active research in different areas of activity. 2003 was the third anniversary of the SEIOMM-MSD research calls, while the well-established FAES and Italfármaco research prizes continued, as well as

those from the Lilly Foundation which had been presented at all the congresses.

Apart from research, SEIOMM carried out a number of educational activities, among which was the II Densitometry Course (Bone Measurement Technique Accreditation) in April 2001 in Barcelona under the direction of Luís del Río.

After many years in the post of editor of the Spanish Review of Bone Metabolic Diseases, Aurelio Rapado was succeeded by Manuel Diaz Curiel, and later, by Federico Hawkins Carranza jointly with Esteban Jódar Gimeno as editorial secretary. On a negative note, the lack of original articles put the continued publication of the review at serious risk.

The number of members of the society increased progressively from its foundation. In this period there were 317 members.

At the National Assembly held in Seville in 2000 it was agreed that members of the Society who retired would become Emeritus Members. In this period a congress was organised in Ciutadella (Menorca) in 2001, and another in Maspalomas (Gran Canaria) in 2003. The V Monographic Meeting was held in Seville (2000) on the theme of "Osteoporosis in males". This meeting was coordinated with the annual meeting of FHOEMO. The VI Monographic Meeting took place in Toledo (2002).

During this period it was proposed that the outgoing president would become part of the following board of directors, since the experience they had gained in the previous years could be useful.

From November 2003 until October 2007

At the General Assembly held on 21st November2003 in Maspalomas (Gran Canaria) a new board of directors was appointed. This consisted of Nuria Guañabens Gay (President), Manuel Muñoz Torres (Vice President), Carlos Gómez Alonso (Secretary) and Lius del Río Barquero (Treasurer).

In relation to the Society´s finances, the meeting of the board of directors held in Cáceres in 2004 discussed the difficulties in carrying out financial management within a proper legal framework. In order to facilitate this, an agreement was reached between the board of directors and Pharma Consult for the management of the congresses. The SEIOMM would receive the benefits of the congress, once the corresponding share for the local committee organiser and for FHOEMO had been discounted, with the accounting and financial management of all the expenditure and income being the responsibility of Pharma Consult S.A.

During this period there was new revision of the articles of association, which occurred at a meeting called in Madrid to coincide with the congress of 2005. The following changes were approved:

- The national congress to be held annually, with the monographic meetings ceasing.
- The term of office of the board of directors was reduced from 4 years to 3.
- The maximum period for those representing SEIOMM on other societies would be 3 years.
- The election of president a year before the change of board of directors, to enable them to familiarise themselves with the essentials of SEIOMM.
- That the procedures of the scientific programmes be regulated.

A key activity was the updating of the guide to clinical practice for postmenopausal osteoporosis and its broadening to include corticoid and male osteoporosis. A scientific committee was appointed, coordinated by Jesús García Macías and formed of Guañabens Gay, Muñoz Torres, del Río Barquero and Díaz López. The updated guide was presented at the SEIOMM congress of 2007.

The relationships with other societies continued. Xavier Nogués Solán was the delegate for SEIOMM to the European Society for Calcified Tissue and Luis del Río to SIBOMM, continuing his previous relationship with the IOF and FHOEMO.

At the 2007 SEIOMM congress a round table was organised jointly with the ASBMR and a SEIOMM round table was organised at the IBMS congress in Montreal in June 2007, as well as at periodic meetings with the IOF.

The review Calcified Tissue International was added to previous subscriptions to foreign reviews such as Bone and Osteoporosis International, to which members of the SEIOMM had free access during this period.

In 2004 SEIOMM began awarding grants to members for their attendance at the annual congress of the ASBMR. These grants have continued to the present day,

The SEIOMM congresses took place in Madrid (2005), in Malaga (2006) and in Valencia (2007). In this period there was already only one monographic meeting (the final one) which took place in Cáceres on the theme of "Bone formation from the basic to the clinical". A new course for accreditation in densitometry was run by Luis del Río and Xavier Nogués. The reissue of the course on techniques for the measurement of bone mass, with the collaboration and accreditation of the International Society of Clinical Densitometry was proposed.

The prizes from Italfarmáco, communicated orally and

THE SOCIETY, ALONE OR IN COLLABORATION WITH THE PHARMACEUTICAL INDUSTRY, STIMULATED ACTIVE RESEARCH IN DIFFERENT AREAS OF ACTIVITY. 2003 WAS THE THIRD ANNIVERSARY OF THE SEIOMM-MSD RESEARCH CALLS, WHILE THE WELL-ESTABLISHED FAES AND ITALFÁRMACO RESEARCH PRIZES CONTINUED, AS WELL AS THOSE FROM THE LILLY FOUNDATION WHICH HAD BEEN PRESENTED AT ALL THE CONGRESSES

through posters, from FAES Research 2004-2005, from SEIOMM-MSD, and the SEIOMM prize for the youngest researcher, continued.

The number of members increased up to 364 during this period.

From October 2007 to October 2010

The board of directors during this period was formed by Manuel Sosa Henríquez (President), Javier del Pino (Vice President), María Jesús Gómez de Tejada Romero (Secretary) and Esteban Jódar Gimeno (Treasurer).

At the last Assembly of his period in office the president, Manuel Sosa Henríquez gave a summary of the activities carried out, stating that they had they had met all the targets set:

- Provision of regular detailed information on the management of the Society mainly through bulletins and through the web.

- Creation of a new web site, which since then has been kept updated.

- In 2009 the Spanish Foundation for Bone and Mineral Metabolism Research (Fundación Española de Investigación Ósea y Metabolismo Mineral [FEIOMM]) was founded, associated with SEIOMM, its first president being Manuel Sosa Henriquez, with social objectives and dissemination activities appropriate to foundations. In terms of the Foundation's operational functions, FEIOMM research grants were created aimed at basic, genetic and clinical research.

- Development of new Articles for SEIOMM, and coordinated with those of FEIOMM.

- The production of the Society's own review (Review of Osteoporosis and Mineral Metabolism) given the difficulty in moving forward on this before.

- Stimulating and facilitating research, with almost 120,000 euros available for grants and the creation of 10 working groups. In 2009 the SEIOMM's committee of experts published the "Guide to clinical practice in postmenopausal osteoporosis, osteoporosis due to corticoids, and in men" (Rev Osteoporos Metab Miner 2009 1;1 53-60).

Up to this time, the administrative work of the Society had been carried out by someone with the trust of the Secretary of the board of directors in their home city. This meant that each time the board changed, all the documentation needed to be moved. It was proposed, therefore, that the location of the secretariat be permanent. It was then decided that the company SANED would provide this function and that they would make a person available to SEIOMM who would perform these administrative tasks.

The relationships with the other national and international societies participating in the study of osteoporosis and mineral metabolism continued. Notable among the international societies were the International Bone & Mineral Society, the International Osteoporosis Foundation, the European Calcified Tissue Society, the International Society for Clinical Densitometry and the Iberoamerican Society of Osteoporosis and Mineral Metabolism. Adolfo Díez Pérez was appointed as a member of the board of directors of the International Bone and Mineral Society, and Manuel Díaz Curiel and Nuria Guañabens Gay to the scientific committee of the International Osteoporosis Foundation. Cooperative research work was carried out with other scientific societies, such as the "Consensus Document on Vitamin D" and the "Study of the Prevalence of Non-vertebral Fractures in Patients in Treatment with Corticoids". The board of directors aimed to keep all the associates informed and an email address was approved for the dissemination of specific information. In addition, a SEIOMM Information Bulletin was created, which started to be produced in 2009 on a quarterly basis, through which provided concise information to all the associates on all matters regarding SEIOMM in the previous quarter, with the intention that all associates would be fully informed and up to date with everything related to SEIOMM through the web site.

In a restricted zone of the web site, access was given to Navibone, which allowed access to various reviews of interest such as Osteoporosis International, Calcified Tissue International and the Journal of Bone and Mineral Metabolism. In addition, by being members of the IBMS, thanks to funding from the Nycomed Laboratories, SEIOMM members had access to the reviews Bone and BoneKey. Also, thanks to the work of Adolfo Díez Pérez, access was also available to the review Progress on Osteoporosis.

The documents most consulted through the web site were SEIOMM´s clinical guides, followed by the information bulletins and the NOF Guides. The "Spanish Review of Bone Metabolic Diseases" had to cease publication due to financial difficulties and a lack of original articles. In 2009 the "Spanish Review of Osteoporosis and Mineral Metabolism" was produced, replacing the aforementioned publication, issued three times a year, the third edition each year dedicated to recording the communications presented at the SEIOMM congress. Manuel Sosa was (and continues to be) the editor of the review. In addition to the printed form, an online version was produced, with its own web site accessible through a link from the SEIOMM web site. The Review was made open access for better dissemination, and is available in two languages: Spanish and English.

During this period, the following projects were established: a clinical case competition promoted by the Nycomed Laboratories; a blog on the web site which could be called "Talk to an expert"; an on-line course on Medicine and Law. A collaboration agreement was signed between the IBMS and SEIOMM aimed at developing an IBMS-SEIOMM joint round table, each society providing 50% of the costs. The first of these round tables was held in 2009 during the Santander conference on the theme "primary hyperparathyroidism". Drs Jan Bolersven from Norway and Serge Ferrari (Vice President) participated on behalf of the IBMS, and

Drs Manuel Muñoz Torres, Estebán Jódar Gimeno and Manuel Sosa Hénriquez on behalf of SEIOMM. This collaboration has continued at each year´s congress to the present day.

The number of members increased, reaching 447 in this period.

In November 2008 the congress was held in Oviedo in October 2009 the XIV congress in Santander, and in 2010 in Salamanca. In 2008 the congress of the European Calcified Tissue Society took place in Barcelona, A new course for accreditation in "Techniques for the Measurement of Bone Mass" was organised, led by Luis del Río and Xavier Nogués.

At the Salamanca congress Juan José García Borrás requested that it be put on record that: "support be given to retired members who, due to the lack of support from the pharmaceutical industry do not attend the congresses. Given the favourable financial policy which has been put in place by the board of directors and the creation of the Foundation, and since some funding has been requested for basic research, I ask that the interests retired members be taken into account, and that they are brought to the congresses, and that at the next executive meeting considers this plea and brings its decision to the next Assembly".

During this period the FAES FARMA-SEIOMM prizes were awarded. Seven ASBMR grants continued and a new Amgen-SEIOMM prize established. At the Santander congress of 2009, the Italfármaco Laboratories were honoured for having supported the prizes for the best oral and poster communications at the SEIOMM congresses for more than 15 years without interruption.

Carlos Lozan Tonkin, Jordi Farrerons Minguella and Juan José García Borrás were awarded honorary membership.

From October 2010

The board of directors during this period is made up of Javier del Pino Montes (President), Josep Blanch Rubió (Vice President), María Jesús Moro Álvarez (Secretary) and Carmen Valero Díaz de Lamadrid (Treasurer).

It was stated at the congress in Coruña in 2011 that FEIOMM had responsibility for the income and expenditure of the congresses, which means that some of the benefits which are obtained from them should be used to accomplish the action plans of FEIOMM, as well as funding its internal functions.

At this congress it was agreed to increase the membership fee which had remained unchanged for 15 years. After a number of interventions, the change from 27 to 35 euros in 2012, and then to 40 euros in 2013, was approved. At a meeting during this period some working committees were established in accord with the objectives developed using the Metaplan method, a method of group moderation for problem-solving. The following groups were approved: economic management, training and congresses, research committee, external relations and working groups. Based on these objectives a strategic plan was developed with a horizon of 2014.

In addition, a group of senior members of SEIOMM was formed on the initiative of, and in collaboration with, Dr Juan José García Borrás who was appointed as coordinator of the group.

Relationships with other scientific societies continued. In collaboration with the Spanish Society for Osteoporotic Fractures (SEFRAOS) and other related societies the "Blue Book on Osteoporotic Fractures in Spain" was produced. A collaboration agreement was signed with AEEM to collaborate in scientific activities related to postmenopausal osteoporosis. SEIOMM maintained its relationships as part of SIBOMM and the IOF. In relation to the IBMS, due to a change in its communication strategy, it swapped its relationship with Elsevier for one with Nature, and stopped receiving Bone, this being substituted by BoneKey.

With regard to research, the ASBMR grants continued to be given and a revision was announced of SEIOMM´s Guides to Clinical Practice, coordinated by Jesús González Macías.

In this period the SEIOMM congresses were held in La Coruña (2011), Cuenca (2012) and in Tarragona (2013). In 2012 a SIBOMM congress was held in Madrid.

During this period improvements were made to information systems and information technology in relation to Web 2.0, including restructuring and redesigning the SEIOMM-FEIOMM and Review (Review of Osteoporosis and Mineral Metabolism) web site, and the creation of SEIOMM groups on Facebook and Linkedin. A cloud computing service was initiated for the use of members. The BIDI code was incorporated in the documents of the congresses and other SEIOMM documents. SEIOMM and FEIOMM were put on a sound footing, with the establishment of their own headquarters (Paseo de la Castellana 135, Madrid) and an executive secretary (Dª Lorena Herrero).

A group of senior members and a young researchers group have been formed, with meetings being organised post ASBMR.

The Cuenca congress approved the modification of the articles of association which would introduce the following changes:

1. A new home for SEIOMM headquarters.
2. Change to the closure of the accounts to enable the most up to date financial report to be given to the Assembly on June 30th each year.
3. An addition to the board of directors of two voting members was proposed, as well as creating the position of President Elect (the future President) to be incorporated into the board of directors, so that the strategy and activities of the Society may be planned with a horizon of two periods of office.

Note: this historical summary will be updated with the passage of time and as new data becomes available.