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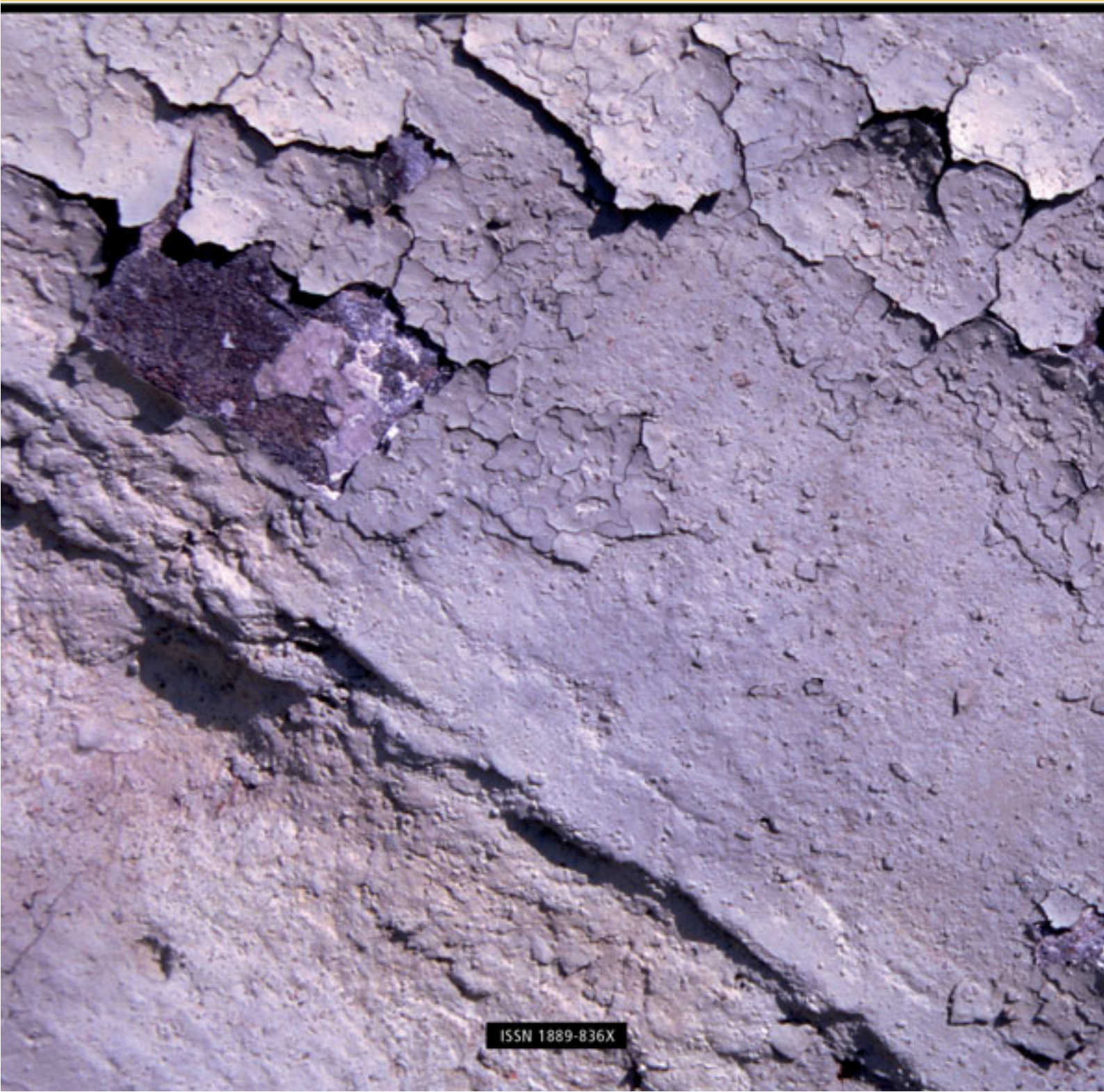

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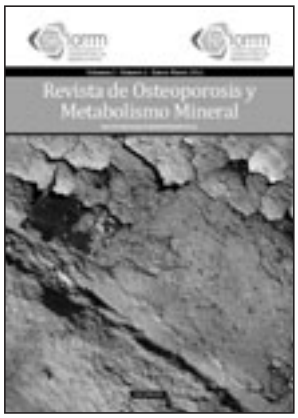

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SUMMARY

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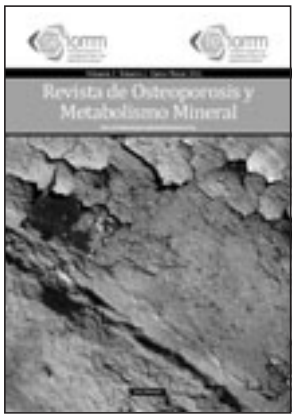

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Erratum

Vol.2 Nº 2, Originals: "Vertebroplasty and kyphoplasty as a treatment for osteoporotic vertebral fractures". The correct name is **Riancho Moral JA**, not Riancho del Corral JA , as was published in error.

Maxillary osteonecrosis: new evidence regarding its etiopathogeny

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Maxillary osteonecrosis (MON) is a disease which has appeared recently as a serious complication in patients suffering from neoplasms or other chronic diseases. MON has been associated with the use of powerful diphosphonates, for which reason many authors have named the disease secondary osteonecrosis of the mandible due to biphosphonates[1-5].

This is a relatively new disease, which means that there is not yet unanimity on many of its aspects. For a start, there is no clear and universally accepted definition of MON. A panel of experts from the American Society of Bone and Mineral Research (ASBMR)[2] recently recommended using the definition "an area of exposed bone which persists for more than 8 weeks in the absence of earlier irradiation and/or metastasis in the mandible". The American Academy of Mouth and Maxillofacial Surgeons published a similar definition: a patient may have MON if they comply with 3 requirements: 1) current or previous use of biphosphonates; 2) the presence exposed or necrotic bone for a minimum of 8 weeks; and 3) an absence of maxillary radiotherapy. At this point should insist that the correct name for the disease is maxillary necrosis and not necrosis of the mandible, given that there is frequently also affectation of the upper maxilla[6].

It used to be thought generally that MON was directly related to the use of biphosphonates, above all with those that are most powerful and administered intravenously, such as zoledronic acid or pamidronate. Thus, prestigious authors such as Reid have described MON as "a complication in the use of high doses of biphosphonates, which is characterised by the appearance of exposed bone in the oral cavity"[7].

The pathogeny of MON is unknown up until now, for which reason various theories relating to this have been developed[1,6,8,9]. Given that it has not been possible to identify one single cause, many authors have implicated various etiopathogenic factors, some of which may act in conjunction. In one way or another, the biphosphonates have always been present in these etiopathogenic theories. For example, one of these is based on the bone toxicity of biphosphonates, according to which the drug would accumulate in the bone in sufficient quantities to be directly toxic to the oral epithelium[1]. This would result in inadequate healing of lesions in the soft tissues, such as those observed in invasive dental procedures or by traumas produced by poorly-fitted prostheses, which could result in a secondary infection in the underlying bone[10]. However, against this theory, we have the fact that only the maxillas are affected, and not other bones where biphosphonates act equally. Another slightly different theory is that the biphosphonates accumulate in the alveolar bone, both in the upper maxilla and in the mandible, producing toxicity in the region of the soft tissues instead of in the bone[7].

However, against the theory that biphosphonates are the cause of MON we have, firstly, the fact that up to 25% of cases of MON have been described in patients who had not received this drug[6,8,10-14]. Secondly, many other risk factors have been described for this disease, such as diabetes *mellitus*, taking

corticoids, immunosuppressive treatment and rheumatoid arthritis, to name but a few[6,11,15,16]. These etiopathogenic factors are not mutually exclusive. In fact it is possible that MON could be a disease with a multifactorial etiopathogeny. On the other hand, it should also be taken into account that in up to 70% of cases there has been a dental intervention, such as teeth extraction, implants, etc.[3-6,8,10,14,16].

Finally, MON has been described in patients receiving denosumab, a monoclonal antibody against the protein RANKL, in 2 studies carried out in patients affected by cancer and who were randomly chosen to receive this drug, or zoledronic acid. In the first report, the prevalence of MON was 1.1% in those patients receiving denosumab and 1.3% in those who received zoledronic acid[17]. In another study, the prevalence of MON was 2% in those receiving denosumab and 1.4% in those who received zoledronic acid[18].

In conclusion, the new descriptions of MON in patients receiving denosumab ought to drive us towards re-examining the etiopathology of MON. Perhaps this disease is caused by an excessive repression of bone remodelling in patients with neoplasms, in whom the dose of these antiresorptive drugs used greatly exceeds that recommended for osteoporosis.

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Changes in bone microarchitecture in rheumatoid arthritis. Study using microCT

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Summary
Introduction: The objective of this study is to analyse the bone microarchitecture in rheumatoid arthritis (RA) in a series of biopsies of the iliac crest carried out previously in patients not having had earlier treatment with glucocorticoids, using microCT analysis.
Material and method: 14 bone specimens were obtained, taken from the iliac crest of patients with RA with no previous treatment with glucocorticoids. None of these patients was diagnosed with a disease or was taking medicines which could compromise bone mineral metabolism. A complete clinical history was taken, and a blood analysis carried out, including the rheumatoid factor. The specimens were embedded in methyl-methacrylate and studied with a microCT eXplorer Locus SP scanner. The acquisition parameters were: 80 kVp/80 μA, thickness of aluminium filter:10^{-3} inches, FOV ≈ 2x2 cm, mode of acquisition of 360°, 720 views, 4 frame averages/view, exposure time 1.700 ms, voxel resolution: 28 μm. A region of interest (ROI) was selected by means of interpolation, avoiding cortical bone. An automatic segmentation process (thresholding) was used to differentiate and segment the hematopoietic bone tissue. The microarchitectural parameters were generated automatically by computer using parallel-plate algorithms. The results were compared with 14 specimens from healthy controls of similar age and sex using Student's test for unpaired samples. The statistical significance was $p< 0.05$.
Results: The fraction of bone volume (BV/TV) was significantly lower in those patients with RA than in the healthy controls ($p< 0.05$). The trabecular thickness (Tb.Th) was higher in the controls. The trabecular separation (Tb.Sp) was higher in those specimens with RA ($p< 0.05$). The trabecular connectivity (Tb.N) was significantly greater in the control specimens ($p< 0.05$).
Conclusions: The patients with RA have worse trabecular bone quality and low trabecular connectivity. The microCT scanner is a quick and powerful tool for the study of trabecular microstructure.

Key words: *Rheumatoid arthritis, Microarquitecture, Bone, Computed Tomography.*

Introduction

Osteoporosis is a global health problem[1]. It has been defined by the National Institutes of Health as "a disease characterised by low bone mass and a deterioration in the microarchitecture of bone tissue which drives an increase in bone fragility and a consequent increase in the risk of fracture"[2]. It is for this reason that alterations in trabecular bone are not only characterised by reductions in bone mineral density (BMD), but also by changes in bone quality, a term which encompasses microarchitecture, bone turnover, microfractures and bone mineralisation[3].

Rheumatoid arthritis (RA) is a chronic inflammatory disease with autoimmune origins and unknown etiology which mainly attacks the synovial joints, producing arthritis. In patients with RA, reductions in BMD have been described in two forms: juxta-articular osteoporosis (one of the earliest findings) and generalised osteoporosis, in locations distant from the inflamed joints. To date, different series of patients with RA have been described with a great prevalence for generalised osteoporosis[4-13,34], as well as an increase in the risk of fracture[14-16]. The factors most determinant of bone loss in these patients appear to be a reduction in physical activity in the most advanced stages of the disease[10,11,17], as well as chronic treatment with glucocorticoids[7-9,18,19]. In addition, low levels of vitamin D have been associated with prolonged periods of confinement to bed, with those with very limited functionality, and with diets poor in calcium[20,21,39,40]. On the other hand, in recent years there is more and more discussion regarding the role played by pro-inflammatory cytokines such as TNF-α and IL-1, which have been shown to increase osteoclast resorption by the differentiation of synovial microphages into osteoclasts[22-26].

To date, few histomorphometric studies have been carried out in patients with RA. Mellish et al. studied 48 bone specimens from patients with RA who had not been treated with corticoids, finding a lower fraction of bone volume and a lower trabecular thickness than in the controls, findings which suggest that RA not treated with steroids is associated with premature bone loss. These results were only significant in women[35]. Pérez-Edo et al. described an association between hypovitaminosis D and a reduction in bone turnover in transiliac bone biopsies of patients with RA, confirming findings published by Compston et al. in 1994[21,44]. Hanyu et al. found a reduction in trabecular thickness and in bone connectivity in menopausal patients with RA compared with controls of similar age with osteopenia[45]. Laan et al., on their part, studied different cohorts of patients with RA treated with steroids, finding a lowering of cortical and trabecular BMD in the lumbar spine, which was partially reversible by the interruption of the corticoid treatment[29-31]. Summarising, it appears that the decrease in bone mass in patients with RA is of multifactorial etiology, notable among which being the effect of the pro-inflammatory cytokines and prolonged treatment with glucocorticoids. Despite the fact that conventional histomorphometry allows us to identify this type of osteoporosis, it is an invasive examination, which makes the search for non-invasive alternatives a fundamental objective. Except for the conventional histomorphometric studies, to date no studies have been published which have specifically looked at the trabecular microarchitecture in osteoporosis through multiplanar three-dimensional techniques such as micro-CT or p-QCT (Peripheral Quantitative Computerised Tomography), techniques which allow the measurement of the trabecular (and cortical) microarchitectural parameters in the radius and distal tibia in a non-invasive way[28].

The principal objective of this study is to evaluate the discriminative capacity of microCT to differentiate between patients with RA but without corticoid treatment and healthy controls using previously carried out biopsies of the iliac crest. These bone specimens come from the documentary records of biopsies of the pathological anatomy service of the Hospital del Mar. Our hypothesis holds that the bone samples of those patients with RA will show a deterioration in their bone quality.

Material and method

A total of 66 patients who met the 1987criteria of the American Rheumatism Association for the diagnosis of RA[36] were randomly chosen from the totality of patients of the rheumatology service of the Hospital del Mar and the Hospital de la Esperanza in Barcelona. None of these patients had other diseases or were taking any medicine with could affect bone metabolism, with the exception of 22 patients who were receiving oral corticoid treatment at low doses (< 8 mg/d of prednisone) over a period of 47 ± 61.8 months (range 6-240 months), with an accumulated dose of 6.34 ± 8.76. The remaining patients (44) had never started corticoid treatment. All the patients were in treatment with non-steroidal anti-inflammatories (AINEs) and 67% were receiving treatment with anti-rheumatic drugs of the DMARD (Disease-Modifying Anti-Rheumatic Drugs) type. The same diagnostic protocol was carried out in all patients, which included a complete clinical history, with a particular emphasis on the existence of diseases which might affect bone metabolism and the use of drugs toxic to bone tissue.

A complete biochemical profiling was carried out, including parameters for inflammatory activity. The degree of functionality was measured by means of the Steinbrocker functionality index[37]. The BMD was measured in the lumbar spine using densitometry (DXA)[38] in 41 patients (34 women and 7 men) using a Hologic QDR-1000 (Hologic Inc. Waltham, MA, USA) densitometer. The precision of the apparatus is 0.45% with an *in vivo* coefficient of variation of 1.2% in the lumbar spine.

The most significant clinical and epidemiological data of all the patients with RA initially chosen for the study are shown retrospectively in Table 1.

Performance of bone biopsy: fourteen bone biopsies were obtained from patients (4 men, 10 women), diagnosed with RA without receiving glucocorticoidal treatment, from the documentary records of biopsies of the pathological anatomy service. These bone specimens are the same as those used by Pérez-Edo et al.[21] in their study. There were no significant differences between these patients and the rest of the patients who had followed steroid treatment in terms of age (59.1 ± 10.7 vs 59.9 ± 12.6 years; $p< 0.05$) and the Steinbrocker index (2.2 ± 0.6 vs 2.4 ± 0.6; $p< 0.05$). Each transiliac bone biopsy was obtained using local anaesthetic with a Bordier-Meunier trepan of 8 mm interior diameter (Lepine, Lyon-Cedek, France)[32]. Each specimen was fixed in 70% ethanol, dehydrated in decreasing concentrations of ethyl alcohol and embedded in a cylinder of methyl-methacrylate of 2 cm diameter. Sections 5 μm thick were obtained by mycrotomy (Supercut2050, Reichert Jung, Germany), subsequently stained with Von Kossa's stain and Goldner´s trichrome.

Finally the following histomorphometric statistical parameters were calculated: trabecular bone volume (BV/TV_H; %) and average trabecular thickness ($Tb.Th_H$; μm) by direct microscopic measurement. Derived parameters such as average trabecular density ($Tb.N_H$; μm^{-1}) and average trabecular separation (Tb.SpH; μm) were calculated according to the following formulae[22]:

$$Tb.N = (BV/TV)/Tb.Th$$

$$Tb.Sp = (1/Tb.N) - Tb.Th$$

We described retrospectively the histomorphometric values obtained from the 14 specimens with RA: BV/TV_H (%): 13.52 ± 5.39; $Tb.Th_H$ (μm): 152.44 ± 37.87; $Tb.Sp_H$ (μm): 1157.3 ± 639.84 and $Tb.N_H$ (μm^{-1}): 0.8650 ± 0.2617.

Acquisition of images using microCT: The bone specimens embedded in methyl-methacrylate were introduced into a sample cylinder, and secured with a strip of polyethylene foam to ensure their immobilisation. The capture of the images was carried out with the microCT for specimens eXplore Locus SP (GE Healthcare). The data was collected using the following parameters: voltage of tube: 80 kVp; current of tube: 80 μA, thickness of aluminium filter: 0.010 inches, FOV ≈ 2x2 cm^2 depending on the size of the specimen, mode of acquisition in 360°, 720 views (projections), increment of 0.5° between each projection, 4 images/projection, exposure time: 1,700 ms. The scanning time for each specimen was approximately 2 hours, plus time for reconstruction of 1 hour.

The volumetric data were reconstructed to a resolution of 28-μm isotropic voxels (2.2 x 10^{-5} mm^3 per voxel) using Feldkamp's conical algorithm. 28-μm was chosen to improve the signal-noise quotient of the images obtained, to reduce the scanning time and to lessen the volume of data obtained. The analysis of the images and the generation of the microarchitectural parameters were carried out using MicroView© (GE Healthcare) software.

Due to the fact that the volume of trabecular bone tends to vary, especially sharply decreasing towards the endosteal surface, the region of interest (ROI) to be quantified was selected in trabecular bone using two different methods: one restricting the analysis to only the central trabeculae of the biopsied sample, and the other including all the trabeculae from the endosteal surface. The first method used a cylindrical ROI aligned parallel to the external cortical surfaces with a diameter exactly 50% of the distance between both endosteal surfaces. In the second method a curved outline (spline fitting drawing) which encompassed the combination of trabeculae in each of the cuts was used, the ROI being created subsequently through interpolation. The cortical bone was excluded from the analysis in both methods. In order to avoid artefacts the study of sections or slices near to the edges of the cut were omitted.

For each ROI the bone tissue was segmented from the bone medulla by means of a software application which differentiates the intensity of each of the voxels (bimodal histogram thresholding). The microarchitectural parameters BV/TV_{CIL}, $Tb.Th_{CIL}$, $Tb.Sp_{CIL}$ and $Tb.N_{CIL}$ (for cylindrical ROI) and BV/TV_{SPL}, $Tb.Th_{SPL}$, $Tb.Sp_{SPL}$ and $Tb.N_{SPL}$ (for curved ROI) were calculated automatically using the same parallel-plate algorithms mentioned earlier for conventional histomorphometry, recalculating that Tb.Th was determined by means of an image-processing algorithm included in MicroView©. The stages in the acquisition and processing of the images are summarised in Figure 1.

The Euler-Poincaré number and the Euler volume ($EulerV_{CIL}$ and $EulerV_{SPL}$) were also calculated. All the histomorphometric and microCT results of the patients with RA were compared with a control group of similar sex and age made up of bone biopsies from 14 healthy donors from the documentary records of biopsies from the pathological anatomy service.

Statistical analysis: The data were compiled on a spreadsheet (Microsoft Excel 2002) and were analysed statistically using JMP software (version 5.1.2, SAS Institute Inc. Cary, NC, USA). A basic descriptive statistical study was carried out, applying the Shapiro-Wilk normality test for continuous variables (sample size ≤ 2,000). The statistical significance was set at $p< 0.05$, and the results were expressed as an average ± SD. The comparisons of the microarchitectural data obtained in patients with RA and in healthy donors were carried out using the Student's t test unpaired for multiple comparisons.

Results

In the end, a total of 14 bone samples were included from 10 women and 4 men. No significant differences were found in age between the patients and healthy donors ($p< 0.05$). Even though the volume of the ROIs generated was between 5 and 5.4 times greater in the curved outline than in the cylindrical, a close concordance between both

Table 1. Clinical and epidemiological data for all patients with RA (n= 66)

	Men (n=16) Average ± SD (range)	Women (n=50) Average ± SD (range)
Age, years	57.6 ± 12.2 (34-74)	60.5 ± 11.9 (33-85)
Years since the menopause; n=43	---	16 ± 9.6 (0-45)
Body mass index	24.9 ± 3.1 (18.1-32.5)	25.5 ± 4 (16.8-38)
Duration of the disease, months	68.1 ± 78.05 (6-240)	84 ± 87.6 (6-360)
Prednisolone, accumulated dose (g)	12.0 ± 14.2 (1.1-34.5)	5.3 ± 7.3 (0.7-28)
Steinbrocker's index	2.1 ± 0.5 (1-3)	2.4 ± 0.5 (1-3)
Rheumatoid factor (IU/mL)	521 ± 640 (69-2015)	353 ± 514.2 (65-2150)
VSG (mm/1ºhour)	31.3 ± 16.6 (4-60)	41.7 ± 23.2 (7-85)
PCR (mg/dL)	1.2 ± 0.9 (0.5-3.6)	2.9 ± 2.8 (0.5-10.2)
25-OH-vitamin D, ng/mL	12.57 ± 14.9 (2.2-48.9)	7.45 ± 5.62 (1.5-33.6)
Lumbar DXA (g/cm^2)	0.764 ± 0.118 (0.491-0.935)	0.592 ± 0.129 (0.091-0.792)

methods (ROICIL and ROISPL) was found for all microarchitectural parameters (r^2= 0.83-0.91) especially for Tb.Sp and Tb.N (r^2= 0.91). All the results obtained through microCT in patients and controls for each model of ROI are described below in Table 2.

The trabecular bone mass measured by the two methods (BV/TV_{CIL} and BV/TV_{SPL}) in patients with RA was significantly lower than in the control group (p< 0.05). $Tb.Th_{CIL}$ in the specimens with RA was lower than in the control group, but this difference was not significant (p= 0.83). At any rate, in calculating $Tb.Th_{SPL}$ the result was on the margin of statistical significance (p= 0.06), probably due to the inclusion of thicker peripheral trabeculae at the time of the selection of the ROI. It is probable that this justifies the slightly (although not significantly) higher values for BV/TV and Tb.Th when the same specimen was analysed using both models of ROI selection.

As was expected, those specimens with RA obtained higher values for $Tb.Sp_{CIL}$ and $Tb.Sp_{SPL}$ than the healthy controls (p= 0.028 and p= 0.013, respectively). TbN, or trabecular number, is a parameter which represents the average number of trabecuale per μm. A expected, the controls had higher values for this parameter, but this difference was only statistically significant when the curved ROI model was used (p= 0.027). The volumetric reconstructions of three cylindrical bone cores from patients with RA and from one control exemplify visually the predominance of the trabecular structure in each group (Figure 2).

As has been mentioned earlier, the Euler volume measures the connectivity by unit of volume. As expected, the healthy controls had Euler volumes higher than the patients with RA in both models (EV_{CIL} p< 0.01; EV_{SPL} p= 0.031). In the specimens with RA, a moderate-to-high relationship was found between EV_{SPL} and $Tb.N_{SPL}$ (r^2= 0.69; p< 0.01).

Discussion

To date, earlier studies have clearly demonstrated the association between RA and a reduced bone mineral density, but none had examined directly the microarchitectural changes in humans using a technique such as microCT. In this study we have used this three-dimensional technique to determine whether the trabecular microstructure in transiliac biopsies of patients with RA differ from those from healthy donors. The results show a lower fraction of trabecular bone volume (BV/TV) and a lower average trabecular thickness (Tb.Th) in the specimens with RA in comparison with the controls, as well as

Table 2. Structural parameters of the patients with RA and their controls measured using microCT

	Parameter		RA (n=14)	Controls (n=14)	p*
ROI cylindrical (ROI_{CIL})	Volume of ROI (mm^3)	Total	23.55 ± 11,5	52.47 ± 53.47	
		Only bone	4.33 ± 2.51	13.29 ± 12.68	
	BV/TV_{CIL} (%)		18.92 ± 6.29	25.15 ± 7.46	0.024
	$Tb.Th_{CIL}$ (μm)		123.33 ± 16.91	136.46 ± 21.41	0.083
	$Th.Sp_{CIL}$ (μm)		593.84 ± 249.91	426.73 ± 99.72	0.028
	$Tb.N_{CIL}$ (μm^{-1})		1.52 ± 0.43	1.82 ± 0.31	0.051
	EV_{CIL}		3.15 ± 2.24	5.94 ± 2.42	0.004
ROI curved (ROI_{SPL})	Volume de ROI (mm^3)	Total	154.52 ± 108	154.52 ± 108	
		Only bone	20.90 ± 8.13	40.26 ± 26.4	
	BV/TV_{SPL} (%)		20.44 ± 6.22	27.45 ± 7.27	0.011
	$Tb.Th_{SPL}$ (μm)		130.04 ± 13.68	140.29 ± 13.97	0.060
	$Th.Sp_{SPL}$ (μm)		554.95 ± 203.51	393.03 ± 101.86	0.013
	$Tb.N_{SPL}$ (μm^{-1})		1.56 ± 0.40	1.95 ± 0.47	0.027
	EV_{SPL}		83.33 ± 2.66	6.92 ± 5.28	0.031

*Significant if $p < 0.05$

a higher average trabecular separation (Tb.Sp) for both models of ROI selection. With respect to the parameters for connectivity, the control specimens showed higher values of trabecular density and Euler volumes. These findings are consistent with the existence of an advanced trabecular osteoporosis in these patients free of treatment with steroids, and reflect an altered bone quality and poor trabecular connectivity related to the continuous inflammatory stimulation which occurs in RA.

It was decided to select two different ROIs (cylindrical and curved) for each specimen since in earlier publications there was no preference for either of the two types. The two ROI models were useful, but the curved outline achieved higher levels of statistical significance due to the inclusion of the entire trabecular volume (that is to say, the analysis was not restricted only to the core of the biopsy). This fact suggests that the central trabecular region of the cylindrical ROI does not adequately reflect the microstructural changes in osteoporosis, and that the peripheral trabecular regions should be considered in this type of study since they are also important when studying the microarchitecture.

However, our greatest limitation in this study was the small number of patients and controls, due to the inherent difficulty in obtaining bone biopsies. For this reason it is probable that the differences between the values of some microarchitectural parameters between the samples from patients and controls only show a statistical trend and not a clear statistical significance. This is one limitation already known in this type of study, due to the fact that this type of biopsy is difficult to obtain since patients do not tend to willingly accept invasive procedures such as a biopsy. Another significant limitation was the generation of the curved ROI based on a method of interpolation starting from a freehand selection in each of the cuts carried out. However, we believe that this is a relative problem, considering that it concerns a methodology necessary at the time to avoid the inclusion of cortical bone.

In conclusion, we believe that microCT is a relatively new imaging technique which permits a complete quantification of the trabecular microstructure, being more rapid than conventional histology and permitting a non-destructive examination of the bone specimen before the pathological analysis. Nevertheless, perhaps the most important of its limitations is that even though it allows the non-destructive examination of a bone specimen, it remains an invasive technique for the patient which therefore, in real life, does not allow diagnosis nor

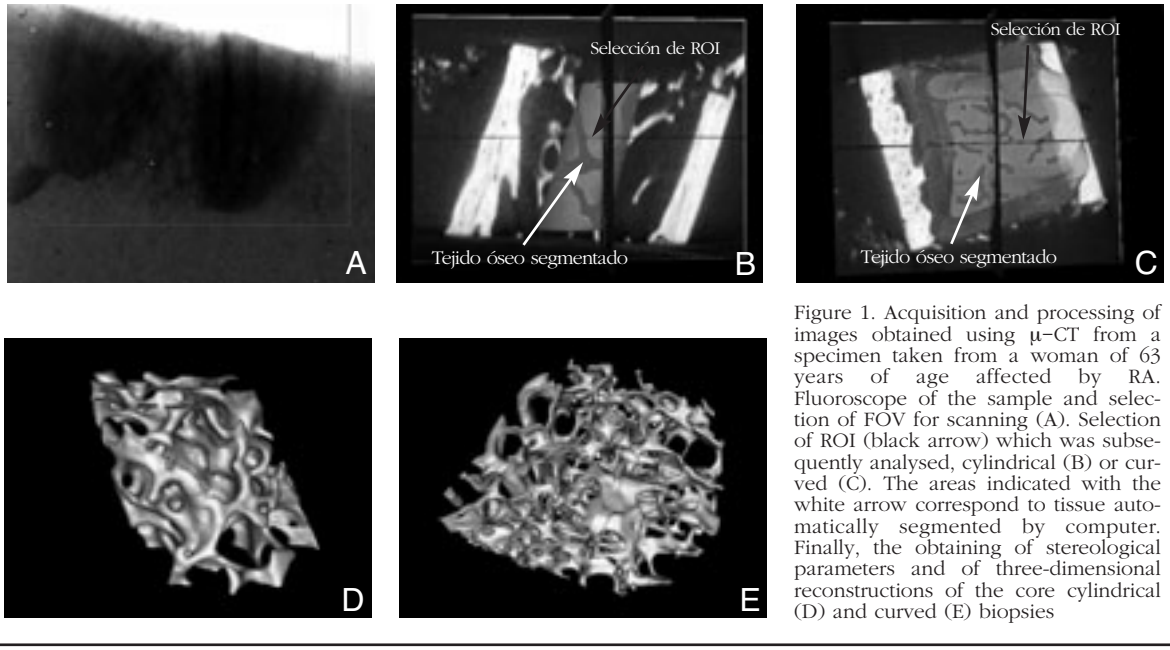

Figure 1. Acquisition and processing of images obtained using μ–CT from a specimen taken from a woman of 63 years of age affected by RA. Fluoroscope of the sample and selection of FOV for scanning (A). Selection of ROI (black arrow) which was subsequently analysed, cylindrical (B) or curved (C). The areas indicated with the white arrow correspond to tissue automatically segmented by computer. Finally, the obtaining of stereological parameters and of three-dimensional reconstructions of the core cylindrical (D) and curved (E) biopsies

post treatment follow-ups to be carried out in a routine way. Therefore we believe that an important role is played by a non-invasive technique such as p-QCT, which has been shown to distinguish, *in vivo*, between osteoporotic patients and healthy controls, to predict the risk of fracture in patients with osteoporosis, and which has demonstrated excellent correlation with results obtained by microCT[47-49]. The absence of specific studies around bone microarchitecture using p-QCT in adult patients with RA should also be mentioned.

Therefore, we have focused on the deterioration of bone microstructure in patients with RA, since: 1) they are patients who are accustomed to being treated with glucocorticoids, a fact which aggravates bone deterioration even more; and 2) the increase in the risk of fracture of the hip and/or vertebrae should be taken into account since when they occur they are a further aggravation for the patient who is already has limited functionality due to their underlying disease. Also, we believe that more studies will be required in the future due to the incidence in the population of bone fractures and their economic implications, and that these studies should be carried out using three-dimensional multiplanar techniques such as microCT or p-QCT, since both have been shown to characterise bone microarchitecture sufficiently well.

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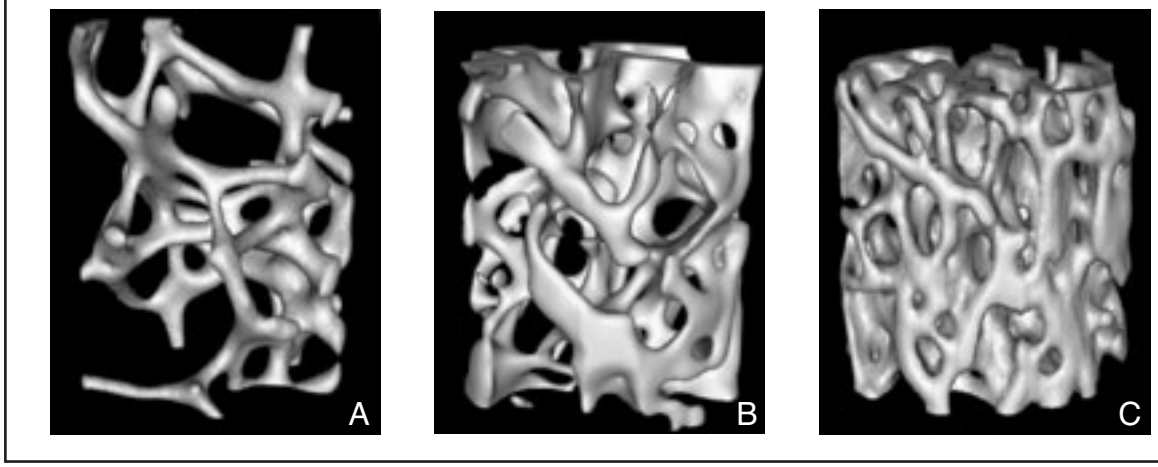
Figure 2. Trabecular microstrucutre of transiliac biopsies obtained by microCT with an isotropic resolution of 28 μm. A: cylindrical cores from patients with RA C: cylindrical core from a healthy control. The ROI volume selected was the same in the three cases. (A) Age: 68; BV/TV_{CIL}: 8.8%; $Tb.Th_{CIL}$: 118.43 μm; (B) Age: 54; BV/TV_{CIL}: 17.98%; $Tb.Th_{CIL}$: 124.59 μm; (C) Age: 38; BV/TV_{CIL}: 26.3%; $Tb.Th_{CIL}$: 137.56 μm



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Evaluation of the risedronate efficiency 75 mgs versus generic alendronate 70 mgs, in women with post-menopausal osteoporosis and previous vertebral fractures in Spain

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Summary

Introduction: The objective is to assess the cost-effectiveness of risedronate 75 mg 2 consecutive days/month vs generic alendronate 70 mg weekly, during one year in 75 years old females with post-menopausal osteoporosis and previous vertebral fracture.

Methods: A cost-effectiveness analysis under Health National System perspective has been developed to assess clinical (hip fracture prevention and quality adjusted life years gained) and economic consequences (€ 2010) during 5 years following one year treatment with both alternatives. Drug effect has been considered during the one year of drug administration. Epidemiology data and unitary costs were derived from Spanish literature.

Results: In a cohort of 1.000 females, (75 years old) with post-menopausal osteoporosis and vertebral fractures, risedronate 75 mg vs alendronate avoid 10 hip fractures, with 9.983€/hip fracture avoided cost. Aditional QALY gained are 4 with an incremental cost of 99,83€. Incremental cost-effectiveness ratio (ICER) is 24.957€ per QALY gained with risedronate 75 mg vs generic alendronate 70 mg.

Conclusion: In the treatment of females with post-menopausal osteoporosis and previous vertebral fracture, risedronate 75 mg 2 consecutive days/month compared to generic alendronate 70 mg weekly is an efficient strategy in Spain.

Key words: *Osteroporosis, Risedronate, Alendronate, Costs.*

Introduction

Osteoporosis constitutes a significant public health problem, with a great clinical and economic impact[1]. In Spain, 25% of women aged between 60 and 69 years, and 40% between 70 and 79 years, have osteoporosis[2].

A Spanish study carried out locally, found that the prevalence of vertebral fracture in people over 50 years varies between 17.4 and 24.6% depending on the radiological criteria used, this prevalence increasing with age. In fact the number of fractures practically doubles for each 10 years of age[3].

Within osteoporotic fractures, hip fractures are those with the strongest direct link to osteoporosis, due to their serious clinical consequences, their higher requirement for days of rehabilitation and costs of hospitalisation[4,5]. It is estimated that there are, globally, 1.6 million hip fractures annually, which could reach 4.5 million in the year 2050[5,6].

The biphosphonates are considered to be the medicines of first choice in the treatment and prevention of osteoporotic fractures[7], but a significant percentage of women with osteoporosis discontinue treatment, or do not adhere to it[8], due to the dosage, frequency of administration and the occurrence of adverse events. The discontinuation and lack of adherence to treatment are associated with an increase in the risk of fractures[9-11] and in health costs[12]. The relationship between the cost of treatments for osteoporosis and the results obtained by their use (number of fractures avoided and survival in quality adjusted life years) is a relevant factor in taking decisions in clinical practice[13].

The aim of this evaluation has been to estimate, from the health perspective, the Incremental cost-effectiveness *ratio* (ICER) relationship between the biphosphonates 75 mg risedronate for 2 consecutive days/month and 70 mg generic alendronate weekly, administered for a year, in women over 75 years of age with OPM and PVF.

Methods

Patients

The profile of the population analysed in this economic evaluation is: women of 75 years of age, with a bone mineral density of ≤ -2.5 SD (T-score < -2.5) and with FVP.

The case base of the analysis centres on a hypothetical cohort of 1,000 patients, although a sensitivity analysis was also carried out which showed the results applied to the female Spanish population from 65 to 80[14], to which was applied the rate of osteoporosis[15], which were weighted into 8 different strata due to the presence or not of PVF[16].

Compared treatments

The alternative therapies compared were: 75 mg risedronate for 2 days consecutively/month for a year, against 70 mg generic alendronate weekly over a year.

Effectiveness of the medicines

The evaluation of the efficiency of medicines requires the estimation of their effectiveness. In this case, the data on effectiveness are obtained from a sub-analysis of the REAL (the RisedronatE and ALendronate study) study[17]. The REAL study[18], is a retrospective observational cohort study in which are compared the effectiveness of weekly administration of alendronate with risedronate in the reduction of vertebral and hip fractures. The effectiveness of generic alendronate included in the economic evaluation was considered to be equivalent to the original alendronate which was administered in the REAL study[18].

Economic analysis

The calculation of the efficiency comparison between risedronate and alendronate was carried out by means of the ICER[19] relationship between the two alternatives using the following formula:

$$ICER = \frac{\text{COST OF RISEDRONATE} - \text{COST OF ALENDRONATE}}{\text{EFECTIVENESS OF RISEDRONATE} - \text{EFECTIVENESS OF ALENDRONATE}}$$

The costs of each of the therapies include the total costs of treatment and of fractures.

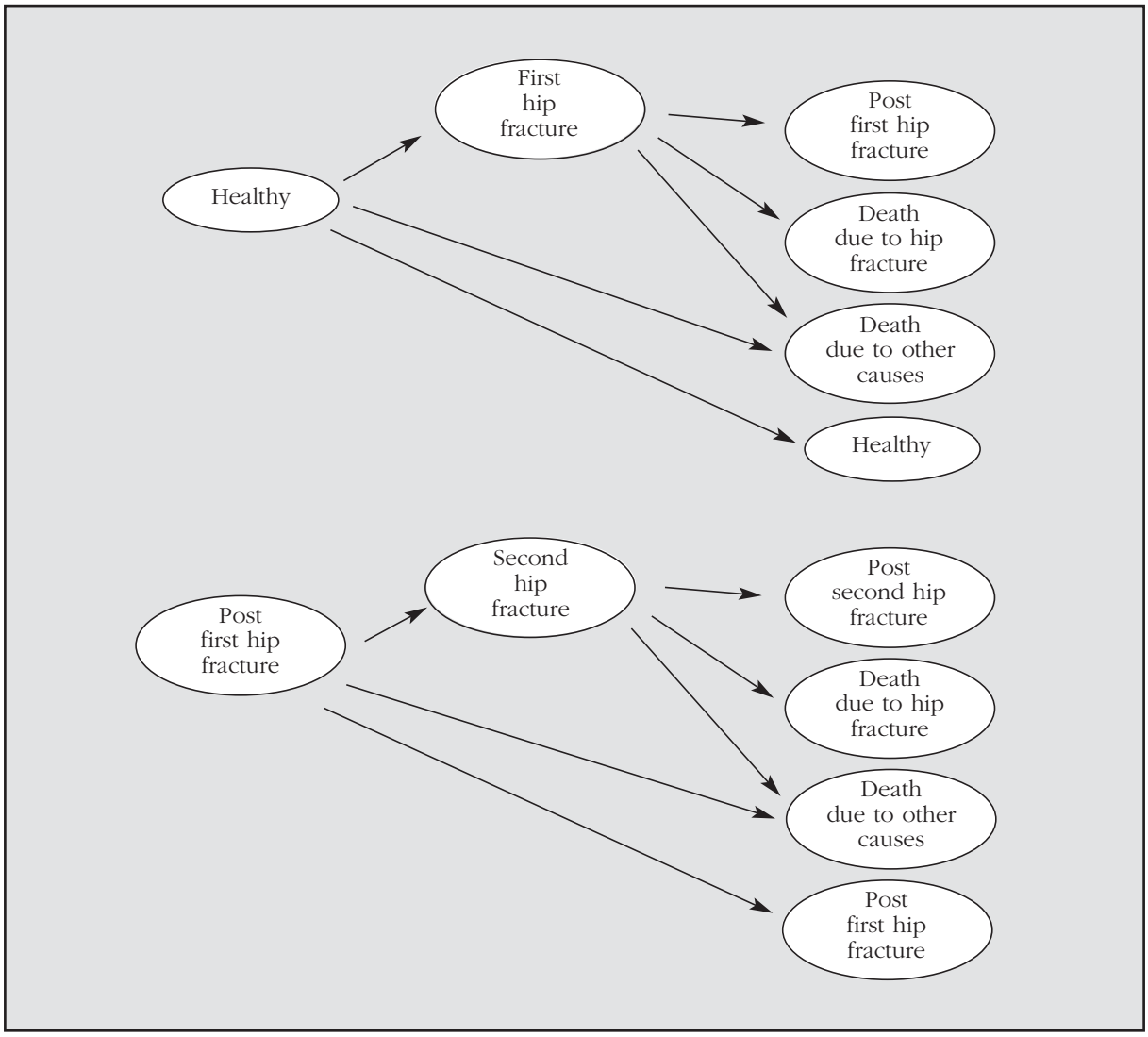
To measure the effectiveness, the number of hip fractures avoided (using the incidence of fractures according to age and the efficacy of each medicine), and life years for quality adjusted life years (QALY) gained by each alternative, was used.

To determine whether the adoption of an alternative has a reasonable increased cost in relation to the increase in effectiveness achieved, in the cost-utility analysis the maximum efficiency or cost threshold was defined as that cost which it was prepared to be paid for each additional unit of effectiveness achieved with one therapeutic option compared with another. In this study the efficiency threshold was considered to be 38,000€ per quality adjusted life year. This value was obtained by updating to the year 2010, using the general consumer price index[20], the normally accepted threshold value for economic evaluation in Spain, (30,000€ per year of life gained in the year 2000)[21], and which agrees with the recommendations for Spain of other authors which place the threshold in a range between 30,000 and 45,000€ for each quality adjusted life year gained[22]. In addition, a threshold has recently been established in a series of countries, among which Spain is included, for health interventions indicated for the treatment of osteoporosis[23]. This threshold, specific for the interpretation of results in osteoporosis in Spain, has been positioned at 47,000US$, equivalent to 34,768€, (using an exchange rate of 1 euro = 1.3518 US$, at 15th May 2009; ECB)[24].

Model

The economic analysis of risedronate compared with alendronate was carried out using Markov's model, which had allowed the estimation of the long term (5 year) clinical and economic consequences of the administration of the two treatments compared with a hypothetical cohort of 1,000 patients.

Figure 1. Description of Markov model



The Markov models are characterised by their requirement for the definition of different states of heath between which the patients may move. The model used in this study includes 4 different health states:

-Healthy (not having suffered any hip fractures).

-Hip fracture (first or second).

-Post-fracture of the hip (first or second).

-Death (whether due to hip fracture, or for other reasons).

Figure 1 shows a schematic representation of the model used.

Among the premises contained in the model, notable is the fact that discontinuations in treatment have not been taken into account, which means that the pharmacological cost of the therapy evaluated refers to the pharmacological cost of a complete year of treatment for each patient. In addition, any residual effects of the drugs have not been considered, rather, it has been assumed that the medicines only had an effect during the year of administration.

Estimation of costs

All the costs included in the analysis are given in euros (€, at 2010 value). The evaluation was carried out from the perspective of the Spanish National Health System, which means that only the direct health costs associated with the therapies have been considered.

The pharmacological cost was calculated from the retail cost plus VAT of the medicines, for generic alendronate, taking into account the stipulations of the Law of Royal Decree 4/2010[25]. The cost of hip fractures was obtained from the literature[26].

Table 1 includes the values of the relevant parameters and the unit costs used in the analysis.

In agreement with current recommendations[27] a discount rate of 3% has been applied to the costs and benefits.

Sensitivity analysis

The sensitivity analysis to confirm the stability of the model was carried out, having:

-Obtained results for the Spanish female population of between 65 and 80 years of age (from 8 different strata), with OPM, weighted with/without PVF, and taking into account mortality due to hip fracture[28].

-Considered the residual efficacy during the year following the end of the year of treatment.

Results

The administration of 75 mg risedronate for 2 consecutive days/month for a year in a cohort of 1,000 women of 75 years of age with OPM and PVF avoided 10 more hip fractures than the administration of 70 mg generic alendronate weekly for a year.

The cost of each additional hip fracture avoided with 75 mg risedronate vs alendronate is 9,983€.

In the cohort of 1,000 women 2,919 QALYs were achieved with 75 mg risedronate, compared with 2,915 with alendronate, which means an additional gain of 4 QALYs with the risedronate therapy, with a total increased cost of 99.83€ . The cost for each gain in QALY with risedronate as against alendronate is 24,957.50€ (Table 2).

The results in the Spanish population females of between 65 and 80 years of age with OPM, aggregated and weighted as a function of 8 different strata, with or without PVF, show that the increase in cost per QALY gained with 75 mg risedronate for 2 consecutive days/months is cost-effective in comparison with 70 mg generic alendronate weekly, being 32,827 per QALY.

The cost/additional QALY of risedronate, as against alendronate, is 13,374€/QALY in the population with PVF and 41.481€/QALY in the population without PVF.

When the residual effect of the therapies after the end of the year of treatment is taken into consideration, the cost per hip fracture avoided with 75 mg risedronate for 2 consecutive days/month as against 70 mg generic alendronate weekly is 3,266€ and the cost per QALY gained is 8,065€/QALY with risedronate vs alendronate.

The results in the Spanish population between 65 and 80 years of age, weighted as a function of 8 different strata, with or without PVF, taking into consideration the existence of residual efficacy, estimate that the cost/additional hip fracture avoided is 12,241€ and the cost/QALY is 25,488€/additional QALY with 75 mg risedronate vs generic alendronate.

Table 3 shows the detailed results of all the sensitivity analyses carried out.

Discussion

Osteoporosis, in recent years, has consolidated its position as one of the major socio-health problems in Spain, both due to its high prevalence and for the economic costs which it generates.

Various studies have provided evidence that therapy with risedronate reduces the risk of fracture in women with osteoporosis[29-32], even in the first 6 months of treatment, giving it an added advantage over other biphosphonates[18].

In women over 75 years of age with OPM and PVF therapy with 75 mg risedronate for two consecutive days/month is more effective than therapy with 70 mg generic alendronate weekly, since more hip fractures are avoided and the patient benefits from a greater number of quality adjusted life years.

The efficiency of the treatments for osteoporosis, that is to say, the relationship between their cost and the health benefits resulting from their use (reduction in risk and number of fractures avoided, and survival in quality adjusted life years), should be a key factor in taking decisions in normal clinical practice.

In comparison with 70 mg generic alendronate weekly, 75 mg risedronate for 2 consecutive days/month, is an efficient therapy (cost-effective alternative). The study was based on an efficiency threshold of 38,000 euros per quality adjusted life year gained, derived by updating threshold of Sacristan et al. in values for 2009, of 38,220 euros[21], and the average of the threshold range established by De Cock et al., of 37,500 euros[22]. These values are close to the threshold determined for Spain in the treatment of osteoporosis of 34,768 euros[22]. The authors of this international study recommend the use of this threshold in the pharmacotherapeutic guides, in combination with algorithms for the prediction of risk of fractures, to be used in taking decisions with the aim of carrying out an efficient selection of patients suitable for treatment. The efficiency threshold varied between the different countries as a function of the availability of funding for each quality adjusted life year, the costs associated with fractures and the costs of health interventions used to reduce the risk of fracture[23].

The results, aggregated and weighted in 8 strata representative of women of between 65 and 80 years of age according to the rate of osteoporosis and the incidence of PVF in Spain, confirmed the robustness and consistency of the results.

When the residual effects of the therapies at the end of the year of treatment are taken into consideration, the cost-utility of 75 mg risedronate vs alendronate is only 8,065€/QALY and continues to be below the accepted efficiency threshold.

This analysis considers treatments of a complete year for each of the therapies. Adherence, with its two facets: compliance and persistence, is a key factor for being able to extrapolate the efficacy of the biphosphonates demonstrated in the clinical trials into clinical practice[33,34], since the inadequate adherence to treatment has been associated with increase of 17% in the risk of fracture 10 and even 37% in the risk of hospitalisation for any cause[35].

In addition to deteriorations in the state of health, poor compliance and low persistence are also associated with a reduction in the efficiency of the therapies[36]. Adequate compliance, with rates from 50% and mainly of 75%[37], are directly related to changes in bone mineral density in those patients, which as an important marker for bone turnover, is considered a good predictor for the reduction in risk of fractures. Adherence is therefore a challenge for clinicians involved in the treatment of osteoporosis[32]. Those medicines with the simplest and most time-spaced dosage regimens are better accepted by patients, ensuring greater compliance with the therapies[38,39].

Table 1. Principal variables of case base of model

Parameter	Value	Reference
Epidemiological data		
Rate of osteoporosis in Spain		(2)
60-69 years	24.29%	
70-80 years	40.00%	
Incidence of hip fractures (expressed per 10,000 inhabitants)		(15)
50-69 years	13.8	
70-74 years	30.5	
75-79 years	65.1	
80-84 years	124.4	
85-89 years	213.2	
90-94 years	302.2	
95-100 years	432.8	
Prevalence of vertebral fracture		(16)
50-54 years	11.50%	
55-59 years	14.60%	
60-64 years	16.80%	
65-69 years	23.50%	
70-74 years	27.20%	
>75 years	34.80%	
Mortality in year following a hip fracture	Relative risk	(28)
50-54 years	8.26	
55-59 years	6.69	
60-64 years	5.44	
65-69 years	4.41	
70-74 years	3.58	
75-79 years	2.74	
80-84 years	2.09	
85-89 years	1.74	
>90 years	1.46	
Effectiveness (reduction in hip fractures)		
Risedronate	50%	(18)
Alendronate	10%	(18)
Cost data		
Hip fracture (first year)	7,438€	(26)
Hip fracture (successive years)	1,487.66€	(26)
75 mg risedronate – retail cost, plus VAT/day	1.1553€	
70 mg alendronate – retail cost, plus VAT/day	0.5507€	(25)

Table 2. Results of the cost/utility analysis in the case base (women older than 75 years of age with postmenopausal osteoporosis and previous vertebral fracture)

	Risedronate	Alendronate	Risedronate vs Alendronate
Hip fractures (per 1,000 women)	99	109	10
QALY (per 1,000 women)	2,919	2,915	4
Total costs (€/per 1,000 women)	1,354,200	1,254,370	99,83
Avoided cost per hip fracture (€)			9,983
Cost per QALY gained (€)			24,957.50

QALY: quality adjusted life year

The premise of total adherence to treatment adopted in the current analysis makes a conservative assumption for risedronate, since its monthly administration has demonstrated significant improvements with respect to the weekly administration of alendronate in adherence to treatment with biphosphonates in women with OPM, with a compliance of 74% with monthly risedronate, as opposed to 66% with weekly therapy with alendronate[40].

The consideration of a higher adherence to risedronate therapy would not have been able to have been extrapolated from the effectiveness data from the REAL study[18], which excluded the same proportion (41%) of patients in both treatment groups for not complying with the minimum period for adherence established in the trial's protocols (3 months).

The efficiency of 35 mg risedronate as opposed to 70 mg of generic alendronate, both administered weekly, has previously been established in the Spanish environment[41]. Possible methodological differences, as well as the reference years for the costs, and differences in medical practice are a barrier to direct comparisons with estimates of efficiency obtained in other countries. Even so, illustratively, it has been found that monthly therapy with risedronate is considered to be cost-effective in comparison with weekly alendronate, by other authors, with estimates of 9,476$/QALY (US)[42].

The model used in this work, informed by the REAL study, has been used in economic evaluations of risedronate vs alendronate in other environments with women over 65 years of age, reaching similar conclusions in terms of efficiency to those we obtained in our analysis, with values of 3,877$/additional QALYs obtained by risedronate compared with alendronate in Canada (values for 2006)[43] and dominant in studies carried out in Italy (values for 2006)[44], and Germany (values for 2008)[45].

As limitations and possible bias in this economic evaluation, should be mentioned the inherent theoretical nature of any type of modelling which, on occasions, does not give results which reflect clinical practice.

The validity of an economic model is conditional on the quality of the data on which it is based. In our case, the principal source of information was the REAL study, a retrospective observational study with a level of data lower than that of a clinical trial, due to the possible existence of differences in the characteristics of the cohorts which are compared. However, the use of data from randomised clinical trials is also arguable, due to the rigidity of the inclusion criteria which do not make them representative of normal clinical practice, principally when data from multinational studies are used in economic evaluations at a local level[46].

In conclusion, our results demonstrate the efficiency of therapy with 75 mg risedronate for 2 consecutive days/monthly compared with 70 mg generic alendronate weekly in the treatment of women over 75 years of age with OPM in Spain.

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Table 3. Results of the analyses of sensibility realized

Without residual efficacy			**Cost/QALY**
	Spanish population (a) (b)		
		Results (population with PVF) (c)	
		Cost/avoided hip fracture (with FVP)	6,322€
		Cost/QALY (with PVF)	13,374€
		Results (population without PVF)	
		Cost/avoided hip fracture (without FVP)	29,997€
		Cost/QALY (without FVP)	41,481€
		Weighted results with/without PVF	
		Cost/avoided hip fracture	22,707€
		Cost/QALY	32,827€
With residual efficacy			
	Hypothetical cohort (1,000 women > 75 years of age with PVF)		
		Cost/avoided hip fracture	3,226€
		Cost/QALY	8,065€
	Spanish population (a) (b)		
		Results (population with PVF) (c)	
		Cost/avoided hip fracture (with FVP)	3,089€
		Cost/QALY (with PVF)	6,503€
		Results (population without PVF)	
		Cost/avoided hip fracture (without FVP)	16,313€
		Cost/QALY (without FVP)	33,935€
		Weighted results with/without PVF	
		Cost/avoided hip fracture	12,241€
		Cost/QALY	25,488€

(a): female population in 2009[14]
(b): rate of osteoporosis[2]
(c): rate of incidence of previous vertebral fracture in women[16]
QALY: quality adjusted life years
PVF: previous vertebral fracture

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Preliminary study of osteoblasts in peripheral blood in the population of infants and adolescents*

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Summary
The presence of osteoporosis in adult life is conditional on the adequate development and formation of bone during growth in infancy and adolescence and the successive loss which occurs throughout life. Knowledge regarding bone tissue cells and their precursors in stages of growth is scarce, given the difficulties in obtaining samples of this tissue. Recent studies suggest a method of obtaining osteoblast line cells from peripheral blood. The main objective of this work has been to quantify the osteoblast line cells in the peripheral blood of infants and adolescents, as well as noting any possible differences according to the stage of growth.
38 subjects were studied, 16 children (between 4 and 12 years of age) and 12 adolescents (aged between 12 and 18 years). Osteoblast precursor cells in peripheral blood were analysed using the flow cytometry technique. The preliminary results show higher levels of preosteoblastic cells in the youngest age group: 4.17% ± 0.92 vs 2.03% ± 0.48, p= 0.021. There is a negative correlation between the percentage of pre-osteoblastic cells and age r= -0.488 and weight r= -0.530, p< 0.05. In summary, this technique allows us to quantify preosteoblasts in peripheral blood, and we show that they have a higher percentage, the lower the age, during the period of infancy and adolescence.

Key words: *Osteoblasts, Peripheral blood, Infants, Adolescents.*

Introduction

Osteoporosis is a disease of adult life characterised by the presence of non-traumatic fractures, and among the determining factors of this lower bone resistance one of the most important is the lower bone mineral density of the skeleton. Bone mass develops over a lifetime. During the period of growth bone is formed until it reaches a peak in young adulthood, and subsequently, from 35-40 years of age it is physiology which produces a decrease in bone mass. Therefore it is possible to arrive at a significant reduction in bone mineral density due to two fundamental reasons: low levels of bone formation during the growth period, or excessive bone loss in adulthood[1].

Up to the present time, the majority of the research studies have been centred on the adult stage of bone loss, and little is known about its development and formation at an intimate level during the growth stage of infancy and adolescence, given that for this is would be necessary to carry out bone biopsies, which means carrying out a process which is painfully invasive and not often allowed at this stage of life. However, the physiological knowledge of bone metabolism during the period of growth is of great importance, given that on this depends the bone "capital" which an individual ends up acquiring.

Currently we know that the osteoblast and osteoclast precursor cells, in addition to residing in the bone medulla, are capable of being mobilised through the peripheral blood to be directed to zones in which there is active bone remodelling[2]. The problem is that with the techniques which were being used, the number of osteoblast precursors circulating in the blood described to date, has always been very low and difficult to detect[3,4]. Kosha et al.[2] suggested the possibility of using the technique of flow cytometry, with antibodies for proteins specific to bone (osteocalcin and alkaline phosphatase) to better identify the pre-osteoblast cells circulating in the peripheral blood, and in addition, that if these osteoblast cells have a physiological role in the formation of bone, it is expected that their concentration increases in conditions of bone formation[5,6].

Our principal aim has been to quantify the osteoblast line cells in peripheral blood in healthy children and adolescents, as well as understanding possible differences according to stages of growth and gender.

Material and method

Group studied

We studied 38 subjects (whose parents/guardians had previously given their consent, once informed about the study) divided into two groups according to age: group A (4-12 years), 16 children (7 female, 9 male); group B (13-18 years), 12 adolescents (2 female, 10 male). The period of study was during the year 2008 and the subjects came from the follow up clinic for healthy children or from the ophthalmic service of the Virgen Macarena University Hospital. For all subjects, after carrying out a questionnaire on current and previous state of health, the following actions were carried out: determination of height, weight and body mass index (BMI). In addition, a sample of peripheral blood was taken to determine levels of calcium and bone alkaline phosphatase (BAP) and the levels of pre-osteoblast cells circulating in the blood.

None of the subjects selected had diseases related to bone metabolism, or were taking medicine.

Extraction of mononuclear cells

To obtain the mononuclear cells we diluted the total blood with PBS (1:1). The diluted blood was added to a Ficoll-Hypaque solution 1:2 and centrifuged (1,250 xg, 20 mins 4º C). The ring formed at the interphase contains the mononuclear cells, which are collected and washed with PBS. Subsequently we carried out hypo-osmotic stress with distilled and deionised H_2O to lyse the contaminated red globules, and NaCl at 1.8% was added to re-establish the isomolarity, then after washing with PBS the cells were counted with a haemocytometer, determining their viability by exclusion with Tripan Blue.

Flow cytometry

For the cytometric analysis we incubated the cells with the anti-osteocalcin-ficoeritrine antibody and we selected the reading channel corresponding to the light emitted. The positive population will be identified as cells which express specific levels of fluorescent activity compared with the non-specific autofluorescence of the control isotopes. The cells identified will be expressed as a percentage of the "gate" selected initially corresponding to the area of the lymphocytes-monocytes.

Collection and analysis of data

All the experiments were repeated three times, accepting the value of the arithmetical mean of the repeated exercises. The quantitative values were expressed as an average ± SD.

The software package SPSS v17.0 was used for the management of the statistical results. The ANOVA test was used to compare quantitative variables and Pearson's correlation coefficient for correlation between variables.

In all cases, the level of significance was considered to be 5% ($p<0.05$).

Results

We would like to make it clear that we present here preliminary results, given that at present we are continuing to increase the sample size for all the groups. The average age of the group of children was 9.05 ± 3 years, of whom 7 were girls and 9 boys, of comparable ages. In the group of adolescents, the average age was 14.16 ± 1.2 years, with 2 girls and 10 boys. The characteristics of both groups are given in Table 1. We did not observe significant differences in the vales if BMI, calcium or alkaline phosphatase between the two groups studied, or within the same group, between genders.

The osteoblast line cells were quantified according to the percentage of cells positive for osteocalcin circulating in the peripheral blood, using flow cytometry.

The group of children between 4 and 12 had a significantly higher percentage of pre-osteoblast cells (pre-OB) in their peripheral blood (4.17% ± 0.92) than that found in the adolescents over 12 years of age (2.03% ± 0.48 p= 0.021) (Figure 1).

In analysing the data by sex we did not find differences within the same group. Specifically, in group A the levels of pre-OB were slightly higher in males (4.5% ± 0.9) than in the females (3.34 ± 0.9), even though this difference was not statistically significant. And for group B, the quantity of pre-OB cells was also very similar (1.95 ± 1.96 in boys; 1.06% ± 1.03 in girls) (Figure 2).

We have confirmed the fact that there is a negative correlation between the percentage of osteocalcin-positive pre-osteoblast cells circulating in the peripheral blood, and age (r= -0.488 and p= 0.005), as well as between the number and body weight (r= -0.530, p= 0.035).

Table 1. Anthropomorphic characteristics and blood biochemistry parameters related to calcium metabolism of the groups studied. The results are expressed as an average ± standard deviation

Variables	Group A (4-12 years) n=16	Group B (13-16 years) n=12
Age (years)	9.05 ± 3	14.16 ± 1.2
Size (cm)	136.5 ± 20.5	158.5 ± 9.1
Weight (Kg)	39.26 ± 18	53.5 ± 10.7
BMI (Kg/m^2)	19.69 ± 4.5	21.1 ± 2.55
BOA (U/L)	550 ± 181	576 ± 79.7
Calcium (mg/dl)	9.7 ± 0.38	9.8 ± 0.37

BMI: body mass index
BOA: bone alkaline phosphatase

Discussion

In this work we show that, using flow cytometry techniques, it is possible to quantify osteoblast line cells in the population of young people. In adults it had been indicated that the quantity of these cells was practically undetectable, while increases were seen in patients with bone fractures[3,4]. We consider that the availability of this powerful technique enables us to evaluate in children and adolescents highly important factors related to bone metabolism, an evaluation which would be difficult to carry out in any other way, due to the need to analyse samples obtained through bone biopsies.

We have observed that the number of pre-osteoblasts in peripheral blood is dependent on age, reaching higher values in younger children, ≤ 12 years of age, compared with adolescents of between 12 and 16 years of age. To us, these results could indicate that the more the pre-osteoblast cells in peripheral blood increase the more active is the process of bone remodelling. Children in the most rapid phase of growth have a correspondingly high level of bone remodelling activity, and therefore, a higher number of osteoblast line cells in circulation. Due to this same mechanism, in patients with bone fractures, in whom there is a higher level of bone formation in the area of the skeleton in which the bone callus is formed, has been found a higher percentage of pre-osteoblasts in peripheral circulation, compared with values found in healthy subjects[5].

The number of pre-osteoblast cells in peripheral blood does not only correlate with age, but we have also observed a negative correlation with weight (r= -0.530 p= 0.035). These results may concur because both osteoblasts and adipocytes derive from the same pluripotent mother cells and, as such, a higher differentiation in one direction may be accompanied by a lower proportion of the other cells.

In the developmental stages of an individual, in which the activity of bone formation is higher than the formation of adipose tissue, as is usual in growth during infancy, it is predominantly the cellular signals for the formation and maturation of osteoblasts (an increase in the canonical Wnt signalling pathway with over-expression of Runx2) which need to be activated at the expense of the genesis of adipocytes (reduction in PPARγ2). These data need to be confirmed, but they would relate to a higher level of formation of adipocytes in the bone medulla at the expense of a lower number of osteoblasts, which has been confirmed in the adult population[7].

We found no differences in the quantity of pre-osteoblasts in peripheral blood by gender. During infancy it is known that boys and girls increase bone mass as they grow in height in a similar way. It is from adolescence, under the influence of sexual hormones, when the curves for the increase in BMD are seen to separate, with males achieving higher values[8]. In our study few subjects at this stage were evaluated, and those were mainly female. It is necessary to study a larger population of adolescent girls to reach conclusions about this.

Figure 1. Average value of osteocalcin positive cells for group A (4-12 years) and for group B (13-18 years)

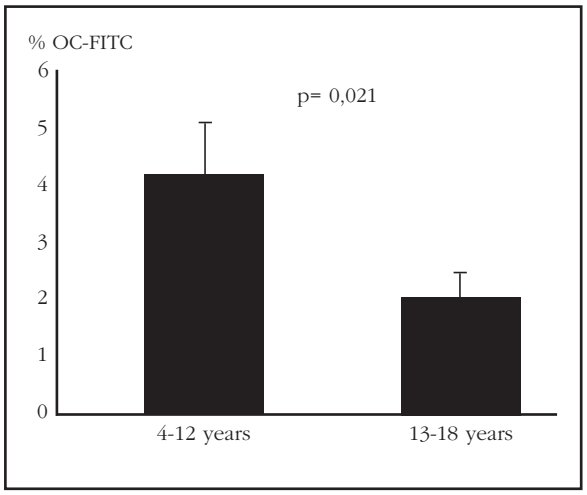
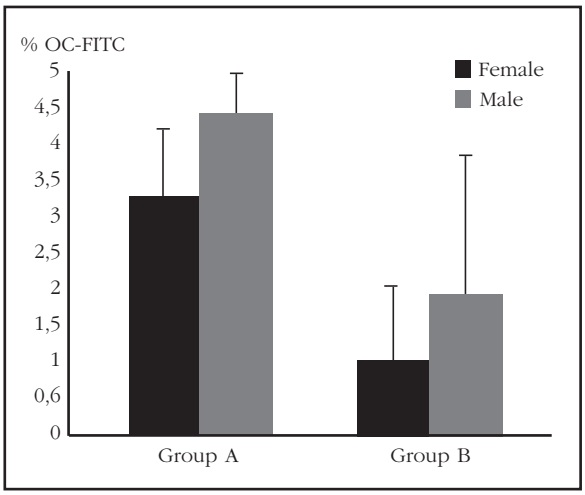


Figure 2. Average value for groups A (4-12 years) and B (13-18 years) separated by sex



We can definitively conclude, despite the small sample size, that by utilising the flow cytometry technique and using osteoblast cell markers, such as osteocalcin, it is possible to quantify a valid percentage of osteoblast line cells in peripheral blood in healthy children and adolescents. In addition, we show that there is a higher percentage of these cells in smaller children, in whom bone formation is more intense, and in inverse relation to body weight.

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Treatment of Paget's disease of bone

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Introduction

Paget's disease of bone (PDB) is a chronic and focussed skeletal disorder, whose cause is unknown. The disease is located in the osteoclasts, which increase in number, size and activity. Bone turnover accelerates, with an increase in bone resorption, followed by excessive and disorganised formation. The result is bone which is not laminar (plexiform bone) highly vascularised, increased in volume, less compact and more susceptible to fracture or deformation. It is usually diagnosed at over 60 years of age, being infrequent below 40 years of age. It slightly predominates in males. It is the most common metabolic bone disease after osteoporosis[1].

It is considered to be a multifactorial disease with the involvement of environmental and genetic factors.

Its main clinical manifestations are bone deformity and pain. During its evolution various complications may appear, the most frequent being degenerative arthropathy in its vicinity, neurological changes due to compression, fractures, cardiac pathology, disorders of the metabolism and of bone remodelling.

The diagnosis is based on clinical manifestations, raised levels of biochemical markers for bone remodelling (essentially, alkaline phosphatase – AP) and radiology.

There is no curative treatment, but the antiresorptives, especially the diphosphonates, are efficacious in controlling the activity and progression of the disease. The therapeutic objectives are to eliminate bone pain, normalise bone remodelling, re-establish normal bone structure and prevent recurrence and complications.

Indications

The treatment of PDB can be divided into symptomatic treatment and specific treatment.

Symptomatic treatment is intended, primarily, to eliminate pain. Simple analgesics, non-steroidal anti-inflammatories, opioids and low doses of tricyclical antidepressives control the pain. In addition, rehabilitation, and mobility aids, such as walking sticks, improve the quality of life of the patient.

In the case of fracture, deformity or pagetic arthropathy surgery may be indicated.

The indications for establishing a specific treatment for the disease are not universally accepted due to the absence of evidence from the controlled clinical trials that medical treatment not only suppresses markers for bone remodelling, but also reduces reported complications.

There are relative and absolute indications, and it is always necessary to take into account the age of the patient, their life expectancy and their clinical state. The specific treatment is aimed at suppressing osteoclast activity in the pagetic lesions.

Salmon calictonin has been used for over 30 years. However, clinical and biochemical remission in PDB is exceptional, since at 6 months a plateau is reached and on suspending the drug a progressive increase in biochemical markers is observed, and nowadays it has been replaced by other drugs.

Since the introduction of the diphosphonates these have assumed a principal role in the treatment of this disease. The drugs used nowadays are aminated diphosphonates, which have a much more intense and much more prolonged antiresorptive action. They slow osteoclast activity to normal levels, are easy to administer and have an acceptable level of tolerance. They do not alter mineralisation, and normalise bone structure at a histological level.

The indications for the treatment of PDB have been listed in various publications[2-11]. They differ from one author to another, but a number of absolute indications have been established, which are not disputed, for the initiation of treatment. On the other hand, there is a series of **relative indications** which are the cause of major discussions, since they are based on criteria which are not supported by sufficient scientific evidence. The clearest and most discussed example is the active asymptomatic disease shown only by biochemical markers or by imaging techniques, in which the treatment is intended to achieve control of the osteoclast activity, and of the disease, as well as avoiding its complications. However, there is no scientific evidence which indicates that their appearance is avoided.

However, treatment in those asymptomatic patients with normal biochemical markers and normal bone gammagraphy, would not be indicated, although radiology shows lesions compatible with PDB[2].

The **absolute indications** established for the treatment of PDB are:

1. In those cases in which the symptoms are caused by the disease, such as: primary bone pain caused by the disease, or due to a pathological fracture, pagetic radiculopathy, arthropathy due to lesions in the neighbouring joints, deafness, neurological compression (especially the medullar) or other neurological symptoms associated with PDB.

2. In the case of programmed orthopaedic surgery on a pagetic bone, in order to minimise bleeding in the active disease.

3. To avoid the hypercalcemia which may occur during prolonged immobilisation.

4. Another indication, for many authors, would be to diminish its local progression and reduce the risk of future complications in those patients with the active asymptomatic disease, and in whom the location of the disease, and its degree of metabolic hyperactivity, may carry a risk of progression and complications.

There is indirect evidence that aggressive treatment of PDB is associated with the prevention of progression, and a reduction in the risk, of future complications. This evidence is:

1. Failures in the treatment of the disease are associated with future destruction of bone and the progression of deformities.

2. Efficacious treatments are associated with the normal restoration of new bone deposits. In addition, a study has shown that facial and cranial deformities improve after treatment.

Hence, in the light of these findings, various authors conclude that good clinical practice would include treatment both symptomatic patients, whose alterations may respond to a reduction in abnormal remodelled bone, as well as asymptomatic patients in whom the disease is active which may happen with future complications.

In 2008, Devogelaer et al.[5], produced a consensus document in which the indications for the treatment of asymptomatic PDB are presented. These indications are:

1. Age of diagnosis before 50 years of age.

2. Location of the bone lesions: in limbs, near the joints, due to the risk of secondary arthrosis; lytic lesions, due to the risk of fracture; in the hip; in the cervical and thoracic spine, due to the risk of neurological complications, spinal stenosis or pagetic steal syndrome; in the cranium, especially locations at its base, due to the risk of loss of hearing and/or other neurological complications.

3. Raised levels of total AP, greater than twice the upper limit for normality.

4. Programmed orthopaedic intervention on pagetic bone.

In 2002 Selby et al.[3], established grades of recommendation for the treatment of symptoms of PDB, according to the level of the evidence which supports it. Thus:

1. Bone pain is a clear indication for treatment (Grade A)

2. Fracture is a complication of the disease. Treatment solely to reduce the risk of fracture, or after a fracture, to improve its repair, is not indicated (Grade C).

3. The effect of antipagetic therapy on bone deformity is not clear (Grade C). However, the use of diphosphonates may be justified in facial deformities due to the disease (Grade B).

4. Different studies have suggested that the diphosphonates improve the osteolytic lesions in PDB. However, the clinical significance is not clear, and no specific recommendations are made for the treatment in osteolytic disease in the absence of other indications for treatment (Grade C).

5. In the prevention of arthrosis, whose presence is increased in PDB, there is no evidence that the treatment prevents its development or progression (Grade C).

6. A very common complication of the disease is deafness, but the effect of antipagetic treatment in the development and progression of deafness is not totally clear. In patients with pagetic affectation at the base of the cranium, treatment would be considered to minimise the risk of loss of hearing (Grade B).

7. The effect of the diphosphonates on the quality of life of patients with PDB has only been evaluated in two studies which did not show statistically significant differences (Grade C).

8. Medullar compression is a relatively rare complication of PDB. Various studies have been published which show that calcitonin and diphosphonates improve neurological function in these patients.

9. It has been suggested that antipagetic treatment may help to reduce bleeding in patients undergoing surgery in pagetic bone. However, this has never been demonstrated in controlled clinical trials (Grade C).

10. A rare complication of PDB is hypercalcemia due to the combination of an increase in remodelled bone and immobility.

11. In terms of sarcoma, there is no scientific evidence that treatment of the disease diminishes its development or progression in PDB (Grade C).

12. Finally, with reference to treatment of PDB in young patients, some experts warn that they should always receive treatment, independently of any other indications. However, there is no scientific evidence to support this opinion (Grade C).

Drugs used in the treatment of PDB

General characteristics

The drugs most used, and of first choice, are the diphosphonates. Their use in PDB began in 1970, and with the appearance of the aminated diphosphonates the therapeutic response has been stronger. They are synthetic analogues of the pyrophosphates and are characterised by their high antiresorptive power in the bone remodelling cycle. Their accumulation in the bone contributes to the maintenance of the reduction in markers for bone turnover for years.

Physical-chemical properties:

All the diphosphonates share a common chemical structure in which a carbon atom is bonded to two phosphate groups (P-C-P), whose negative charge explains its affinity to bone tissue. The power of its action comes from the lateral chains united with a common nucleus, and it has been demonstrated that the presence of nitrogenated compounds in these lateral chains give it its strong activity. Its absorption when taken orally is very low, no higher than 1%, which means that it should be administered after prolonged fasting. Its average blood life is approximately 1 hour, but its level of stable incorporation into the bone is 20% of the dose absorbed. However, its average life in bone is greater than 10 years. The diphosphonates are eliminated through urine, which means that care should be taken in patients with chronic renal insufficiency since that may alter the metabolism of the drug and worsen previous renal function[12].

Action mechanism:

The diphosphonates are selective inhibitors of osteoclast action in the bone remodelling cycle. The effect is achieved both by slowing the differentiation of common precursor cells (haematopoietic stem cells), and by favouring the apoptosis of the mature osteoclasts.

There are two principal action mechanisms. The older and less powerful diphosphonates are captured by the osteoclasts and converted into toxic analogues of ATP. However, the most powerful diphosphonates act by inhibiting farnesyldiphosphate synthase (FPP-synthase), an enzyme of the cholesterol synthesis pathway derived from mevalonate. These diphosphonates, which contain nitrogen, indirectly suppress the geranylgeranylation process of the proteins, which in turn inhibits osteoclast activity.

Lately, other actions of diphosphonates have been described which involve the osteoblast-osteocyte cell line. *In vitro*, the diphosphonates have been shown to have a protective action on the integrity of the bone matrix through an inhibition of the apoptosis of the osteocytes. Recent studies show that the diphosphonates may act to facilitate the recruitment of the osteoblasts, as well as stimulating in the aforementioned cell line the production of an antiresorptive compound: osteoprotegerin[13].

Method of administration:

Given that the diphosphonates have a low oral absorption, they should be administered after a prolonged period of fasting. They should be ingested with a sufficient quantity of water (100 ml or more) in order to favour their dispersion in the stomach. The taking of other liquids or foods should be avoided, for at least half an hour after their administration. In addition, patients should remain upright, preferably standing, during this period, in order to avoid gastro-oesophageal reflux, and its potential lesions. In some patients hypocalcemia and vitamin D deficit is observed, which may result in mineralisation disorders, for which reason calcium and vitamin D supplement should be given.

Secondary effects:

In general, these are well-tolerated drugs, if they are administered correctly. In respect of oral diphosphonates, the most frequent secondary effects are those which affect the higher digestive system: erosions, gastric ulcers, and, in more serious cases, oesophagitis and oesophageal stenosis. Less frequent are those adverse ocular effects such as conjunctivitis, scleritis, uveitis...

Notable among the adverse effects of intravenous diphosphonates are: phlebitis, which may appear in up to 18% of patients; transitory febricula and shivering (10-41%); pseudo-flu syndrome (20%) hypocalcemia (5-17%), which can be avoided by administering 1 gram of calcium a day orally, for 7-14 days following the administration of the treatment.

Drugs approved in Spain:

Etidronate was the first diphosphonate used in PDB. The dose used was 5 mg/kg weight/day for 6 months. It achieved a reduction in bone pain in approximately 50% of patients, and the reduction in bone turnover varied between 40% and 60%. At the end of treatment a reactivation was observed for a few months, and in some, a resistance to the drug in later treatments was observed[12,14-16].

Tiludronate is 3-10 times more powerful than etidronate. The optimum dose for the treatment of

PDB is 400 mg daily, taken orally, over 3 months. A reduction in AP has been seen, which varies between 30.5% and 76.1% depending on the study, and a normalisation of the values of AP varying between 27% and 38% at the end of treatment, remaining normalised after a year in 69% of cases[17-21].

The response to tiludronate usually appears during the first 3 months and may last 18 months. It is recommended that a new cycle of treatment is not repeated before 6 months have passed.

Risedronate: the recommended dose is 30 mg/day over 2 months. It reduces by 60-70% the levels of markers for bone turnover in most patients, and its effects continue for 2 years after the end of treatment[13,22-28].

Various studies published have shown that risedronate is efficacious in the reduction of pain, and even in its disappearance. In terms of radiology, there is evidence of a reduction in osteolytic activity in the first six months of treatment, which was correlated with the markers for the activity of the disease[29]. In the histological analyses, the formation of lamellar bone was observed, without evidence of a mineralisation disorder in the bone tissue not affected by Paget's.

The use of **pamidronate** is exclusively intravenous. The total dose approved is 180-210 mg, in two forms of administration: one is a dose of 30 mg once a week for 6 weeks; the other is an initial dose of 30 mg, followed by 60 mg every 2 weeks until the dose is complete. It achieved an alleviation of pain in 70% of patients, and remission, with suppression of bone resorption following the reduction in AP. This reduction is produced more rapidly than with other diphosphonates and the remission time is greater in cases of low activity. The remission according to the series was 50% after 2 years and 25% after 4 years. This suppression has been evaluated by bone gammagraphy with a reduction in capture. Histologically, there was a reduction in remodelled bone, with formation of laminated bone and with no alterations in bone mineralisation[30-35].

Zoledronate, recently incorporated into the treatment of PDB, is a third generation diphosphonate, and the one which currently has the greatest antiresorptive power. A second atom of N achieves a radical imidazole heterocycle. The dose is 5 mg i.v., administered in a single infusion. In the most important study carried out, zoledronate was compared with risedronate in 350 patients; they considered there to have been a clinical response when the level of AP was reduced by more than 75% from its initial level, or was normalised. A response of 96.6% was observed with zoledronate and of 74.3% with risedronate, with a normalisation of AP of 88.6% and 57.9% respectively. After six months of treatment, the loss of response was 0.9% for zoledronate and 25.6% for risedronate ($p< 0.001$). In the long term control zoledronate maintained bone turnover within margin of reference values during the 24 months after the start of treatment[36-38].

Other diphosphonates being used in PDB, or in the experimental phase:

Among these drugs we have one approved in other countries, and others in experimentation.

Clodronate: this is marketed for tumoral hypercalcemia. In the clinical trials the doses used have varied between 400 and 2,400 mg/day. The optimum dose was 800 mg/day over 6 months, taken orally. Its efficacy is similar to that of etidronate[39].

Alendronate: this is an aminodiphosphonate. Its usual regime is 40 mg/day, taken orally over 3-6 months. It produces a normalisation in biochemical markers in more than 50% of cases. It is not indicated for the treatment of PDB in our country, but it has been approved by the FDA.

Neridronate: there are few studies available on this drug. There is a study with 32 patients to whom were administered i.v. neridronate, which observed a normalisation of AP of 65%, with the response being maintained over 12 months[40].

Ibandronate: a clinical trial with 24 patients to whom were administered 2 mg i.v. of this drug observed a normalisation of AP of 45%, with the response maintained over 12 months[41].

Olpadronate: there are also few studies carried out on this drug. Administered at a dose of 200 mg, orally over 12 days, it showed a normalisation of AP of 87%, with the response maintained for 12 months in 60% of patients[42].

Future therapies: new treatments for PDB continue to be studied, among which is subcutaneous recombinant **osteoprotegerin**. Recently a study has been published in which two twins with juvenile PDB were used, which observed a suppression of bone resorption[43].

Monitoring of treatment

In symptomatic cases, the improvement of clinical manifestations is the essential parameter to be taken into account as the indicator of therapeutic efficacy. Although improvements in radiological lesions and a reduction in gammagraphy capture have been suggested, all authors accept the convenience of following the therapeutic response through markers for bone turnover. Among these are:

1. Total blood AP, the most-used biochemical marker[1]. It has good reproducibility and sensitivity and, although the ideal is the normalisation of these parameters, in the last clinical trial a good clinical response was considered to have occurred when there was a reduction of at least 50-75% in values prior to treatment. Some authors recommend determining the level of AP every three months during the first 6 months and then at intervals of 6 months.

2. Biochemical markers for bone resorption, such as deoxypyridinoline, and other more modern markers (CTx, NTx), respond more rapidly to treatment. Their nadir is within the month of initiation of treatment. The correlation between these markers and total AP is good[3].

Bone gammagraphy is not a good method for monitoring the response to treatment, since there

is a considerable delay, of approximately six months, between the biochemical response and the bone gammagraphy. In addition, patients are exposed to radiation. However, in monostotic PDB, and in those patients in whom pain persists in spite of the normalisation of the biochemical markers, repeating the bone gammagraphy may be useful[3].

Indications for renewing treatment of a patient with PDB

Giving a further cycle or dose of treatment in PDB is recommended when there is a relapse in the disease or when the symptoms persist. In the case of pain, it should be confirmed that it is of pagetic origin, completely discounting other causes of pain[3].

Although there is no supporting evidence in the clinical trials carried out, it is generally accepted that an increase in AP of 25% from the baseline or the upper limit of normality after normalisation indicates a significant biochemical relapse[2,3,5].

Other authors also recommend returning to treatment when lytic lesions reappear in the large bones[2,5].

The effect of the diphosphonates appears at 3 months from the initiation of treatment and their maximum effect is at 6 months, therefore it would appear logical not to initiate another treatment until this six months has elapsed.

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Dyslipidemia and bone metabolism. A common bond of the osteoporosis and the atherosclerosis?

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Summary
The magnitude of the public health problem related to cardiovascular disease (CVD) and osteoporosis has been widely documented in the medical literature in the last decades, and common pathogenic links have been recently proposed. Dyslipidemia is one of the most important risk factors in the genesis and development of atherosclerosis, and therefore of CVD, which remains the leading cause of cardiovascular mortality in western countries. On the other hand, osteoporosis and its more serious consequence; fracture, represent a true epidemic nowadays. In this context, the relationship between dyslipidemia and bone metabolism has been addressed by several investigators, although results have been inconsistent. The purpose of this paper is to review the medical literature about the possible association between dyslipidemia and several aspects of bone metabolism.

Key words: *Dyslipidemia, Arteriosclerosis, Cardiovascular disease, Osteoporosis, Fracture, Bone mineral density, Bone turnover markers.*

Introduction

The common pathogenic basis of most cardiovascular diseases (CVD) is arteriosclerosis, a natural multifactorial process, in whose origin are implicated various risk factors, with dyslipidemia being one of the most significant. Similarly to arteriosclerosis, osteoporosis has a high prevalence in the population, with significant associated morbimortality. It is for these reasons that there is great interest in studying the possible associations between the two, with the aim of promoting more strongly primary preventative activities and prioritizing interventions against the risk factors for both diseases.

The relationship between arteriosclerosis and osteoporosis appears to go further than a mere coincidence of common risk factors. What is more, in recent years, the possibility has been raised of the existence of pathogenic links and physiopathological interactions between bone metabolism and risk factors for CVD. This fact has been endorsed by the discovery of some of the molecular action mechanisms of the statins and the biphosphonates[1], to which are attributed antiatherogenic effects by means of a reduction in the accumulation of lipids and of fibrosis in the atheromatous plaques, as well as the inhibition of extra-bone calcification[2]. On the other hand, the statins inhibit the limiting step of the biosynthetic route of cholesterol: the conversion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, reducing its synthesis, and that of isoprenoids, which also affect osteoclast function, which is an effect in common with the aminobiphosphonates (Figure 1)[3,4,5].

Recently, it has been proposed that dyslipidemia could be a common risk factor for CVD and osteoporosis. *In vitro* studies have shown that the products of lipid oxidation in the subendothelial space of the bone arteries inhibit osteoblast differentiation[6], and that hyperlipidemia strengthens the activity of the osteoclasts[7]. Also notable is the presence of products of the oxidation of the low density lipoproteins (LDL-C) in the arteriosclerotic plaques[8]. There has been discussion of a similarity between the processes of bone mineralisation and of vascular calcification, and the factors which may influence the development of both, such as, for example, the presence of oxidised LDL , with its high atherogenic potential[6]. Although the precise intrinsic mechanism for this nexus is not yet known, recently it has been confirmed that the high density lipoproteins (HDL-C) have a regulatory effect on osteoblast differentiation and on vascular calcification. In fact, the prolonged treatment with HDL-C inhibits the calcification of the vascular cells and osteogenic activity induced by inflammatory cytokines, such as the interleukins 1β y 6[9]. In addition, osteoclast activation appears to be favoured by other inflammatory cytokines[10], such as colony stimulating factor type 1 (CSF-1), tumour necrosis factor α (TNF- α), and the receptor activator of ligand NF-κB (RANKL), also present in the arteriosclerotic plaque[11]. In accordance with what has been mentioned earlier, the statins could be useful drugs in the treatment of osteoporosis, since they do not only share with biphosphonates anti-inflammatory properties, but also possess modulatory characteristics in relation to bone formation and resorption[12-14].

However, the studies carried out with the aim of establishing a clear pathogenic nexus between the alteration in the parameters of lipid metabolism, bone mineral density (BMD) and/or osteoporotic fractures, have not been conclusive.

The purpose of this work is to carry out a review of the studies most relevant to the possible association between dyslipidemia and different aspects of bone metabolism, specifically: bone mineral density, the markers for remodelled bone, the calciotropic hormones and osteoporotic fractures. Finally, we produced a brief outline of the repercussions of some of the principal drug treatments (specifically, statins and biphosphonates) in both diseases.

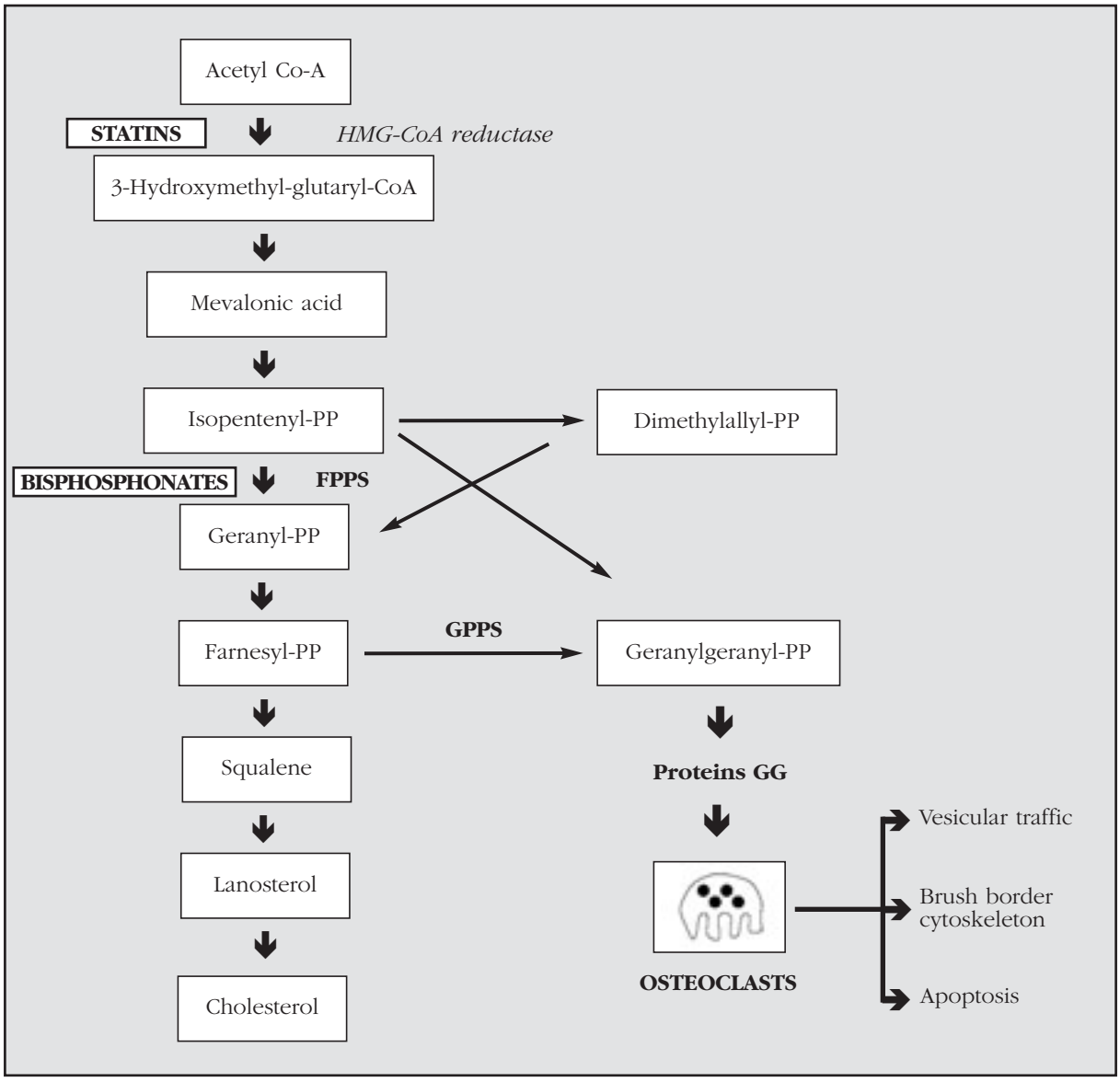
Alterations to lipid metabolism and bone mineral density

Most of the studies have explored this association in postmenopausal women, and in addition, many of them have included patients being treated with hypolipidemic drugs, for which reasons the results have been less than consistent. The main question should probably be whether or not there exists a direct relationship between bone mineral density and blood lipids, or if this hypothetical association is due to confusion factors (principally their estrogenic state, in the case of the women).

So, the changes in the parameters of lipid metabolism have been related to BMD in different works, although with, in many cases, contradictory results. In the Framingham cohort Samelson et al.[15] studied 712 women and 450 men with ages of between 32 and 61 years. In the first period of study (1953-55), a densitometric analysis was carried out in the hip, lumbar spine and distal radius, as well as laboratory tests and surveys of cardiovascular of risk factors, which were repeated in the study's second phase (1988-89). No significant association was found between blood levels of cholesterol in both sexes and the BMD in the areas under consideration, save in the radial diaphysis, where the association with total cholesterol was inverse in the group of males. This study concluded that blood levels of total cholesterol did not appear to have a significant influence on the BMD, neither in men, nor in women.

Poli et al.[16], in a study which included 1,303 postmenopausal women, observed that those with blood levels of LDL-C ≥ 160 mg/dL had more than double the probability of having lumbar osteopenia than women with lower levels of LDL-C. Yamaguchi et al.[17] found an inverse association between levels of LDL-C and BMD in the forearm and the spinal column, and a direct association between HDL-C and BMD in the aforementioned areas in 214 postmenopausal Japanese women. They observed also that women with earlier verte-

Figure 1. Metabolic pathway of cholesterol. Involvement of statins and aminobiphosphonates



PP: pyrophosphate; FPPS: farnesyl pyrophosphate synthase; GPPS: geranyl pyrophosphate synthase; HMG: hydroxyl-methyl-glutaryl; CoA: coenzyme A. Adapted from Hernández et al.[1]

bral fractures had lower levels of triglycerides than the women without fractures.

Makovey et al.[18], carried out a longitudinal study in 497 female twins with ages of between 20 and 81 years (224 at the premenopausal stage, and 273 in the menopause; 156 in treatment with hormone replacement therapy (HRT), and 117 without it). They examined the influence of age, menopausal status, and HRT on blood cholesterol and BMD (measured in the lumbar spine, total hip, femoral neck and whole body, by means of double X-ray absorptiometry – DXA). They observed an inverse relation between levels of total cholesterol and of LDL-C with BMD in the lumbar spine and in the total body measurement, in the postmenopausal women, in addition to a negative relationship between HDL-C and BMD in the hip, which appeared to be modified by HRT.

Nuzzo et al.[19], investigated bone quality in 256 postmenopausal women stratified according to the absence (total cholesterol < 200 mg/dl; n= 180) or presence (cholesterol total ≥ 200 mg/dl; n= 76) of hypercholesterolemia (in turn, divided in subgroups as a function of whether they were receiving dietetic treatment or treatment with statins). The study was carried out using ultrasound (QUS) in the proximal phalanges of the hand, observing a statistically significant reduction in the velocity of the ultrasound (AD-SoS) in subjects with hypercholesterolemia, per se, could be a risk factor for bone deterioration and that the statins could have a protective effect on the bone, independently of the intake of calcium.

In a study of 52 postmenopausal women who were overweight, Orozco et al. 20 observed that those patients with an atherogenic lipid profile (total cholesterol ≥ 240 mg/dl or LDL-C >160 mg/dl) had a lower BMD in the lumbar spine and the femoral neck, as well as a higher risk of osteopenia, in comparison with those patients with a normal lipid profile, suggesting a possible association of hyperlipidemia with osteoporosis.

However, Solomon et al.[21], in a work which included 13,592 participants in the NHANES III study (1988-1994), and excluding those subjects receiving hyperlipidemic therapy, did not find any significant relationship between the parameters of lipid metabolism and BMD measured in the hip with DXA.

Adami et al.[22], studied this relationship with two cohorts of subjects: one clinical cohort which included 236 pre- and postmenopausal women of between 35 and 82 years of age who had attended a clinic specialising in osteoporosis, and a populational cohort (265 males and 481 females aged between 68 and 75 years). In the clinical cohort there was evidence of a negative relationship between lumbar and hip BMD and levels of HDL-C, and a positive one with levels of blood triglycerides. In the community cohort the same correlations were found between these lipids and BMD in the hip and that measured in the whole body. In both, the relationship between the lipid profile and bone mass continued to be significant after adjusting for body mass index and weight.

In the Hertfordshire cohort[23] in Great Britain, which included 465 women and 48 males, a direct association was observed between the lumbar and total hip BMD and the levels of triglycerides in both sexes, as well as an inverse relationship between HDL-C and lumbar BMD in males, and BMD in the total hip in both sexes. However, these associations were neutralised by adjusting for the percentage of body fat. No associations were observed between BMD and total cholesterol or LDL-C.

In a recent work which analysed 289 males included in the Camargo cohort[24], we observed a direct association between blood levels of total cholesterol, LDL-C and the quotient LDL-C/HDL-C and BMD in the lumbar spine and hip. There was no evidence of any relationship with HDL-C or triglycerides. After controlling for confusion variables it was seen that the males with hypercholesterolemia had a higher BMD in measurements taken in the hip, with respect to those normocholesterolemic males. In addition, in the bone ultrasound study, a positive correlation was found between levels of triglycerides and the LDL-C/HDL-C relationship and the broadband ultrasound attenuation (BUA), and between the total cholesterol/HDL-C quotient and the quantitative ultrasound index or consistency index (QUI) and the BUA. Only one other work, published by Buizert et al.[25], has analysed the value of ultrasounds in patients with dyslipidemia. These authors found a positive association between the total cholesterol/HDL-C quotient and the SOS and BUA, and an inverse relationship with HDL-C in both sexes. Our data, and those of Buizert et al., indicate that a high "good/bad" cholesterol quotient may not only be in relation to BMD but also to bone quality.

Alteration of lipid metabolism, markers for bone remodelling and calciotropic hormones

Various studies, *in vitro* and with animal models, have shown some harmful effects of dyslipidemia on bone metabolism[22]. The *in vitro* studies, for example, have indicated that the osteoblast differentiation is inhibited by the products of lipid oxidation[26]. Recently, the participation of the mevalonate pathway has been proposed in both the synthesis of cholesterol and the regulation of the proliferation or apoptosis of bone cells[27]. Also, the regulatory role of the LRP5 gene in osteoblast proliferation[28], whose mutation causes a significant reduction in BMD both in rats and in humans[29]. The same has been suggested of the mutations in the gene LRP6, a homolog of LRP5, demonstrating its role in the reduction in bone mass in rats[30] and its genetic link with early coronary disease, metabolic risk factors and osteoporosis in humans[31]. In addition, Parhami et al.[32] demonstrated that hypercholesterolemia increases osteoclast activity and the reduction in BMD in rats.

Markers for bone remodelling

In this context, there are few works which have studied the effects of hypercholesterolemia or lipid parameters on markers for bone remodelling (MBR), and the results have also not been very consistent, and have even been contradictory. Majima et al.[33], analysed the blood levels of alkaline phosphatase, bone alkaline phosphatase (BAP), and collagen type 1 N-terminal telopeptide (NTx) in 281 Japanese patients with hypercholesterolemia and 267 controls. In the women, there was evidence of values of BAP significantly higher in the cases than in the controls. The levels of NTx in those subjects with hypercholesterolemia were significantly higher than those of the controls, in both sexes. In addition, blood levels of BAP and NTx in males showed an inverse correlation with HDL-C, whilst this correlation was direct with total cholesterol and LDL-C in the case of the women. In both sexes, the relationship between the MBRs and the lipid profile continued to be significant after adjusting for confusion variables. These data indicate an elevation in levels of MBR in dyslipidemic patients independent of sex.

Although the studies are difficult to compare for obvious reasons, our data in the study of the Camargo cohort do not support these findings and, in fact, we found lower blood levels of PINP and β-CTX in those individuals with hypercholesterolemia with respect to the controls, although the difference did not reach statistical significance. However, in stratifying by age, the blood levels of both MBRs were significantly lower only in those

Table 1. Principal studies which relate the use of statins with the risk of fracture

Author[Ref]	Population	Study	Cases	Controls	Treatment	Results (OR with CI 95%) after adjustment
Meier et al.[50]	♂ and ♀ 50-89 years (Great Britain) N=27,319, average age 77/76 years	Cases and controls	3,940 subjects with previous bone fractures in any location	23,379 subjects without a history of fracture	Statins, fibrates, other lipid-lowering	↓risk of fractures with statins (OR 0.55; 0.44-0.69) No effects with other hypolipidemics (OR 0.87; 0.7-1.08)
Wang et al.[49]	♂ and ♀ >65 years (US) N=6,110, average age: 82/82 years	Cases and controls	1,222 subjects with hip fracture	4,888 subjects without fracture	Statins	↓risk of hip fracture (OR 0.29; 0.10-0.81) ↓risk of hip fracture (OR 0.50; 0.4-0.76) ↓risk of hip fracture (OR 0.57; 0.40-0.82)
Chan et al.[51]	♀ >60 years (US) N=3,675, average age: 77/76 years	Cases and controls	928 ♀ with fracture in any location	2.747 ♀ without fracture	Statins	♀ ≥13 pharmacological dispensations of statins: ↓risk of fracture 52% (OR 0.48; 0.27- 0.83) ♀ <13 dispensations: no effect
Ray et al.[53]	♂ and ♀ with an average age of 62 years (US) N=34,584, average age: 62/62 years	Retrospective study Tennessee Medicaid Programme Cohort	12,506 subjects on statins, 4,798 subjects on other hypolipidemics 17,280 subjects without hypolipidemic treatment		Statins, other lipid-lowering	RR in subjects using statins: 0.62 (0.45-0.85) RR using other hypolipidemics: 0.44 (0.26-0.95) Statins are not better than other hypolipidemics in ↓the risk of fracture
Scranton et al.[65]	♂ and ♀ older than 65 years (1998-2001) in US N=91,052, average age: 65/59 years	Retrospective study US Veteran Population Cohort	86,731 ♂ and 4,321 ♀ (28,063 in treatment with statins, 2,195 with other hypolipidemic treatment) Risk of certain fractures by diagnosis		Statins, other lipid-lowering	↓risk of fracture 36% (OR 0.64; 0.58-0.72) in subjects in treatment with statins in comparison with other hypolipidemics (32%, OR 0.67; 0.50-0.91)
Bauer et al.[66]	8 observational studies (4 prospective studies - SOF, FIT, HERS, Rotterdam-) 2 clinical trials	Meta-analysis	SOF: N=9,704 ♀, average age 75/77 years, cases=1,083, follow up 4 years FIT: N=6,459 ♀, average age 69/69 years, cases 1,241, follow up 3.6 years HERS: N=2,763 ♀, average age 66/67 years, cases=271, follow up 4.5 years Rotterdam: N=4,878 ♀, average age 66/72 years, cases=726, follow up 5.3 years		Statins	Observational studies: users of statins for hip fractures: OR 0.43 (0.25-0.75) and non-vertebral fracture: 0.69 (0.55-0.88). Clinical trials: use of statins for hip fracture: OR 0.87 (0.48-1.58) and non-vertebral fracture: OR 1.02 (0.83-1.26)
Toh et al.[67]	Database: Medline, Embase and Cochrane N=522,507 subjects	Meta-analysis 15 articles (6 case-control studies, 8 cohorts –6 prospective, 2 retrospective–, 4 *post hoc* analyses of randomised controlled trials)	N=522,507 subjects, with 109,919 fractures included in the analysis		Statins	↓risk of fracture OR=0.77; 0.66-0.90 [use of statins vs non-use]. The protective effect of statins was found in case-control studies (OR=0.62; 0.45-0.85) and in cohort studies (OR=0.77; 0.59-1.00), not in randomised clinical trials. ↓risk of hip fracture: OR=0.58 (0.46-0.74), vertebral column: OR-0.65 (0.48-0.88), other locations: OR=0.77 (0.6-1.00). The evidence does not support the use of statins in the prevention of fractures: what is missing is an association in clinical trials, heterogeneity in observational studies, confusion factors and possible publication bias

patients with hypercholesterolemia of between 70 and 74 years of age[25]. Neither did Brownbill et al.[34], in a transversal analysis of 136 healthy postmenopausal women, with no hypolipidemic treatment, find any association between the MBRs (blood osteocalcin and NTx in urine) and BMD.

Calciotropic hormones

With respect to the relationship between vitamin D and CVD, the results are again contradictory. On the one hand, an excess of vitamin D favours the development of arteriosclerosis in animal models[35], while on the other, its deficiency is related to ischemic cardiopathy[36]. Other authors did not find an association between vitamin D and vascular disease[37].

Something similar occurs with parathyroid hormone (PTH). Hangstrom et al.[38], in the ULSAM study, carried out in 958 males, observed a direct relationship between levels of PTH and cardiovascular mortality. Whilst Reis et al.[39], in a transversal study carried out in 654 subjects aged between 55 and 96 years, did not find an association between blood levels of PTH and carotid arteriosclerosis. In a cohort of 410 males and 660 females, participants in the Rancho Bernado study, the same authors found evidence of the existence of a direct relationship between levels of PTH and metabolic syndrome in the males[40].

However, we have not found any works which explicitly analyse the possible relationship between vitamin D and/or PTH with lipid profile or dyslipidemia. In the NHANES III study, already mentioned[22], levels of 25OHD did not vary in any of the quintiles of total cholesterol, LDL-C or HDL-C in the blood. The levels of PTH were not measured. In the Camargo cohort, also, no significant differences were found with respect to blood levels of 25OHD or intact PTH in males with or without hypercholesterolemia, which suggests perhaps that these calciotropic hormones do not play a significant role in the association between bone and lipid metabolisms[24].

Alterations in lipid metabolism and bone fractures

Over the last few decades epidemiological studies have provided evidence of an increase in mortality by CVD both in patients with osteoporotic fractures[41], and in non-fractured subjects with reduced bone mass[42]. The mechanism which underlies the relationship between cholesterol and osteoporotic fracture may be directly related to the contribution of the cholesterol metabolism to bone structure.

Once again, the relationship found in those few relevant publications, between alterations in lipid metabolism and fractures, is not very conclusive.

Yamaguchi et al.[18] analysed the lipid profile in 214 postmenopausal women and its relationship to BMD and the presence of vertebral fractures. They observed a direct relationship between HDL-C and BMD in the lumbar spine and forearm, and a positive association between triglyceride values and previous vertebral fractures.

Another nested case- control study from the SOF[43] cohort, which analysed 271 women with fracture of the proximal femur (n= 133) and radiological vertebral fracture (n= 138), did not find any association between blood levels of total cholesterol, LDL-C or HDL-C, and the incidence of vertebral or hip fracture, once the statistical model was adjusted for age and body weight.

Ahmed et al.[44], studied the effect of some components of metabolic syndrome, among them lipid profile, on the risk of non-vertebral fracture in a prospective cohort of 12,780 males and 14,211 females of between 25 and 98 years of age, followed for 6 years (1994-2001). They observed that the low levels of HDL-C protected against the risk of fracture in obese females and males.

Sivas et al.[45], reviewed the relationship between lipid profile, osteoporotic vertebral fractures and BMD in 107 postmenopausal women, aged between 45 and 79 years. They analysed lateral dorso-lumbar X-rays, BMD in the proximal femur, radius and lumbar spine by means of DXA, and lipid profile (total cholesterol, triglycerides, LDL-C and HDL-C). The values of the first three lipid parameters were lower in those postmenopausal women who had at least one vertebral fracture in comparison with those who had none, this relationship staying the same after adjusting for the principal confusion variables (age, duration of the menopause, BMI, among others). An increase of 1 mg/dl of total cholesterol reduces the risk of having a vertebral fracture by 2.2%, there being also a weak association between the levels of total cholesterol, LDL-C and BMD in the distal radius.

In the Camargo cohort study we did not observe any association between blood lipids and earlier vertebral fractures. However, we found that blood levels of total cholesterol ($p< 0.03$) and of LDL-C ($p= 0.04$) were lower in males with existing non-vertebral fractures[24].

Impact of the statins on bone metabolism and of the biphosphonates on lipid metabolism

Recent *in vitro* and *in vivo* studies have described possible beneficial effects of statins on the bone[46,47]. In 1999, in a study in rats, it was suggested that the statins could promote osteoblast differentiation through the stimulation of BMP-2[48]. Subsequently, in an observational study an inverse association was found between hip fracture and the use of statins[49]. Since then, various works have analysed the relationship between the statins, BMD and osteoporotic fractures, although with disparate results. A protective effect of the statins on the bone has been observed in different case and control studies[50-52], and in various cohort studies[53,54]. However, the data coming from randomised controlled *(post hoc)* trials[55,56], and other observational studies[57,58], does not show such findings. In Table I are presented the studies most relevant to the possible association between the use of statins and osteoporotic fractures.

Table 2. Studies which relate to the hypolipidemic effect of the biphosphonates

Author[Ref.]	Population	Study	Cases	Controls	Treatment	Results
Celiloglu et al.[60]	72 ♀ (52/51 years) Follow up: 1 year	Prospective	39 ♀ with osteoporosis in treatment with alendronate	♀ 33 without treatment	Alendronate 70 mg/week	Positive effect of alendronate on ApoB/ApoAI quotient ($p<0.01$); ↓reduction in thickness of the carotid intima-media a year from the start of treatment ($p<0.05$)
Guney et al.[63]	49 ♀ postmenopausal (54 years), Follow up: 6 months	Prospective	49 ♀ with osteoporosis and dyslipidemia		Alendronate 10 mg/day	↓CT, triglycerides and LDL-C. No significant differences in HDL-C, ApoA1 nor Apo B
Adami et al.[64]	87 ♀ postmenopausal (53-72 years) Follow up: 1 year	Cases and controls	44 ♀ with osteoporosis in treatment with neridronate	♀ 43 without treatment	Neridronate 50 mg/ 2 months	↑HDL-C in 17-18% at 12 months ($p<0.0001$; ↑ 24% HDL-C/LDL-C at 12 months ($p<0.01$); ↑ApoAI/ApoB ($p<0.001$); ↓LDL-C at 4, 8 and 10 months ($p<0.05$)
Iwamoto et al.[68]	121 ♀ postmenopausal, (69 years) Follow up: 1 year	Prospective	61 ♀ with osteoporosis in treatment with alendronate	61 ♀ with osteoporosis in treatment with alendronate	Alendronate 5 mg/day	No difference in lipid profile of the group in treatment with alendronate

On the other hand, some works have suggested that the biphosphonates, in addition to reducing bone resorption and risk of fracture, could slow the arteriosclerotic process, due to their effect on the synthesis of cholesterol, the progression of inflammation and oxidative stress. Although the majority of the studies in animals show the clear antiatherogenic activity of the biphosphonates, the data in humans are not consistent[59]. In relation to the lipid metabolism, some authors have described a positive effect of alendronate on the ApoB/ApoA-I quotient, and a reduction in the thickness of the carotid intima-media (CIM) in women with postmenopausal osteoporosis[60]. Koshimaya et al.[61], showed evidence in 57 subjects with type 2 diabetes *mellitus* and osteopenia of a reduction in CIM at 12 months from the initiation of cyclical treatment with etidronate. Other recent studies did not find this hypolipidemic effect of alendronate in women with postmenopausal osteoporosis[62]. Guney et al.[63], in an analysis of 49 women with osteoporosis and dyslipidemia, found a reduction in concentrations of total cholesterol, triglycerides and LDL-C 6 months after the start of treatment with alendronate. Another study carried out by Adami et al.[64], showed the hypolipidemic effect of endovenous neridronate, which continued with an increase in HDL-C, of the HDL-C/LDL-C quotient and the ApoA-I/Apo B relationship, as well as a reduction in LDL-C. The main studies which have analysed the hypolipidemic action of the biphosphonates are summarised in Table 2.

Conclusion

The relationship between osteoporosis and dyslipidemia probably goes further than the mere presence of joint risk factors, and in this relationship are probably implicated common pathogenic mechanisms which favour the development of both diseases. Despite the fact that the results obtained by the studies carried out to date are not definitive, future studies should establish the magnitude of this relationship, especially at the level of tissue.

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Sociedad Española de Investigación Ósea y del Metabolismo Mineral (SEIOMM) y Sociedades afines

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Position document on the requirements and optimum levels of vitamin D

Introduction

In the last few years there has been a notable interest in vitamin D, not only due to its crucial importance in bone mineral metabolism, but also for its effects outside the bone, which, every day, are becoming better known.

Similarly, the existence of low blood levels of vitamin D, lower than what is desirable, has been found in different populations, both healthy and sick, and there is a discussion as to what would be the optimum levels of vitamin D in the blood.

For all these reasons, the Spanish Society of Bone and Mineral Metabolism Research (Sociedad Española de Investigación Ósea y Metabolismo Mineral – SEIOMM), jointly with all the scientific societies involved in the study of bone metabolism, have produced this position document on the requirements and optimum levels of vitamin D.

Material and method

The content of this document was developed in the following stages:

a) Meeting of a group of experts in osteoporosis to discuss and agree the relevant clinical questions related to vitamin D (Table1).

b) Creation of a systematic review team, formed by two experts in bone mineral metabolism who carried out the search, a standardised review, critical analysis and tabulation of the articles which had been published in Spanish and English between January 2000 and May 2010. The search was carried out using the MeSH (Medical Subject Headings) terms of the National Library of Medicine of the US National Institutes of Health, related to the topic. Using these terms, the following databases were consulted: PubMed, Medline Plus, Cochrane Library, Up to Date and OVID. Similarly, an ascending search was made of the previously published guides to clinical practice relevant to the topic, as well as articles suggested by the group of experts.

c) Those articles which provided the best level of evidence for each of the questions raised were included (Table 2).

d) Subsequently, following on from the results obtain in the search, a draft of the position document was put together by the group of clinical experts to respond to the questions previously formulated and to provide a consensus on recommendations, taking into account social, economic and health repercussions. In cases of disagreement, a majority opinion was formed, leaving the absence of unanimity on record.

PART 1. LITERATURE REVIEW

Introduction

1. Sources of vitamin D

More than 90% of the vitamin D in our bodies comes from the transformation of 7-dehydrocholesterol into previtamin D_3, and subsequently into vitamin D_3 by the action of ultraviolet B radiation from the sun on the skin. There is no danger of vitamin D intoxication due to an excess exposure to the sun, since any excess previtamins and vitamin D synthesised degrade in the skin itself into inactive metabolites.

The rest is obtained through intestinal absorption, either from the diet (although those foods which contain vitamin D do not provide sufficient quantities) or by taking supplements[1].

2. Physiology of vitamin D. Action inside and outside the bone

Vitamin D is actually formed from a family of chemical substances with similar action. But when we talk generically of vitamin D we are referring both to vitamin D_3 (coleclaciferol), and vitamin D_2 (ergocalciferol), the first being part of human physiology, and the second obtained by UV irradiation of ergosterol contained in yeasts. Dietary vitamin D absorbed by the fraction of the kilomicrons or synthesised in the skin, and later also its metabolites, circulate bonded to a transporter protein (DBP). In the liver it undergoes a hydroxylation by the action of 25-hydroxylase to form calcifediol or calcidiol (25 hydroxy-colecalciferol, 25 hydroxy-vitamin D, 25 (OH)D). The calcifediol has a high concentration and a long average life, of two or three weeks, which means that it is used to evaluate the status of vitamin D in the body (see later), and forms the ideal substrate for the formation of calcitriol or 1.25 dihydroxy-vitamin D ($1.25(OH)_2D$), the hormonally active metabolite in the endocrine system of vitamin D[1-3].

The complex formed by calcifediol and its transporter protein, [25(OH)D]-DBP, is bonded with megalin (a protein located in the plasmatic membrane of the renal tubular cells), which it introduces into the cells. Here, the 25(OH)D is released and directed to the mitochondria, where, by the action of 25-hydroxyvitamin D-1α-hydroxylase it is transformed into $1.25(OH)_2D$, which has as its principal endocrine function, the maintenance of calcium homeostasis. This balance, in turn, is fundamental to the various metabolic functions to be carried out normally, for adequate muscular transmission and for bone mineralisation to happen correctly. Its calciotropic function is carried out by acting on the intestinal, parathyroid, bone and renal cells, as we see below[1-3].

In the intestine, the action of vitamin D is fundamental for the absorption of calcium through the saturable transcellular pathway, especially when it derives from foods or compounds with low ionisability. When a deficiency in vitamin D occurs, the absorption of calcium reduces by 15% (and by up to 60% for phosphorus), thus reducing levels of ionized calcium in the blood. This decrease is detected by the calcium sensors in the parathyroid glands, which respond with an increase in the secretion of parathyroid hormone (PTH)[1,4], whose function is to maintain adequate levels of blood calcium, by acting on the kidneys, as we will see later, and in the bone, where it stimulates bone resorption. This final action it achieves by increasing in the osteoblasts the expression of RANKL, which bonds to the receptor, RANK, in the plasmatic membrane of the monocyte precursors of the osteoclasts, inducing their maturation. The mature osteoclasts then bond with the bone surface to initiate their resorptive action by releasing, above all, hydrochloric acid and collagenase. The calcium and phosphorus released in the process pass into the circulation, and thus increase their blood levels[1-3].

In the kidney, the PTH reabsorbs the filtered calcium (both in the distal and proximal tubules) and reduces the reabsorption of phosphorus, leading to phosphaturia and, therefore, hypophosphotemia. Both (PTH and hypophosphotemia) in turn powerfully stimulate the renal production of $1.25(OH)_2D$.

Calcium and phosphorus are essential for mineralisation to happen correctly; when the supply of calcium in the body is inadequate $1.25(OH)_2D$ helps to maintain calcium homeostasis, acting on the receptors for vitamin D (VDR) of the osteoblasts in which it induces in a similar way to PTH the formation of RANKL.

In addition to these endocrine actions, which we could call "traditional" or "classic", and which regulate calcium-phosphorus and bone homeostasis, the vitamin D endocrine system has other auto-paracrine functions in the organism as a whole[1,5]. The majority of tissues and cells, normal or neoplastic, such as muscle, heart, brain, blood vessels, breast, colon, prostate, pancreas, skin and immune system, among others, contain VDR and activator enzymes for 25(OH)D, such as 1-hydroxylase, in those locations not regulated by PTH, to synthesise $1.25(OH)_2D$, and, as happens in the kidneys, inactivator enzymes such as 24 hydroxylase, which catabolises both 25(OH)D and $1.25(OH)_2D$, and ends up forming calcitroic acid, which is soluble in water and biologically inactive.

The $1.25(OH)_2D$ bonds with its VDR with a strong affinity and regulates the transcription of approximately 3% of the human genome. It is involved in the regulation of cell growth and maturation, inhibits the production of rennin and increases the secretion of, and sensitivity to, insulin, modulating the function of activated B and T lymphocytes and the macrophages, among other actions, which means that it has important implications for health[5,6].

Questions raised by the Committee of Experts: search for evidence

1st. Optimum levels of vitamin D

Adequate levels of vitamin D are vital for the correct working of the endocrine system, not only

in the bone, but also in practically the whole organism. The principal indicator for the system is 25(OH)D, the metabolite with the longest average life and an essential substrate for the synthesis of $1.25(OH)_2D$, both in the kidneys as well as in other cells and tissues, which makes it commonly accepted as an indicator for the status of vitamin D[4,7].

A fundamental problem in the determination of 25(OH)D consists in the precision and reproducibility of the methods available for its measurement[6]. For a long time there was no consensus regarding what were the optimum levels of 25(OH)D in the population, although in recent years there has been a growing interest in establishing them. Some studies have shown that with levels of 25(OH)D above 30-40 ng/ml (75-100nmol/l), in adults the maximum intestinal absorption of calcium is achieved[8,9], and at the same time, lower levels of PTH, avoiding the appearance of secondary hyperparathyroidism[10]. It is assumed that children have the same requirements as adults, although no studies have been carried out which confirm this.

Table 1. Questions raised by the panel of experts

1. What are the optimum levels of vitamin D?
2. Are there adequate levels of vitamin D in the Spanish population? - Prevalence of hypovitaminosis D in Spain
3. What are the requirements for vitamin D? a- In the general population b- In specific situations: - In children and adolescents - In the postmenopause - In elderly people c- In pathological situations: - In patients with osteoporosis - In patients with fracture - In patients receiving corticoids
4. Vitamin D and falls, muscle strength and balance - Incapacitated patients
5. Treatment with vitamin D. Alone, or always with calcium? - In the prevention of osteoporosis - In the treatment of osteoporosis itself - Combined with other antiosteoporotic drugs

Generalising from these findings the view is that the optimum requirements of vitamin D are those which permit the maintenance of blood levels of 25(OD)D above 30 ng/ml (75 nmol/l)[11,12].

Faced with these results, there is ever increasing agreement in accepting these levels as those most beneficial to ensure healthy bone[8,13]. Minimum desirable blood concentrations of 25(OH)D for anyone ought to be higher than 20 ng/ml, which would imply an average of around 30 ng/ml in the whole population[14]. Bischoff-Ferrari et al. even suggest that, to ensure other non bone-related health objectives, the optimum levels of 25(OH)D should be higher, between 36 and 40 ng/ml[12]. These data are corroborated by a study carried out in populations highly exposed to the sun, in which it is very difficult to surpass blood concentrations of 25(OH)D of 65-70 ng/ml[15].

The IOF, in its recent recommendations on vitamin D in elderly people[16], advises that these levels be reached, and the NOF recommends that the general public should maintain levels of 25(OH)D above these values (www.nof.org). Below these optimum levels, it is generally considered that there is a deficiency when the levels are between 20 and 30 ng/ml (50 and 75 nmol/l); vitamin D deficiency, observed in individuals with osteomalacia or rickets, appears at values lower than 20 ng/ml (50 nmol/l)[4,8,10,13] (Table 3).

Thus, blood levels of 25(OH)D of between 30 and 75 ng/ml would seem to be the most physiologically appropriate, and as such, the most recommendable. With respect to higher values, in a review of thirty works there was no evidence of toxicity in patients with levels of 25(OH)D below 100 ng/ml. It has been proposed that the minimum threshold for toxicity is above 150 ng/ml (375 nmol/l)[4,9].

2nd Levels of vitamin D in the Spanish population

At present, insufficiency, and frankly, deficiency in 25(OH)D constitutes a pandemic which affects more than half the general population[8,17], and both children and adolescents[18-21], as well as adults[22], postmenopausal women[23] and elderly people[24-26]; in this last group, if they have osteoporotic fractures, the prevalence of hypovitaminosis D reaches 100%[4]. Holick and Chen, in 2008, described vitamin D deficiency as a global health problem with diverse pathological consequences[8], and a recent review carried out by Mithal et al. of studies on hypovitaminosis D across the world concluded that this deficit was emerging globally as a major health problem[27].

This situation is occurring also in Spain, as can be observed from the various studies carried out in this country[28-35]. Despite having a propitious climate which could result in an adequate synthesis of vitamin D by solar exposure, the general levels are similar or even lower than those described in central Europe or Scandinavia in earlier studies[36,37], although the variation in methodologies between laboratories makes a rigorous comparison difficult.

There has been an attempt to explain the paradoxical state of hypovitaminosis D which is obser-

ved in our country, and which is also seen in other Mediterranean countries, by the scarce supply of dietary vitamin D which cannot be compensated for by cutaneous synthesis[38]. On the other hand, it is important to understand that the greater part of the Iberian peninsula is above the latitude of 35° N, which means that the inclination of the sun's rays reduces the possibility of synthesising vitamin D during the months of winter and spring[36].

However, vitamin D deficiency in Spain ought not to be explained solely by geographic factors; some studies have observed low levels of vitamin D in populations with an adequate, or even, abundant, exposure to sun, such as that carried out in habitual surfers in Hawaii, by Binkley et al.[39]. In our country, where there is a significant seasonal variation in levels of vitamin D between the months of greater sunshine (summer-autumn) and those with lower (winter-spring), it has been observed, however, that they scarcely normalise after the first months[28]. This insufficiency is observed in children and young people[29,30], persists in adults[31], in healthy postmenopausal women[32,33] and women with osteoporosis[36], and, logically, is patent in elderly people, both those living in their own homes as well as, even more so, in those living in residential homes[28,34,35].

3rd Vitamin D requirements

It is logical to think that the requirements for vitamin D should be those which maintain the optimum levels of 25(OH)D. However, the quantities of vitamin D which have come to be recommended until recently for the healthy population (200 UI/day from birth until 50 years of age, 400 UI for adults up to 70 years of age and 600 UI daily for those over 71 years of age) seem insufficient for purpose, as various authors have indicated[4,8,40,41]. Coming to the same conclusion are Ginde et al., who carried out a demographic study to look at the trend of vitamin D insufficiency in the population of the US[42] comparing the levels in the population studies in NHANES during the years 1988 to 1994 (18,883 people), with those recorded in people studied during the years 2001 to 2004 (13,369 people), and observing a marked decrease in levels of 25(OH)D over time.

If we take into account the different population groups, various authors already consider it necessary that children and adolescents should acquire 400 UI daily of vitamin D to reach optimum blood levels of 25(OH)D[43]. In 2008, the American Academy of Pediatrics increased the recommended daily dose of vitamin D for children and adolescents to 400 UI, and when this is not achieved through diet and exposure to sun, it should be acquired through supplements[44].

For adults, although the daily acquisition of 400 UI of vitamin D[13] has for a long time been recommended in order to reach optimum blood levels of 20-30 ng/ml (50-75 nmol/l) much higher amounts are needed , approximately 1,700 UI/day[45]. When a daily dose of 1,000 UI is given over 3 or 4 months, blood levels of 25(OH) increase 10 ng/ml, so that a subject with levels of 10 ng/ml would need 2,000 UI/daily to reach the 30 ng/ml considered to be optimum[46]. The fear of toxicity has limited the recommendations of authors, since, as we mentioned in the earlier section, the upper safety limit in order to avoid the risk of producing hypercalcemia is 150 ng/ml (375 nmol/l) of blood 25(OH)D. For some years some authors have already been recommending higher quantities, both in women and men, of between 700 to 1,000 UI[11,13,47,48,49], and others indicate that even higher daily doses, of 1,000 - 2,000 UI[50], and up to 2,600 UI, can be much more effective to achieve more adequate levels of 25(OH)D with no risk of toxicity[11]. In 2007, a panel of experts produced a consensus document for nutritional guides for vitamin D[51], and in it they stated that the maximum safe intake of vitamin D, established at 2,000 UI daily, should be re-evaluated and raised to allow the carrying out of studies which evaluate the effects of high daily doses of vitamin D in the maintenance of better general health. In the same year, Hathcock et al.[52] in a review on the safety of vitamin D based on the risk of hypercalcemia, concluded that the upper limit for the ingestion of vitamin D in adults should be 10,000 UI daily. This indicates that the safety limit is much higher than any of the recommended quantities. Very recently, the IOF in its position document recommends doses of 800-1,000 UI/day, although with subjects at risk of low blood levels of 25(OH)D (obesity, osteoporosis, malabsorption, low exposure to sunlight, etc.) these daily doses should rise to 2,000 UI[16].

In postmenopausal women, the same as with elderly people, both populations with a high risk of bone loss, the quantities which the experts recommend become higher, between 2,000 and 3,000 UI/day[26,53,54]. Bacon et al. evaluated the safety and effectiveness of high doses, such as 500,000 in a single dose, an initial dose of 500,000 UI and 50,000 UI monthly for maintenance, or 50,000 UI monthly, showing that they were both safe and effective[57].

Up until now, we have talked of the desirable requirements for healthy subjects; evidently, patients with osteoporosis should be considered in a special way, since in these patients vitamin D plays an important role in the etiopathogeny of the disease. Even though it is not clear whether vitamin D supplements alone, are sufficient to treat osteoporosis, it is recognised worldwide these patients should be supplied with sufficient quantities of vitamin D, which in the majority of cases they do not acquire through diet and exposure to sun. Later we dedicate a specific section to treatment of osteoporosis with vitamin D and its complication, fractures. Related to both osteoporosis and vitamin D, we should not forget that those patients receiving corticoid therapy. In these patients, the action of the drug results in a lower intestinal absorption of calcium, along with a higher rate of urinary elimination, which produces secondary hyperparathyroidism Although the studies carried out with regard to its effectiveness in preventing bone loss or fractures are highly heterogeneous and involve low

numbers of patients (and are therefore not generally conclusive), it is generally recognised that vitamin D (combined with calcium) should be prescribed in all those patients in long term treatment with corticoids at high doses, with the aim of maintaining bone metabolism; although it is also generally considered that they should not be prescribed alone, but with an antiosteoporotic drug (biphosphonates, teriparatide), especially when dealing with patients with high risk of fracture[56-63].

There is neither agreement nor unanimity regarding the dose of vitamin D which patients in treatment with glucocorticoids ought to receive. In the Guide on Corticoid Osteoporosis, published by the Spanish Society of Internal Medicine in 2007 it is recommended that vitamin D be administered at a dose of 800-1,000 UI/day, combined with 500-1,000 mg/day of calcium[63].

4th. Vitamin D and falls, muscle strength and balance

Apart from the well known effects of vitamin D on bone metabolism, hypovitaminosis D is also associated with muscular weakness, predominantly in the proximal musculature. It has been demonstrated in experimental studies that the metabolites of vitamin D have an influence on the maturation and function of muscle through the receptors for these metabolites which the muscle cells possess[64]. In a sample of 976 people older than 65 years of age it has been confirmed that their levels of vitamin D were inversely correlated with being in poor physical shape. Given the high prevalence of vitamin D deficiency in the older population, studies aimed at clarifying this correlation appear justified, especially since there is an ever-increasing number of elderly people in whom there will be have to be identified potentially modifiable risk factors for disability[65].

Stewart et al. recently carried out a study in 242 healthy postmenopausal women (aged between 48.8 and 60 years) with the aim of understanding the relationship between levels of 25(OH)D to obesity, risk of falls

Table 2. Levels of evidence. CEBM Oxford

Level of evidence	Type of study
1a	Systematic review of randomised clinical trials, with homogeneity
1b	Randomised clinical trials with narrow confidence interval
1c	Clinical practice ("all or nothing") (*)
2a	Systematic review of cohort studies, with homogeneity
2b	Cohort study or randomised clinical trial of low quality(**)
2c	"Outcomes research" (†), ecological studies
3a	Systematic review of case-control studies, with homogeneity
3b	Case-control study
4	Case series or cohort and case-control studies of low quality (‡)
5	Opinion of experts without explicit critical validation, or based on physiology, "bench research" or " first principles" (§)

A minus sign (-) should be added to indicate that the level of evidence is not conclusive if:
-Randomised clinical trial with wide confidence interval and not statistically significant.
-Systematic review with heterogeneity statistically significant.
(*) When all the patients die before a specific treatment becomes available, with which some patients survive, or when some patients used to die before its availability, and with it, none die.
(**) For example, with follow up low than 80%
(†) The term "outcomes research" makes reference to cohort studies of patients with the same diagnosis in whom the events which occur are related to the therapeutic measures which they receive.
(‡) Cohort study: without clear definition of the groups compared and/or without objective measurement of exposures and events (preferably blind) and/or without identifying or adequately controlling known confusion variables and/or without complete or sufficiently prolonged follow up. Case-control study: without clear definition of the groups compared and/or without objective measurement of exposures and events (preferably blind) and/or without identifying or adequately controlling known confusion
(§) The term "first principles" makes reference to the adoption of a specific clinical practice based on physiopathological principles.

Table 3. Valuation the levels of 25 (OH) D serum

	ng/ml	nmol/l
Adequate vitamin D levels	> 30 ng/ml	> 75 nmol/l
Vitamin D insufficiency	20 - 30 ng/ml	50 - 75 nmol/l
Vitamin D deficiency	< 20 ng/ml	< 50 nmol/l

1 ng/ml equivalent to 2.5 nmol/l

and muscular weakness. 19.4% had values of 25(OH)D < 50 nmol/l (20 ng/ml). For these subjects, a correlation was sought with some indicators for good physical health, such as the android fat mass, lean body mass, balance and the hand grasp strength, the strength of the torso and of the lower limbs. They found that the levels of vitamin D were correlated with all the indicators, except with the strength of the torso and lower limbs, concluding that the blood levels of 25(OH)D can be a contributor to the indices of physical health in healthy postmenopausal women[66].

The aforementioned muscular weakness associated with hypovitaminosis D, if it surpasses a certain limit, can affect functional capacity and mobility, which puts , especially elderly people, at greater risk of falls, and therefore, of fractures. The provision of vitamin D supplements to elderly people in situations of deficiency can improve muscle strength and functional capacity, which results in a reduction in falls and therefore, of non-vertebral fractures[67]. Bunout et al. evaluated the effects of resistance training and the provision of vitamin D supplements on the physical condition of 96 healthy elderly people with low levels of vitamin D, concluding that the addition of this treatment improved their walking speed and stability, while the training improved muscle strength[68].

Some authors have found that in healthy elderly people vitamin D supplements did not prevent a decrease in muscle strength due to age-related involution[64,69]. In a review carried out by Annweiler et al. the results around the association of vitamin D with physical function were contradictory[70]. Dhesi et al. carried out a study in 139 mobile subjects older than 65 years of age with a history of falls and hypovitaminosis (levels of 25(OH)D ≤ 12 µg/l), and to whom were randomly allocated either a single dose of 600,000 UI of intramuscular ergocalciferol, or a placebo. The results showed that at 6 months, the subjects who had received the vitamin D supplement had significant benefits in terms of their physical function, reaction times and balance, although not muscle strength[71]. A more recent study continued the controversy: More-Pfrimer at al. studied muscle strength in 46 institutionalised subjects ≥ 65 years of age, to whom they administered over 6 months either daily calcium plus a placebo, or daily calcium plus oral colecalciferol (initial dose of 150,000 UI monthly for two months followed by 90,000 UI monthly for 4 months), randomly allocated. At 6 months, and without having taken physical exercise, the strength of the hip flexors increased in the group which received vitamin D by 16.4% (p= 0.0001), and the strength of the extensors of the knee by 24.6% (p = 0.0007)[72]. Lips et al. carried out a study in which were assigned randomly a dose of 8,400 UI weekly of colecacliferol, or a placebo, to 226 subjects ≥ 70 years of age whose concentrations of 25(OH)D were between 6 and 20 ng/ml. To evaluate muscle function and balance their mediolateral body sway with eyes open was measured at 8 and 16 weeks with an AccuSway^{PLUS} platform and a battery of short physical exercises (SPPB, Short Physical Performance Battery) carried out. In the results obtained vitamin D did not reduce the mediolateral body sway or improve the SPPB, although by grouping the subjects according to their baseline mediolateral sway, those who had the greatest instability (≥ 0.46 cm) improved significantly when they had been treated with vitamin D for 16 weeks (p= 0.047). It is important to indicate that even though the levels of 25(OH)D increased in patients treated at 8 weeks, they did not reach adequate levels (30 ng/ml) during the whole period of the study (16 weeks)[73].

In terms of its effect on the reduction in falls, the same studies which showed that the vitamin D supplements favoured muscular function and balance, suggest that there should also be a reduction in falls, and therefore, in fractures[71]. Various meta-analyses published in recent years indicate that vitamin D supplements reduce the risk of falls in the elderly[74], although some specify that the dose should be 700-1,000 UI daily, since at lower doses (or blood concentrations < 60 nmol/l) this reductor effect is not produced, which could be as much as 22% (adjusted OR: 0.78; 95% CI: 0.64-0.92) compared with those patients who had received calcium alone or a placebo[75]. This is corroborated in a review of Cochrane carried out by Gillespie et al. who observed that vitamin D supplements do not reduce the risk of falls (RR 0.96; 95% CI: 0.92-1.01), but indicate that they may do so in those with low blood levels of vitamin D[76]. Another review carried out more recently, found that these supplements reduce the rate of falls (rate *ratio*, RaR 0.72: 95% CI: 0.55-0.95), but not the risk of falls (risk *ratio*, RR 0.98; 95% CI: 0.89-1.09) 77. To add more controversy, in a recently published study, carried out in 2,252 women of ≥ 70 years of age who were not institutionalised, to see the effect of a single high dose of 500,000 UI of colecacliferol, it was observed that the group which took the high dose of vitamin D showed an increase in the number of falls and fractures as opposed to the group which had taken the placebo[78].

On the other hand, it has been suggested that there could be an inverse relationship between levels of vitamin D and intensity of muscular-skeletal pain, which means that optimum levels of vitamin D could be useful in patients with secondary pain due to osteoporotic complications[79,80].

In this section we should make special mention of the effect vitamin D supplements can have in patients affected by multiple sclerosis (MS). Originally, the hypothesis that a sufficient supply could prevent the disease was established to explain its geographic distribution; and since then, hypovitaminosis D has been considered one of the environmental risk factors for MS[81-83]. However, recently, studies have been carried out which have shown an association of low blood levels of 25(OH)D with the prevalence of MS, the risk of suffering MS, its incapacity and the frequency between two breaks[84,85].

Table 4. Reference studies with drugs used in the treatment of osteoporosis in postmenopausal women. Principal objective: incidence of fractures

Drug	Study name	Year	First author (ref.)	Group treated	Calcium and vitamin D	Monitoring
Etidronate	---	1990	Storm (101)	Women with OP postmenopausal	Calcium and vitamin D (quantities ND)	3 years
Alendronate	FIT	1996	Black (102)	Postmenopausal women with BMD with FxV/without FxV	Calcium carbonate (500 mg/day of calcium element) and vitamin D (250 UI/day) if diet low in calcium (< 1,000 mg/day)	3 years
Risedronate	VERT	1999/2000	Harris / Reginster (103/104)	Postmenopausal women < 85 years with at least 2 FxV or one FxV and low BMD (T-score < -2)	Calcium carbonate (1,000 mg/day) and vitamin D (500 UI/day) if 25(OH) vit D < than 16 ng/ml or 40 nmol/l	3 years
	HIP	2001	McClung (105)	Women of 70-79 years of age and osteoporosis; or aged ≥ 80 years with at least one clinical risk factor for hip Fx		3 years
Ibandronato	BONE	2004	Chesnut (106)	Postmenopausal women with T-score ≤ -2 in at least one lumbar vertebra and between 1 and 4 FxV	Calcium (500 mg/day) and vitamin D (400 UI/day)	3 years
Zoledronate	HORIZON	2007	Black (107)	Women with densitometric OP with T-score < -2.5 without fractures; or T-score < -2.5 and ≥ 1 FxV	Calcium (100-1.500 mg/day) and vitamin D (400-1.200 UI/day)	3 years
Raloxifene	MORE	1999	Ettinger (108)	Women with ≥ 2 years of menopause with densitometric OP	Calcium (500 mg/day) y cholecalciferol (400-600 UI/day)	3 years
Teriparatide	---	2001	Neer (109)	Postmenopausal women with at least one FxV	Calcium (1.000 mg/day) and vitamin D (400-1.200 UI/day)	3 initial years (19 months)
PTH 1-84	TOP	2007	Greenspan (110)	Postmenopausal women of between 45 and 54 years with T-score < -3; or T-score < - 2.5 plus 1-4 FxV	Calcium citrate (700 mg/day) y vitamin D (400 UI/day)	18 months
Strontium ranelate	TROPOS	2005	Reginsterb (111)	Postmenopausal women with T-score < -2.5; or if > 70 years, also with 1 risk factor for Fx	Calcium (>1.000 mg/day) and vitamin D (400-800 UI/day)	5 years (preliminary 3 years)
	SOTI	2004	Meunier (112)	Postmenopausal women (>5 years), aged > 50, with at least 1 FxV and BMD ≤ 0.840 g/cm^2	Calcium (>1.000 mg/day) and vitamin D (400-800 UI/day)	3 years
Calcitonin	PROOF	2000	Chesnut (113)	Postmenopausal women with established OP	Calcium (1.000 mg/day) and vitamin D (400 UI/day)	5 years

OP: osteoporosis; BMD: bone mineral density; Fx: fracture; FxV: vertebral fracture; ND: unavailable

However, its therapeutic potential in established MS has not yet been sufficiently studied. A recently published work by Burton et al. was carried out in 49 patients with MS of average age of 40.5 years and average blood levels of 25(OH)D of 78 nmol/l, with an average EDSS (Expanded Disability Status Scale) of 1.34. They were randomly assigned to a placebo group (n= 24), or to treatment with a dose which was increased step by step until it reached 40,000 UI/day at 28 weeks, thus rapidly raising their blood levels of 25(OH)D and thereby evaluating its tolerability. The dose was then maintained at 10,000 UI daily over 12 weeks, subsequently being reduced to 0 UI/day (n= 25). During the whole study 1,200 mg/day of calcium was given. In spite of blood levels of 25(OH)D reaching a peak of 413 nmol/l, there were no significant adverse effects. The group treated with vitamin D appeared to have fewer breaks and a persistent reduction in the proliferation of T lymphocytes, compared with the control group. Despite recognising that there were confusion variables in the clinical results, the authors concluded that high doses of vitamin D supplements had an evident immunomodulator effect on the MS, in addition to being safe[86]. All the researchers considered it necessary that more studies be carried out in this area.

5th. Calcium and vitamin D supplements in the prevention and treatment of osteoporosis

There is near unanimity among researchers in concluding that vitamin D on its own is insufficient for the prevention of osteoporotic fractures[87-91], and those who find a positive impact on the risk of fracture indicate that this improves with the addition of calcium, with a reduction of 30% in this risk[92]. The same occurs with calcium supplements, which are considered insufficient in themselves in reducing the risk of hip fracture[93-94], although some have obtained results which indicate that calcium supplements alone are sufficient to reduce the risk of fractures in general[95], and even vertebral fractures[96].

But the majority of studies and researchers concede that supplements of calcium plus vitamin D have a positive effect in the reduction of risk of fractures in the region of 20%[89-91,97]. And this is demonstrated in various meta-analyses on this subject. Thus, Bischoff-Ferrari et al. published a meta-analysis in 2005 in which they analysed the effect of calcium and vitamin D on the prevention of hip and non-vertebral fractures. The authors observed that at a dose of 700-800 UI/day of vitamin D, the reduction in the risk of hip fracture was 26% (relative risk, RR: 0.74; 95% CI: 0.61-0.88) and for non-vertebral fractures, 23% (RR: 0.77; 95% CI: 0.68-0.87), while for lower doses of vitamin D, below 400 UI/day, no protection against fractures was observed[98].

Subsequently, Boonen et al. delved deeper into the earlier meta-analysis of Bischoff-Ferrari and found that in 4 randomised clinical studies, which included 9,083 patients, the relative risk for hip fracture was not statistically significant (RR: 1.10 95% CI: 0.89-1.36). Instead, in the 6 randomised studies in which calcium and vitamin D were administered, which included a total of 45,509 patients, the risk of hip fracture was reduced by 18% (RR: 0.82; 95% CI: 0.71-0.94). No heterogeneity was observed between the studies, and an adjusted indirect comparison of the combined relative risks of both meta analyses found a reduction in risk of fracture of 25% in those patients who had received calcium and vitamin D as against those who had only taken vitamin D (RR: 0.75; 95% CI: 0.58-0.96)[91].

More recently, Tang et al. carried out another meta-analysis using 29 randomised studies which included a total of 63,897 patients, analysing both the reduction in the relative risk of all fractures and the increase in bone mineral density. Studying those publications in which the principal objective was the reduction in risk of fracture, 17 studies were included with a total of 52,625 patients. In these patients a reduction of 12% in the risk of suffering new fragility fractures (RR: 0.88; 95% CI: 0.83-0.95; p= 0.0004) was found, and they concluded that the evidence supported the use of calcium, or calcium combined with a vitamin D supplement in the treatment of osteoporosis in people of 50 years of age or over, and that for a maximum therapeutic effect a dose of 1,200 mg/day of calcium and 800 UI/day of vitamin D was necessary[48].

According to the last clinical practice guides, for it to be able to be effective in the prevention of risk of fracture, the minimum daily dose of supplements recommended are 1,000-1,200 mg of calcium element, plus 800 UI (or 20 µg) of vitamin D_3[99,100]. To guarantee the necessary intake of calcium, should it be needed, it recommended that this be through food, whenever possible.

Administration of calcium and vitamin D jointly with other antiosteoporotic drugs

An adequate provision of calcium and vitamin D is essential when a treatment with any antiosteoporotic drug is prescribed, be it antiresorptive, anabolic or of mixed action. Given the difficulty in achieving this through diet and exposure to the sun alone, it is necessary to administer jointly supplements of calcium and vitamin D. In all the clinical trials which have been carried out with the different antiresorptive drugs to demonstrate their antifractural efficacy, supplements of calcium and vitamin D were administered to all participants, which indicates that this efficacy has not been demonstrated in the absence of correct levels of calcium and vitamin D. In Table 4 we show these trials and the quantities of calcium and vitamin D which were administered[101-113].

2nd PART. RESPONSES TO THE QUESTIONS RAISED. RECOMMENDATIONS

1.- What are the optimum levels of vitamin D?

- We consider that the optimum levels of vitamin D should be between 30 and 75 ng/ml, and that levels below 20 ng/ml are clearly pathological.

2.- Are levels of vitamin D in the Spanish population adequate?

- No. The majority of the Spanish population does not achieve optimum levels of vitamin D. Depending on the type of population studied and the cut off point, the prevalence of vitamin D deficiency (< 20 ng/ml) varies between 30% in young people and 87% in institutionalised elderly people, with the ages in between and the non-institutionalised elderly at between 50% and 70%.

3.- What are the requirements for vitamin D?

- In general, they are those which ensure optimum levels of vitamin D in the blood. The means of acquiring these optimum levels may be through adequate exposure to sun, foods and vitamin D supplements.

- In specific situations, the panel makes the following recommendations, although they take the view that when there is vitamin D deficiency, higher doses are necessary to achieve optimum levels:

- Children, adolescents: 400-600 UI/day.
- Postmenopause: 600-800 UI/day.
- Elderly people: 800-1,000 UI/day.
- Patients with osteoporosis: 800-1,000 UI/day.
- Patients with fracture: 800-1,000 UI/day. On the basis of a high prevalence of serious vitamin D deficiency in patients with osteoporotic fractures of the hip, the panel considers it advisable to perform an assessment of levels of vitamin D, and when this not possible it recommends the use of a higher dose.
- Patients receiving corticoids: 800-1,000 UI/day.

4.- Vitamin D and falls, muscle strength and balance

- The panel estimates that in the special case of institutionalised elderly people, due to the great difficulty in their achieving necessary levels of vitamin D through hygiene-dietetic measures, the requirements should be met through vitamin D supplements.

On the other hand, we cannot conclude that the provision of vitamin D improves muscle strength.

5.- Treatment with vitamin D. Alone, or always with calcium?

a. – In the prevention of osteoporosis:

- The panel considers that the prevention of osteoporosis should be carried out through good hygiene-dietetic habits (adequate exposure to sun, consuming foods rich in calcium). The use of calcium and vitamin D drugs are not indicated for this task, save in those cases where there is a difficulty in obtaining optimum levels of these substances, when they should be supplemented pharmacologically.

b. – In the treatment of osteoporosis in itself.

- The panel considers that there is no evidence that exclusive treatment with calcium and vitamin D has antifractural efficacy, except in specific populations, such as institutionalised elderly people.

c. – Jointly with other antiosteoporotic drugs.

- When using an antiosteoporotic drug supplements of calcium and vitamin D should always be added. However, the panel considers that in those patients in whom there is a guarantee of an adequate provision of calcium in the diet, the use of a calcium supplement is not necessary.

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