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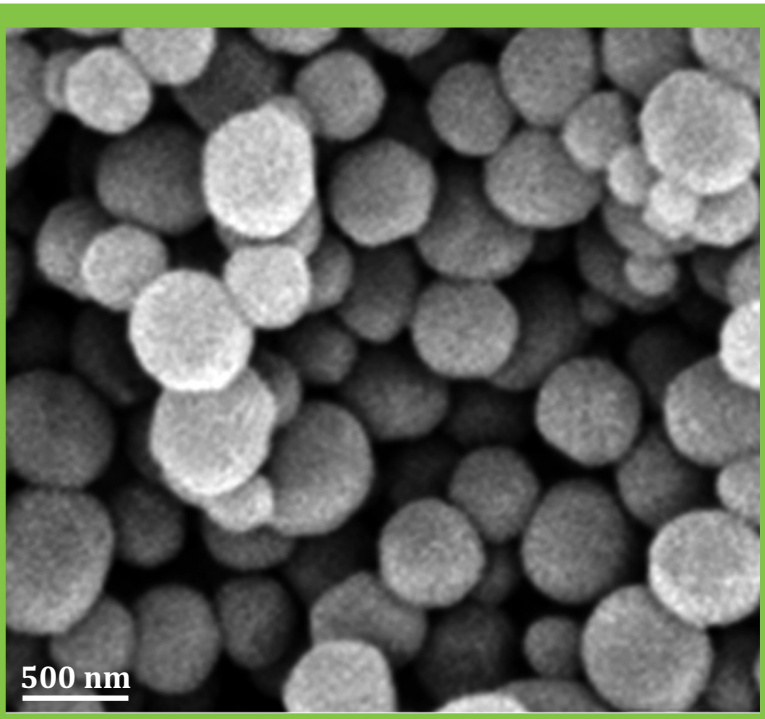

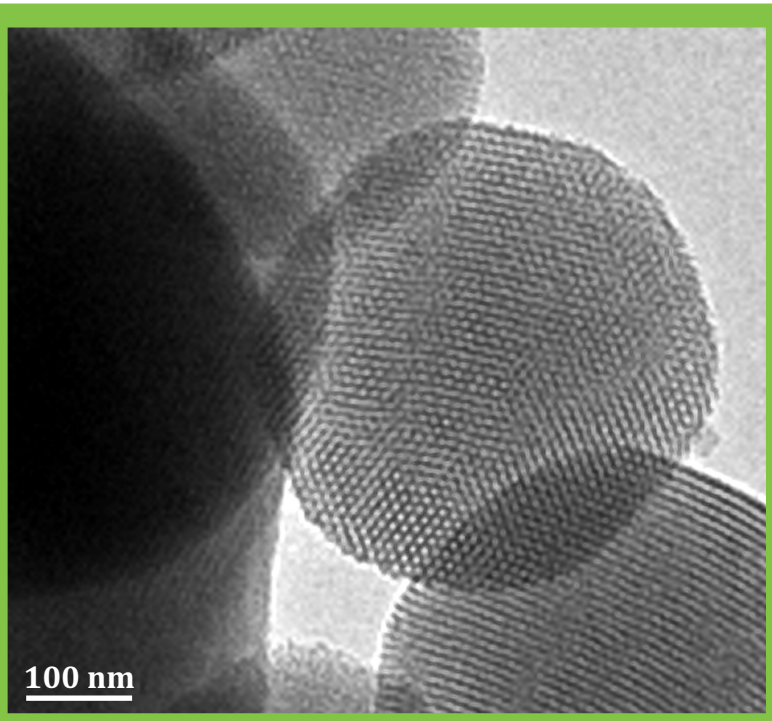

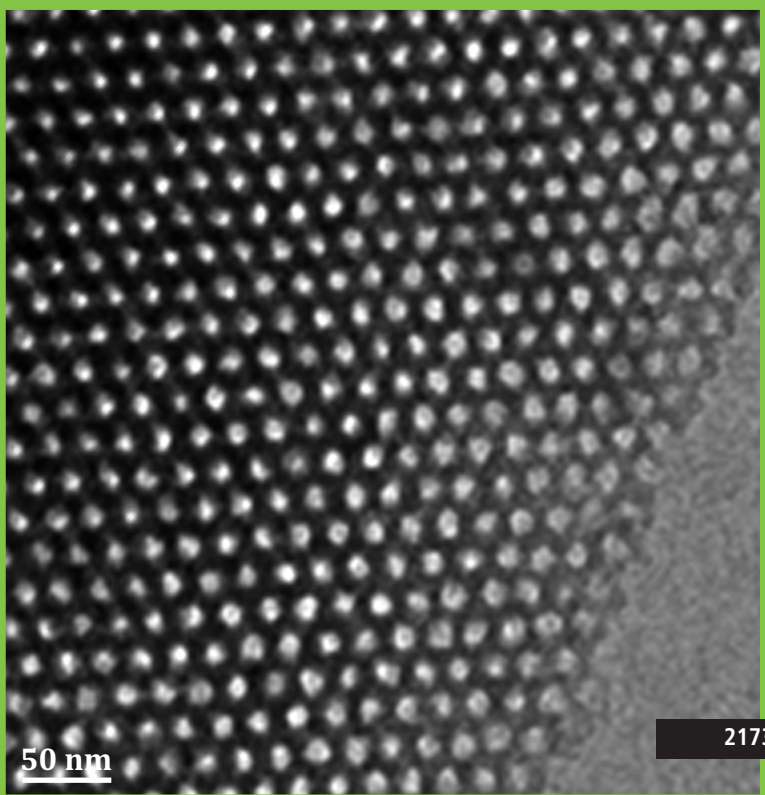

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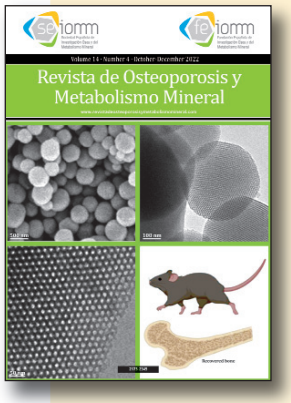

Our cover: Images of mesoporous silica nanoparticles taken by scanning and transmission electron microscopy.
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Summary

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Mesoporous silica nanoparticles and osteoporosis

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Nanomedicine

The application of nanotechnology in medicine has given rise to a new discipline: nanomedicine, which, as we can imagine, is a multidisciplinary field where many main actors take part: engineers, physicists, chemists, biologists, doctors and even legislators[1]. Nanomedicines are so popular today among the scientific community due to a series of factors, among which we would highlight the control over the pharmacokinetic profile, the protection of transported therapeutic agents against possible degradation within the organism, the possibility of developing targeted therapies towards specific tissues, the possibility of including different therapeutic agents in the same transporter and even the possible inclusion of contrast agents to have a biomedical image for diagnosis. In this sense, among the possible systems of drug release, nanoparticles have acquired great prominence since they present great versatility from the point of view of their composition, shape, size, and external surface[2].

This makes them the focus of a large number of biomedical researches for either the treatment of certain diseases, their prevention, diagnosis, or even in tissue engineering.

Mesoporous silica nanoparticles

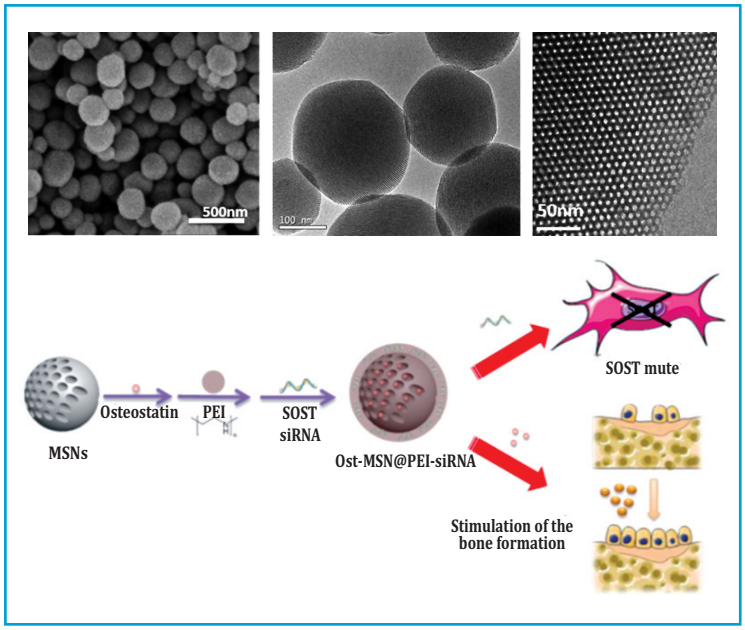
Among all the nanoparticles used in the field of nanomedicine, whether organic or inorganic, mesoporous silica nanoparticles (MSNs) are being widely studied in recent years as transport vectors for therapeutic agents[3].

Their notoriety could be attributed their robustness, their porous system's very high load capacity, their ability to protect drugs against possible degradation and the ease with which their outer surface can be modified[4]. The synthesis of these nanoparticles is based on the combination of 3 methods: the sol-gel process, in which the hydrolysis and condensation of the silica precursors that will form the three-dimensional web of SiO_2 takes place; the use of surfactants as directing agents of the structure as templates, so that the silica condenses around the structures created by these surfactants which, after their elimination, will prompt a network of cavities or mesoporous structures; and the use of high dilution conditions, as proposed by the Stöber method, which allows to obtain spherical shaped nanoparticles of very defined diameters, between 100 and 150 nm, and with pores of around 2 nm, available to be loaded with therapeutic agents. In this sense, the concept of introducing drugs inside the pores of mesoporous materials to later be released into the organism was first proposed by Professor María Vallet-Regí in 2001. This has inspired considerable research conducted by different groups around the world[5]. In this sense, our research group has worked hard in recent years to develop MSNs capable of accumulating in different tissues in a specific way and releasing loaded drugs in response to different stimuli[6] (figure 1).

Treatment of osteoporotic situations

Among all the possible applications, an interesting example in which we have worked on in recent years is the use of mesoporous silica nanoparticles for the possible treatment of osteoporosis[7,8].

Figure 1



Top: Scanning and transmission electron microscopy images of mesoporous silica nanoparticles. Bottom: Strategy to coat the nanoparticles with a cationic polymer to transport the siRNA that will silence the SOST gene and stimulate new bone formation.

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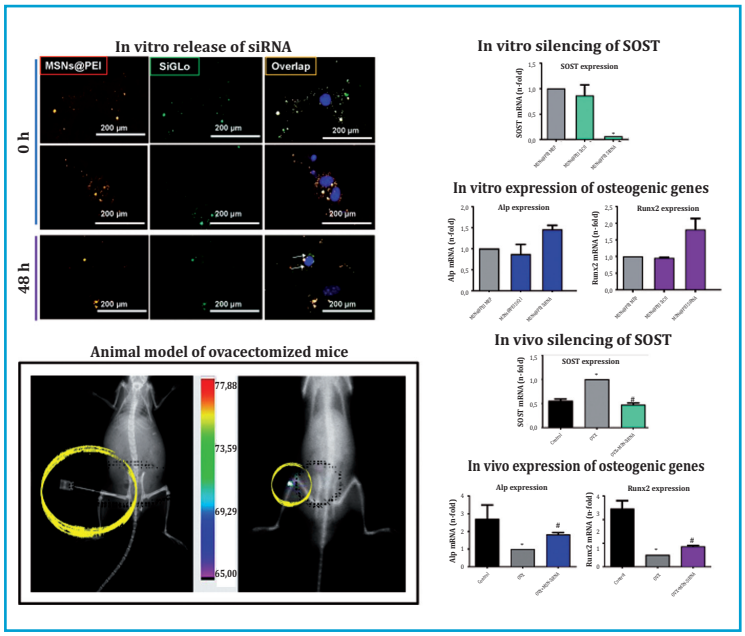
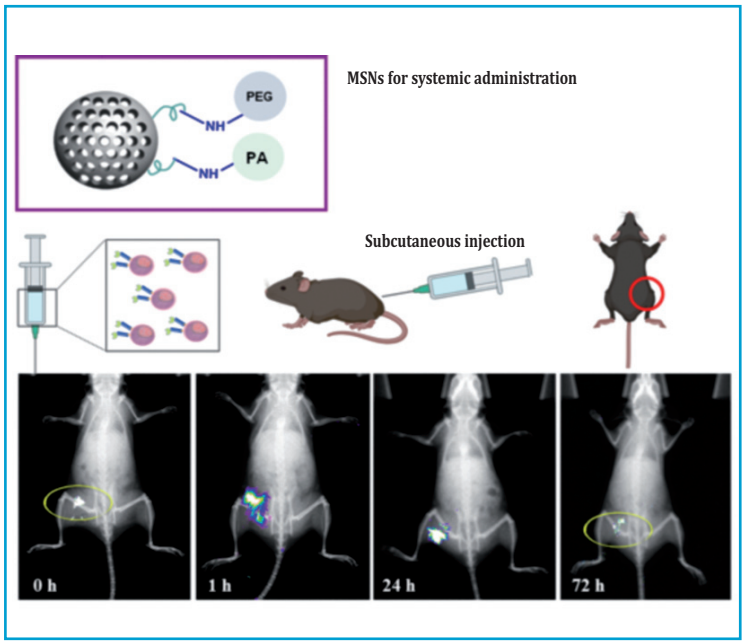
Figure 2



Top: Release and in vitro silencing studies of the SOST gene and the expression of other osteogenic genes. Bottom: Administration of the nanoparticles by femoral bone marrow injection in an osteoporotic female mice model and subsequent in vivo studies of SOST gene silencing and expression of other osteogenic genes.

Figure 3



Top: Design of the nanoparticles for systemic administration by subcutaneous injection. Bottom: Images at different times of the injected animal where the permanence of the nanoparticles in the area up to 72 hours after injection can be noticed.

In an osteoporotic setting, the WNT/β-catenin signaling pathway, which normally participates in the differentiation of mesenchymal stem cells to osteoblasts, is inhibited. This happens due to the overexpression of the sclerostin protein, which is encoded by the SOST gene. The consequence of this inhibition is the reduction of osteoblastic differentiation and, as a consequence, the reduction of bone formation and loss of bone mass so characteristic in osteoporosis. Our working hypothesis has been based on the possibility of silencing the SOST gene using a small interfering RNA (siRNA), in order to reduce sclerostin expression and to be able to reactivate the WNT/β-catenin cycle, and thus reactivate osteoblastic differentiation and consequently increase bone formation, so important in osteoporotic scenarios. However, siRNAs have very short life spans and very poor ability to penetrate cell membranes, so their biggest problem is their administration, and that is where our nanoparticles acquired great relevance. We coated our MSNs with a cationic polymer capable of retaining the siRNA on their surface to be later released, silencing the SOST gene and causing increased bone formation.

After optimizing the system in terms of siRNA design and loading, we found that our nanoparticles were capable, not only, of releasing siRNA inside cells, but also of reducing SOST expression in vitro and increasing other osteogenic factors' SOST expression, such as alkaline phosphatase (ALP) and RUNx2 (figure 2).

We also had very positive results by injecting our nanoparticles into the bone marrow of the femur in osteoporotic female mice, observing the reduction in the expression of the SOST gene and the consequent increase in the expression of certain osteogenic genes.

Taking into account the difficulty of an administration by injection into the bone marrow of the femur could entail in a future clinical application, we decided to redesign the system. To do so, MSNs with high affinity for bone tissue were developed by anchoring bisphosphonates on their surface, which are molecules with a high affinity for the inorganic content of bone, in order to administer them by subcutaneous injection, a much easier method, so the nanoparticles themselves preferentially accumulate in bone tissue. In fact, we observed that after 72 hours of injection, the nanoparticles were still in the area (figure 3).

After different treatments lasting 2 to 3 weeks with successive subcutaneous injections every 72 hours, real-time PCR analysis of the different gene expressions in the bone tissue of osteoporotic animals revealed that we were able, not only of silencing the gene SOST, but also of increasing the expression of various osteogenic genes, such as Runx2, Alp, Osterix and Osteoprotegerin, as well as increasing vascular endothelial growth factor (VEGF), which relates to an increase in vascularization, all this factors pointing at the formation of new bone tissue (figure 4).

In line with the results obtained, the microcomputed tomography analysis revealed significant increases in bone mineral density and bone mineral content, which, together with the quality of the new bone formed, similar

to healthy bone as revealed by trabecular thickness and trabecular separation, gave us significant results in terms of new bone formation and quality in an in vivo model of osteoporotic female mice (figure 5).

This way, this brilliant investigation showed the skills of mesoporous silica nanoparticles in a possible treatment of osteoporosis, proving their capacity to load, transport, protect and intracellularly release a particular type of nucleic acid: a siRNA to silence a specific gene responsible for a malfunction in bone tissue development. These results open the doors for this type of MSNs to be used for carrying a large number of nucleic acids, including all types of RNAs or DNAs, with a wide variety of final applications within the biomedicine of the future.

Acknowledgments: The authors thank Dr. Patricia Mora-Raimundo for her work in this area. Her thesis was based on this type of application of mesoporous silica nanoparticles. The authors acknowledge funding from the European Research Council (Advanced Grant VERDI; ERC-2015-AdG) project number 694160 and the Ministry of Science and Innovation through project PID2019-1064 36RB-I00.

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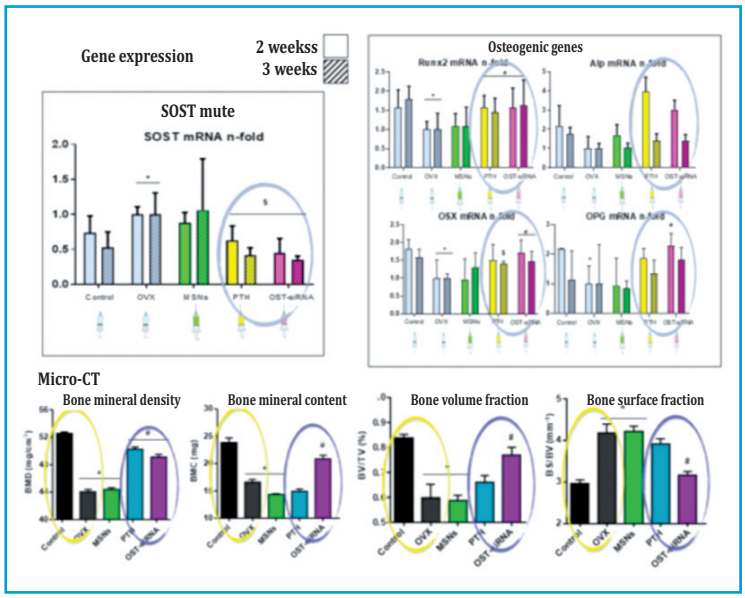
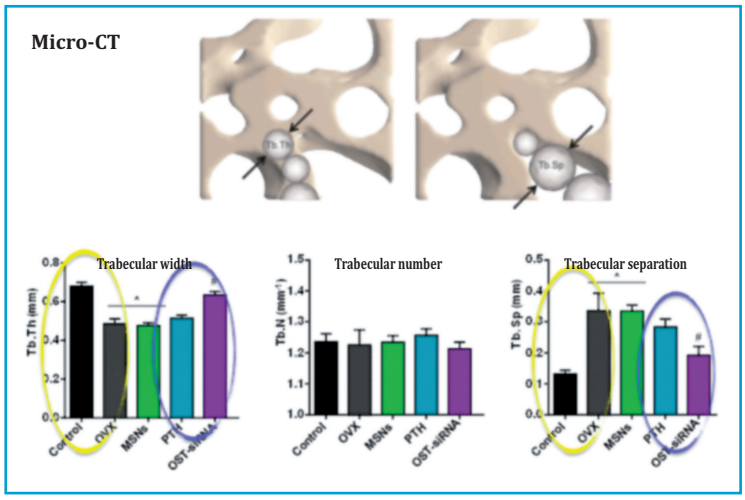
Figure 4



Top: Gene expression results in vivo after administration of the nanoparticles. Bottom: in vivo micro-CT results after administration of the nanoparticles.

Figure 5



In vivo micro-CT results after administration of the nanoparticles

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Teriparatide in the substitutive treatment of chronic hypoparathyroidism. About a case

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Summary

Objetive: To report experience in the use of teriparatide as an effective replacement therapy for chronic hypoparathyroidism.
Material and methods: The clinical case of a patient with post-surgical chronic hypoparathyroidism who previously presented difficult control with conventional treatment (calcium salts and calcitriol) is presented, for which teriparatide was started as a substitute treatment.
Results: The patient presented analytical values of phosphocalcic metabolism compatible with normality from the 4th week of treatment with teriparatide, allowing the suspension of previous treatments and maintaining good control one year after the change in therapy.
Conclusions: Teriparatide is an effective option for treating chronic hypoparathyroidism. We have observed a latency phase until the hormonal effect begins, so we recommend frequent analytical monitoring and gradually reduce treatment with calcitriol and calcium salts for adequate control.

Key words: teriparatida, hipoparatiroidismo, crónico, postquirúrgico, tratamiento.

Introduction

The clinical management of chronic hypoparathyroidism (using calcium salts and active vitamin D analogs) is currently less common than other hormone replacement treatments in endocrinological deficits, where treatment is based on deficient hormone administration. The treatment objectives indicated in the clinical practice guidelines mark albumin-adjusted calcium target levels at or slightly below the lower limit of the reference range (0.5 mg/dL) in order to avoid chronic complications derived from conventional chronic treatment (hypercalciuria due to the lack of effect of PTH on renal calcium reabsorption), with associated nephrolithiasis and calcifications at various levels (central nervous system, ophthalmological, renal,etc)[1,2].

In the past two decades, studies have been carried out to establish the effectiveness and safety of replacement therapy for hypoparathyroidism with PTH 1-84 (parathyroid hormone) and PTH 1-34 (teriparatide) due to poor clinical and biochemical control of chronic hypoparathyroidism with conventional treatment[3-5]. However, NATPAR® (PTH 1-84) has received the resolution of NO FINANCING IN SPAIN[6].

Clinical case report

We present the case of a 28-year-old man with postoperative chronic hypoparathyroidism since the age of 17 after total thyroidectomy for T4N1bM1 papillary thyroid carcinoma.

He was treated with calcium carbonate, a dose ranging between 6 and 8 grams of elemental calcium, calcitriol with a dose ranging between 0.5 and 1.5 mcg per day, cholecalciferol in a variable dose to maintain calcidiol levels between 20-30 ng/ml and magnesium salts, between 8 and 16 mEq of magnesium element daily. As initial complications, he suffered several crises of tetany

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secondary to discontinuing treatment. Once compliance with calcium salts and active vitamin D analog stabilized, he presented high phosphate levels (around 5.8-7.2 for reference values of 2.5-4.5 mg/dl), with calcium phosphorus above 55 on more than one occasion. Hyperphosphataemia was controlled by reducing the doses of calcitriol and spacing calcium carbonate doses to every 4 hours, managing to optimize its chelating effect. It appeared, however, due to the interference of calcium salts in the absorption of levothyroxine, great difficulty in adjusting the suppressive dose despite administering it weekly to minimize the effects of calcium salts on the intestinal absorption of thyroid hormone.

In this context, the indications of Brandi et al.[2] to start parathormone replacement therapy were reviewed and it was decided in 2018 to request PTH 1-84 as compassionate use treatment in this patient, which was not authorized after the 2019 AEMPS resolution.

Alternatively, and after reviewing the existing scientific evidence[3,4] it was decided to use subcutaneous (sc) teriparatide as an alternative. In these studies, the replacement dose reportedly ranged between 0.3 and 0.8 mcg/kg day.

Prior to commencing treatment, the patient was being treated with calcitriol 0.5 mcg daily, calcium carbonate 2g/8h, magnesium 16 mEq daily with the following analytical control: calcium adjusted to albumin 7.64 mg/dl (8.2-10.2), phosphate 5.9 mg/dl (up to 4.5), phosphocalcium: 46.02

Following the REPLACE[5] study indications and the technical data sheet for PTH 1.84[7] treatment was started with teriparatide 20 mcg sc at night, reducing the dose of calcitriol by 50% (to 0.25 mcg/day) and calcium carbonate (to 1 g every 8 hours) and the magnesium salt dose to 4 mEq daily. Analytical controls were carried out every 72 hours, experiencing a significant decrease in albumin-adjusted calcaemia (6.4 mg/dl) during the first week, maintaining similar levels of phosphate (5.7 mg/dl) and with normal figures. of magnesium (1.83 mg/dl), so the dose of teriparatide was doubled to 20 mcg sc every 12 hours. According to a recent study, a dose of 20 mcg teriparatide does not seem to be enough to control the condition[8].

With the change, there was a progressive rebound in the analytical values, presenting the following analytical values 7 days after starting teriparatide 20-0-20 (mcg): albumin-adjusted calcium 8.8 mg/dl (8.4 -10.2), phosphate 4.8 mg/dl (2.4-4.4), magnesium 1.62 mg/dl (1.6-2.6) with detectable urinary magnesium, normal renal function and calcitriol 32.7 ng/dl. It was decided at that time to suspend calcitriol and magnesium salt, leaving the patient under treatment with teriparatide 20 mcg sc every 12 hours and calcium carbonate 1 g/8 hours orally. After four weeks, he presented a practical normalization of the analytical values, so the calcium carbonate was suspended, leaving only teriparatide 20 mcg sc every 12 hours. In the following controls, the patient presented analytical values of phosphocalcic metabolism compatible with normality until reaching the year of treatment without presenting adverse effects, presenting a good tolerance of the drug with the 20 mcg/12h dose (table 1).

Discussion

Replacement treatment for chronic hypoparathyroidism with intact parathyroid hormone (PTH 1-84) or its active fraction (PTH 1-34) represents a novel aspect in managing this condition. The PTH 1-84 molecule has a complete clinical development (phases I, II, III, IV) based on the REPLACE studies and FDA approval for its use, while the management of chronic hypoparathyroidism with teriparatide lacks a similar clinical development, with only phase III studies to date. Knowledge about its effects in the management of this disease is based on results in series of patients with congenital hypoparathyroidism. There are no clinical studies directly comparing both molecules. In our case, teriparatide dose escalation was necessary to maintain calcaemia within target ranges, maintaining a replacement dose within the ranges described in the literature

Table 1. Evolution of the different parameters studied according to the prescribed treatment

Treatment followed	Time	Calcium (mg/dL)	Albumin (mg/dL)	Ca ajusted albumin (mg/dL)	Phosphate (mg/dL)	Magnesium (mg/dL)	FG (mL/min/ 1.73m²)
Calcitriol 0.5 mcg daily + Calcium carbonate 2 g/ 8h, + Magnesium salt 16 mEq/day	**0**	7.8	4.2	7.6	5.9	1.72	90
Teriparatide 20 mcg/24h SC + Calcitriol at 0.25 mcg/day + Calcium carbonate at 1 g every 8 hours + Magnesium salt 4 mEq/day	**1st week**	6.7	4.4	6.4	5.7	1.83	90
Teriparatide 20 mcg/12h SC + Calcium carbonate at 1 g every 8 hours	**5th week**	8.8	4.3	8.5	4.8	1.62	90
Teriparatide 20 mcg/12h SC	**10th week**	9.1	4.3	8.8	4.5	1.68	90
Teriparatide 20 mcg/12h SC	**Year**	9.1	4.2	8.9	3.9	1.63	90

(0.3-0.8 mcg/kg/day). This dose escalation was also seen in the REPLACE study for PTH 1-84 (77% of patients needed to increase the dose of PTH 1-84 from 50 mcg daily to 75 or 100 mcg daily to achieve the primary objective (reduction of the dose of calcitriol and calcium salts by 50%, maintaining good biochemical control). Independence from conventional treatment is also described with both molecules (43% of the patients treated with PTH 1-84 in the REPLACE study were able to discontinue the treatment with calcitriol maintaining calcium supplements providing less than 500 mg of elemental calcium per day).

Indeed, our patient responded well to treatment with teriparatide that allowed us to progressively suspend the calcitriol and magnesium salt treatment until finally suspending calcium carbonate and maintaining a normalization of the analytical values, remaining only with teriparatide 20 mcg sc every 12 hours. In the following controls, the patient presented analytical values of phosphocalcic metabolism compatible with normality until reaching the year of treatment.

As a limitation of our study, we note that the effect of teriparatide on calciuria in our patient has not been quantified, an aspect of great clinical relevance in managing these patients. Furthermore, the effects of teriparatide on bone and mineral density and markers of bone remodeling have not been evaluated.

Conclusions

Teriparatide has been an effective option for the replacement treatment of chronic hypoparathyroidism using a dose that corresponds to that described in the literature (0.5 mcg/kg/day, divided into two doses). A latency period has also been observed until the full effect of the medication is reached, which has allowed us to completely suspend the previous treatment, requiring frequent monitoring and gradual de-escalation of treatment with calcitriol and calcium salts for adequate control.

Conflict of interests: The authors declare no conflict of interest.

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Low-density granulocytes: A new marker of bone deterioration in patients on peritoneal dialysis

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Summary

Objetive: In kidney patients, bone-metabolic disease, systemic inflammation and malnutrition exacerbate the risk of vascular calcification (VC) and morbidity and mortality. Given the strong association between VC and fragility fractures, the objective of this study is to assess the contribution of the major determinants of VC to bone deterioration in patients on peritoneal dialysis (PD).

Methods: In 31 non-diabetic patients on PD (>6 months), markers of alterations in bone metabolism, vascular damage, inflammation and malnutrition, and their impact on bone deterioration (radiological osteopenia and/or history of fragility fracture) were studied.

Results: In these patients (20 men and 11 women; age=54±15 and 60±11 years, respectively (p=0.24)), the prevalence of fragility fractures was 5% in men and 27% in women. Bone deterioration was greater in older people, females, high Charlson and Kauppila indexes, lower muscle mass and with expansion of a highly inflammatory subpopulation of immature low-density granulocytes (iLDG). A logistic regression analysis showed that bone deterioration risk is more influenced by the female sex than by age and that, of the multiple factors associated with greater bone deterioration studied, only the expansion of iLDG estimates the risk of bone alterations in these patients regardless of age and sex.

Conclusion: The expansion of iLDG provides an accurate biomarker for the diagnosis of bone deterioration and to monitor strategies that attenuate its progression in PD patients of any age and sex.

Key words: Fragility fractures, bone metabolism, vascular calcification, inflammation, cardiovascular risk, malnutrition.

Introduction

The concept of metabolic bone disease associated with chronic kidney disease (CKD-MBD, Chronic Kidney Disease-Mineral and Bone Disorder) to refer to bone abnormalities (osteodystrophy), laboratory (calcium, P, vitamin D, parathyroid hormone –PTH–, fibroblast growth factor 23 –FGF23– and Klotho) and vascular (VC) or soft tissue calcification that occur in kidney patients has been established since 2007 and converge in an increase in cardiovascular risk (CVR), risk of fracture and, in short, an excess of morbidity and mortality[1,2].

Chronic inflammation plays a very important role in the development of vascular disorders in kidney patients and previous studies identified the importance of low-density granulocytes (LDG) in vascular calcification in dialysis patients compared to the control population[3], as well as in CVR of patients with lupus[4].

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The association between vascular calcifications and fragility fractures is established both in the general population and in kidney patients. Severe aortic calcification is associated with a greater number and severity of fractures[5]. This is a worrying fact since cardiovascular disease (CVD) is the primary cause of death in patients with CKD, this being 15 to 30 times higher than that of the general population when adjusted for age[6]. The pathophysiology of the so-called "bone-vessel axis" is complex and several factors are involved, including age, malnutrition and systemic inflammation, a non-traditional CVR factor aggravated in kidney patients due to accelerated aging, with an increase in cells senescent that promotes an inflammatory state that "synchronizes" the deterioration of multiple organs and systems, conditioning the grouping of various degenerative diseases[7].

In patients on peritoneal dialysis and hemodialysis, there are changes in leukocyte subpopulations such as decreases in angiogenic T cells (Tang), protectors of vascular homeostasis, increases in immuno-senescent cells (CD4+CD28null)[8], as well as an aberrant expansion of a subpopulation of immature low-density granulocytes (LDG CD14-, CD16-, CD15+) related to a greater propensity for VC in advanced CKD[3].

The increase in bone fragility observed with age (senile/postmenopausal osteoporosis) develops independently of CKD. Thus, it can be present in patients with CKD, with normal or slightly reduced renal function, and even coexist with CKD-MBD after being established. Added to this is the effect of CKD itself on bone quality and microstructure, previously defined as renal osteodystrophy, together with factors associated with uremia that some authors describe as uremic osteoporosis[9]. In addition, to the usual risk factors for osteoporosis in the general population, disorders in nutritional status, physical activity, the underlying disease itself and the taking of drugs that interfere with bone metabolism are added in CKD[10].

In renal patients, the three factors frequently coexist: malnutrition, inflammatory state and atherosclerosis, known as MIA syndrome, with a great negative impact on survival[11]. The evidence suggests that the decrease in food intake and the increase in acute phase reactants and inflammatory cytokines can be seen from early stages of CKD[12]. The same happens with atherosclerosis[13]. This combination results in marked cardiovascular damage that determines hospital admissions and mortality.

Our study is aimed at evaluating the contribution of changes in some cell populations of the immune system involved in vascular homeostasis and in other CV risk factors, such as malnutrition, to bone deterioration in a cohort of patients undergoing peritoneal dialysis.

Patients, material and methods

Participants

We recruited 31 patients with CKD stage 5 over 18 years of age, undergoing peritoneal dialysis (CKD5-PD) at the Nephrology Clinical Management Unit, Hospital Universitario Central de Asturias (HUCA), Spain) who gave their informed consent. Diabetic patients with a history of cardiovascular events (abdominal aneurysm, intermittent claudication and previous carotid surgery), immunosuppressive treatment, pregnancy, diagnosis of immune-mediated disease, cancer, diabetes mellitus, recent or current infections less than 3 months were excluded. These exclusion criteria limited the ability to recruit patients, but they were chosen with the aim of identifying early inflammatory mediators of vascular damage associated with PD and their possible impact on bone health.

Lab tests

Blood samples were obtained by venipuncture. Automated serum biochemical parameters, lipid analyzes and complete blood counts were performed in the Laboratory of Medicine (HUCA) with routine laboratory methods. Circulating Klotho levels were determined using an ELISA assay (Ref. 27998, human soluble a-Klotho; IBL Immuno-Biological Laboratories Co, JAPAN). For other determinations, serum or plasma samples were stored at -80°C until further analysis. Residual renal function (RRF) defined as mean urea and creatinine clearance ((CCr + CU)/2) in 24-h urine >1 mL/min) was also assessed.

Analysis of cell populations of the immune system in peripheral blood

Peripheral blood samples were immediately processed for peripheral blood mononuclear cells (PBMC) by centrifugation (1900 rpm, 20 min) on Ficoll TM density gradients (Lymphosep, Biowest, Germany) for identification of LDG. The CD4+CD28null population was analyzed directly in peripheral blood samples.

PBMC were treated with FcR blocking reagent (Milteny Biotech, Germany) for 20 minutes at 4°C to prevent nonspecific binding of antibodies to Fc receptors, followed by incubation with CD14 FITC (Immunostep, Spain), CD15 PE-Cy7 (Milteny Biotech), CD16 APC-Cy7 (BioLegend, Germany), CD4 PE (Immunostep) and CD28 APC-Cy7 (Thermo Fischer, Germany) or corresponding isotype control antibodies for 30 min at 4°C. After a wash with PBS. Cell types were analyzed by flow cytometry on a Canto II Flow Cytometer (BD Biosciences) equipped with FACS Diva 6.5 software. The identification of leukocyte cell subpopulations was carried out through the expression of their specific markers. Immature LDGs were detected by their CD14-, CD16-, CD15+ LDG phenotype and their values are expressed as % CD15+. Senescent CD4+ lymphocytes were identified by their CD4+ CD28null phenotype and their values are expressed as % of CD4+, according to the method described by Rodríguez-Carrio et al.[3].

Quantification of circulating cytokines

Circulating levels of IL-10, IL-6, IL-2, TNF-α, and IFN-γ were measured in serum samples using a multiplex assay (BiolegendPlex, BioLegend), following the protocol provided by the manufacturer. Detection limits were 1.2 pg/ml (IL-10 and IL-2) or 2.4 pg/ml (IL-6, TNF-α and IFN-γ).

Bone deterioration

Although only bone densitometry allows accurate quantification of bone mass, patients were stratified considering bone deterioration by virtue of the presence of radiological osteopenia and/or a history of fragility fracture. Plain scans of the dorsal and lumbar spine in lateral projection were evaluated and osteopenia was defined as the decrease in density of the vertebral body of the central trabecular bone and of the horizontal trabeculae, since the vertical ones are affected in more severe osteopenias, with the consequent highlighting of the

cortices. Vertebral fractures were identified following the Genant criteria. Peripheral fractures were confirmed by medical report or review of radiographs. An experienced radiologist carried out the scan readings.

Body composition

Body composition was assessed by electrical bioimpedance (BIA) using Fresenius Medical Care's BCM®, Body Composition Monitor. Measurements were obtained at a frequency of 50 Hz, with the patient in the supine position and after draining the PD fluid. The software returned the data of resistance ®, reactance (Xc), total body water (TBW), intracellular water (ICW), extracellular water (ECW), ECW/TBW, liters (OH), lean tissue mass (LTM Kg), lean tissue index (LTI Kg/m^2), adipose tissue mass (ATM, Kg), fat tissue index (FTI Kg/m^2), body mass index (BMI Kg/m^2) and phase angle (PhA) at 50Hz

PhA is a bioelectrical measure of cellular health that is calculated taking into account the state of hydration and the degree of tissue cellularity; and, it constitutes a marker of sarcopenia, oxidative stress, inflammation and vascular calcification in dialysis patients, and it is the BIA parameter that best predicts survival in CKD[14-19].

Ethical statements

This study has been approved by the Institutional Review Committee (Regional Clinical Research Ethics Committee, reference PI17/02181), in compliance with the Declaration of Helsinki. All participants gave their written informed consent before study inclusion.

Statistic analysis

The descriptive analysis is shown as percentages (%), means (X) and standard deviations (SD). For the analysis of the differences between the clinical and biochemical parameters, and their association with vascular calcification, the statistical tests of T-Student, Chi-square test, multiple logistic regression analysis and non-parametric tests (U-Mann Whitney) when necessary, with a confidence interval (CI) of 95% and considering a value of $p<0.05$ as statistically significant. Statistical analysis was carried out using SPSS 26.0 for Windows.

Results

Our study included 31 patients, 20 men and 11 women, aged 54±15 and 60±11 years, respectively (p=0.24). The prevalence of fragility fractures was 5% in men and 27% in women, all of them with vertebral fractures and, in one of the patients, also with two peripheral fractures.

Table 1 shows the clinical parameters, analytical determinations of bone metabolism related to CKD, as well as the nutritional and inflammatory parameters studied, the etiology of CKD, medical treatments with metabolic-bone repercussions and the differences between patients with or no bone loss. As expected, the patients with bone deterioration were older, with a significant predominance of women [26.32% men and 77.78% women (p=0.01)], with a slightly higher Charlson Index and with a lower LTI. No significant differences were found between patients with or without bone deterioration in the analytical parameters linked to CKD-MBD, including PTH levels, except for soluble Klotho, which was higher in patients with bone deterioration (p=0.03). This lack of linkage may be due to the categorical and inaccurate estimation of bone deterioration and, above all, to the sample size. In addition, until 2017 the relevance of DXA in the systematic evaluation of patients with CKD-MBD was not included and it was not part of the routine of clinical parameters, unlike the radiological evaluation[2].

Vitamin D deficiency/insufficiency is notable in both groups. The mean values of 25-hydroxy-vitamin D in patients supplemented with nutritional vitamin D (38.7%) were 8.4 [6.8, 18.3] ng/ml, compared to 9.3 [5.4, 15] ng/ml in those not supplemented, both levels in the range of vitamin D deficiency (<20 ng/ml). In 48.4% of the patients receiving active vitamin D (calcitriol or paricalcitol), the mean vitamin D values were 9.3 [5.4, 12.4] ng/ml, similar to that of the group of patients supplemented with nutritional vitamin D.

As for quantification of vascular calcifications, the Kauppila index was 6 times higher in patients with bone deterioration (p<0.01).

Regarding nutritional parameters, no significant differences were observed in the levels of total protein, albumin, PhA, phase angle or BMI. Only the LTI was lower in patients with bone deterioration.

Of all the leukocyte cell subpopulations studied, a significant elevation was only observed in the population of immature LDG CD14-, CD16-, CD15+ LDG in the group of patients with bone deterioration and, although there was a trend towards higher values in CD4+ CD28null lymphocytes , did not reach statistical significance.

Regarding the etiology of CKD and the medical treatments they were receiving at the time of the study, no differences were found between the patients associated with bone deterioration.

In the logistic regression analysis analyzing bone deterioration as a dependent variable with respect to age and sex, it was the female sex (OR 10.41; 95% CI: 1.52-119.30; p=0.03) variable with greater weight, while age was at the limit of significance (OR 1.09; 95% CI: 1.01-1.22; p=0.05).

When the independent contribution of each of the identified risk parameters to bone deterioration was analyzed, adjusting for age and sex, only immature LDG (LDG CD14-, CD16-, CD15+) turned out to be independent predictors both in raw data and adjusted for age, by sex and by both together (table 2). It should be noted that no differences in circulating neutrophils were observed between patients with or without bone deterioration. The CD14-, CD16-, CD15+ LDG immature LDG subset also did not correlate with absolute neutrophil counts (p>0.05, data not shown).

It is also important to note that all the changes in the differentially expressed parameters in patients with bone deterioration, such as the increases in the Charlson or Kauppila Index and in the density of immature LDG CD14-, CD16-, CD15+ LDG, as well as the LTI are correlated significantly with age (r=0.68, p=0.00; r=0.48, p=0.01; r=0.36, p=0.04; r=-0.40, p= 0.02) (figure 1).

Discussion

This study shows for the first time the association between the expansion of a subpopulation of immature LDG CD14-, CD16-, CD15+ LDG and bone deterioration in peritoneal dialysis patients. These increases had been previously linked to a greater propensity for vascular calcification.

The number of patients included, although it may seem small, was highly conditioned by the exclusion criteria aimed at selecting patients in whom early markers of in-

Table 1. Clinical, CKD-MBD, nutritional and immunological characteristics in patients with CKD5-DP, discriminated by the presence or absence of bone deterioration

	Yes N=14 (45.2%)	No N=17 (54.8%)	p value
Clinical parameters			
Gender (M/F)	5/9	15/2	**0.02**
Age (years) (mean (SD))	62.6 (9.5)	51.9 (15.3)	**0.02**
Charlson Index (median [IQR])	6 [3, 7]	4 [2, 6]	ns
Time on dialysis (months)(median [IQR])	18 [11, 32]	15 [9, 48]	ns
Residual renal function (Yes/No)	12/2	12/5	ns
CKD-MBD			
Calcium, mmol/L (mean (SD))	2.18 (0.18)	2.18 (0.17)	ns
Phosphorus, mmol/L (mean (SD))	1.69 (0.35)	1.64 (0.42)	ns
Magnesium, mmol/L (median [IQR])	0.81 [0.77, 0.95]	0.87 [0.67, 1.01]	ns
PTH, pg/mL (median [IQR])	386 [230, 453]	300 [214, 600]	ns
Vitamin D 25 OH, ng/mL (median [IQR])	10.1 [5,1, 18,6]	8 [6, 13]	ns
Alkaline phosphatase, U/L (median [IQR])	106 [81, 143]	89 [71, 127]	ns
Klotho, pg/mL (median [IQR])	1.79 [0.82, 2.83]	0.79 [0.64, 1.20]	**0.03**
Kauppila Index (median [IQR])	6 [1, 16]	0 [0, 4]	**<0.01**
Nutritional parameters			
Total protein, mg/dl (mean (SD))	63 (6)	63 (7)	ns
Albumin, mg/dl (mean (SD))	37 (3)	36 (5)	ns
LTI, Kg/m^2 (median [IQR])	13.1 [11.4, 17]	16.3 [14.5, 18.5]	**0.04**
FTI, Kg/m^2 (mean (SD))	14.8 (8.3)	10.1 (6.7)	ns
Phase angle, º (mean (SD))	4.9 (1.1)	5.6 (1.1)	ns
BMI Kg/m^2, (mean (SD))	28.6 (7.1)	27.1 (5.4)	ns
Inflammatory parameters			
CRP, mg/dl (median [IQR])	0.3 [0.1, 1.7]	0.3 [0.1, 0.7]	ns
IFN γ, pg/mL (median [IQR])	7.33 [3.80, 15.50]	7.82 [5.88, 11.19]	ns
TNFa, pg/mL (median [IQR])	8.13 [4.13, 13.07]	8.50 [7.76, 12.48]	ns
IL-2, pg/mL (median [IQR])	1.60 [1.32, 2.30]	1.52 [1.26, 1.91]	ns
IL-6, pg/mL (mean (SD))	11.26 (5.09)	11.31 (9.01)	ns
IL-10, pg/mL (median [IQR])	1.71 [1.36, 2.30]	1.91 [1.36, 4.78]	ns
CD4+CD28null, % CD4+ (mean (SD))	10.88 (4.53)	8.02 (2.97)	ns
Tang, % lymphocytes (mean (SD))	1.65 (0.69)	1.96 (0.71)	ns
Immature LDG[log], % CD15+ (mean (SD))	-0.44 (0.4)	-0.12 (0.4)	**<0.01**
DEFA3 [log] (mean (SD))	0.07 (0.41)	-0.12 (0.67)	ns
Etiology			
Glomerular, %	50%	23.5%	ns
Interstitial nephritis, %	0%	5.9%	ns
Unaffiliated, %	14.29%	35.29%	ns
Others, %	0%	11.76%	ns
Pyelonephritis, %	0%	5.88%	ns
PQHR, %	14.29%	11.76%	ns
Nephroangiosclerosis, %	21.43%	5.88%	ns
Treatments			
Nutritional vitamin D	38%	41.1%	ns
Active vitamin D	50%	47%	ns
Cinacalcet	35.7%	29.4%	ns
Phosphorus binders	64.28%	47%	ns
Corticosteroids	17.6%	18.75%	ns

The variables are expressed as mean±SD, %. The differences were analyzed using the Student's t, Mann-Whitney's U or chi 2 tests according to the normal or non-normal distribution in the analyzed variable. PTH: parathormone; LTI: lean tissue index; FTI: fat tissue index; BMI: body mass index; IFN γ: interferon gamma; TNFa: tumor necrosis factor alpha; IL: interleukins; CD4+ CD28null: senescent cells; Tang: angiogenic T cells; Immature LDG [log]: immature low-density granulocytes: logarithmic transformation; DEFA3[log]: defensin 3: logarithmic transformation; PQHR: hepatorenal polycystosis. Significant values are highlighted in bold.

Table 2. Risk factors for bone deterioration adjusted for age and sex in patients with CKD5-PD

	Raw analysis		Adjusted for age		Adjusted for sex		Adjusted for age and sex	
	Odds ratio [95% IC]	p	Odds ratio [95% IC]	p	Odds ratio [95% IC]	p	Odds ratio 95% IC]	p
Charlson Index	1.4 [0.97-2.01]	0.07	1.13 [0.71-1.82]	0.60	1.52 [0.96-2.41]	0.07	1.22 [0.68-2.21]	0.50
Kauppila score	1.52 [0.96-2.41]	0.07	1.05 [0.93-1.17]	0.44	1.11 [0.98-1.25]	0.10	1.06 [0.93-1.21]	0.39
LTI	1 [0.93-1.07]	0.92	1 [0.93-1.07]	0.94	0.99 [0.91-1.07]	0.78	0.99 [0.91-1.07]	0.78
LDGi [log]	8.57 [2.58-52.39]	**0.00**	10.5 [2.5-92.5]	**0.01**	13.21 [2.71-179.6]	**0.01**	15.6 [2.6-300.2]	**0.02**
CD4+ CD28null	1.27 [0.97-1.68]	0.08	1.14 [0.84-1.54]	0.39	1.31 [0.96-1.79]	0.09	1.18 [0.84-1.66]	0.33
Sex	13.5 [2.15-84.69]	**0.00**	15.63 [2.01-121.47]	**0.01**				
Age	1.07 [1-1.15]	**0.04**			1.09 [0.99-1.19]	0.07		

Logistic regression analysis between each of the risk factors for bone damage without adjustment or adjusted for age, sex or age and sex. CI: confidence interval; p: statistical significance; LTI: lean tissue index; LDG [log]: immature low-density granulocytes CD14-, CD16-, CD15+: logarithmic transformation; CD4+ CD28null: senescent T lymphocytes. Significant values are highlighted in bold.

flammation and their role in VC pathogenesis and bone deterioration could be determined, without interference from other factors processes or in advanced stages.

Regarding the general characteristics of the study population, the distribution by sex is similar to the general distribution of dialysis patients (2/3 men and 1/3 women)[20]. As might be expected, bone deterioration, despite being a qualitative variable, was predominant in females, as was the higher prevalence of fractures.

The risk of fracture increases as chronic kidney disease (CKD) progresses and the type of renal replacement therapy (RRT) influences its behavior. Patients with CKD G3-G5, those on dialysis (G5D) and those who are kidney transplant recipients have a fracture incidence of 2 to 100 times higher than the general population of the same age and sex[2]. Thus, the incidence of fractures triples in hemodialysis (HD) and doubles in peritoneal dialysis (PD) when compared to renal transplantation[21]. In fact, it has been seen that vertebral fractures are prevalent in up to a quarter of patients on HD[22] and a meta-analysis showed that HD increases the risk of hip fracture by 60% when compared to PD[23].

In our study, 13% of PD patients presented fragility fractures, being mostly women (75%). The same influence of sex was seen when assessing osteopenia. By grouping fractures and osteopenia under the term “bone deterioration”, we found that this was three times higher in women, although they represented only a third of the participants. Female sex is known to be a predictive factor for fracture[24]. In the study carried out by Naylor et al. During 3 years of follow-up, it was seen that up to 10% of women and 5% of men with CKD stage 5 had at least one fracture[25].

Along with gender, age was also different among patients with bone deterioration, 10 years more, similar to the DOPPS study data[26]. However, Maravic et al. showed that women on dialysis who present hip fracture are younger than the general population[24]. This fact reinforces the concept that CKD is a conditioning factor for developing early aging that includes the skeleton.

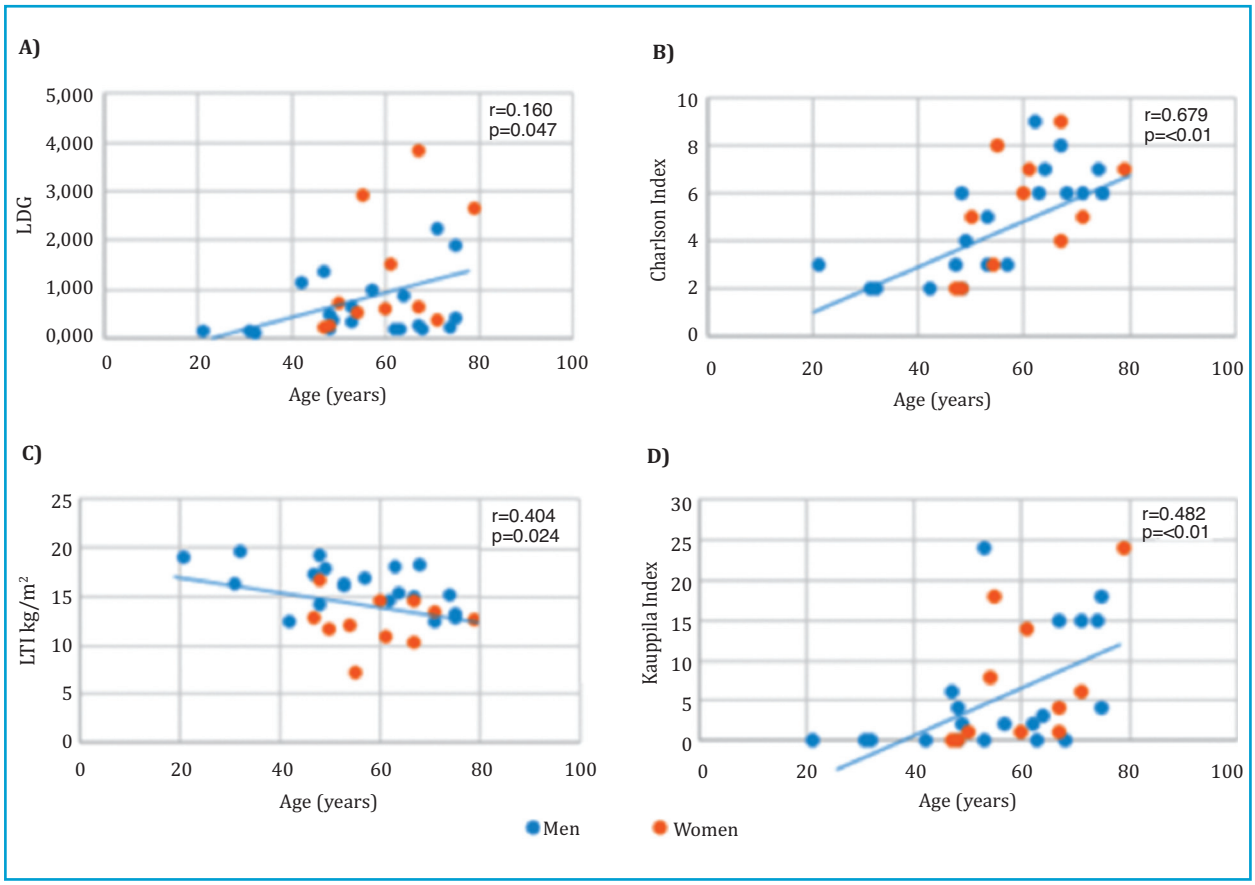
In the general population and in PD, comorbidity has been associated with the risk of fracture[27]. Patients who had bone deterioration presented a higher Charlson Index, these findings being prevalent in men.

On the other hand, the prevalence of malnutrition in PD patients is up to 56%[28]. It has been seen that protein malnutrition predominates in them as a result of low intake, protein loss through dialysis fluid and increased protein catabolism,which in turn leads to adynamic bone disease[29].

In dialysis units, BIA use is becoming more frequent to assess our patients’ nutritional status. We are mainly interested in lean mass, since the association between sarcopenia and osteoporosis, called "sarco-osteoporosis"[30], is known. The risk of fractures is greater due to the risk of falls that conditions the weakness induced by sarcopenia[31,32]. Our study concurs with research showing that the lower the lean tissue index, the greater bone deterioration, primarily in men. Verschueren et al. studied 679 men, among whom 11.9% had sarcopenia and a 3-fold higher risk of developing osteoporosis[33].Therefore, it would be worthwhile to encourage the more widespread use of BIA measurements in nephrology consultations from early stages of CKD.

In our study, the elevated Kauppila Index in patients with bone deterioration is consistent with the concept that bone demineralization is associated with vascular mineralization. However, the Kauppila Index did not have an independent contribution of age and sex as a possible determining factor of bone deterioration. There was also no evidence of an association between bone deterioration and markers of bone metabolic activity, in line with other studies where only PTH was weakly associated with the risk of fractures in dialysis patients[27,34]. Interestingly, soluble Klotho was higher in those with bone deterioration. Although the precise effect of Klotho on bone is unknown, it is known that deletion of the Klotho gene exclusively in osteocytes increases, rather than decreases, the rate of bone formation[35,36]. The impact of local actions of soluble Klotho is an area of great interest in nephrology, but still with many limitations due to the variability among commercial assays available to quantify circulating levels of Klotho. Given the anti-inflammatory and anti-aging actions of circulating Klotho, it would be important to confirm whether changes in blood levels have a favorable influence on attenuating leukocyte actions that affect the propensity for bone deterioration and CV. At this time, the favorable impact of increases in soluble Klotho on immune, renal, and vascular protection has been demonstrated only experimentally.

Figure 1. Association of age and sex with risk factors for bone deterioration in patients with CKD5-PD



Dispersion graphic. The correlation of age and sex with A) LDG: immature low-density granulocytes, B) Charlson Index, C) LTI, lean tissue index, and, D) Kauppila Index is shown. Each circle represents a study subject.

The clearly deficient vitamin D status and similar levels of 25-hydroxy-vitamin D among the patients who received or not supplementation, with respect to those treated with active metabolites, reflect the scant concern of nephrologists regarding the importance of correcting vitamin D deficiency in kidney patients, correction impossible to achieve with the administration of active vitamin D. The levels of 25-hydroxy-vitamin D in patients receiving these treatments rule out a possible induction of the degradation of nutritional vitamin D due to excessive doses of the active form[37] (table 1).

Of the most commonly used immune cell subpopulations to assess vascular damage, only exacerbated expansion of immature LDG CD14-, CD16-, CD15+ LDG was associated with bone deterioration. In the context of systemic inflammation, it has been shown that immature CD14-, CD16-, CD15+ LDGs are released early from the bone marrow through transcortical vessels when there are increases in osteoclast-mediated bone resorption[38]. Although CD14-, CD16-, CD15+ immature LDGs are a minor fraction of neutrophils, in a study of 2586 men, individuals with lower bone mineral density (BMD) had higher neutrophil counts,suggesting an inverse association between bone health and rate of granulopoiesis[39]. In fact, Terraciano et al found that postmenopausal women with low BMD had high concentrations in saliva of a type of defensin released by neutrophils called DEFA1[40].

The contribution of increases in circulating immature LDG CD14-, CD16-, CD15+ LDG to the degree of vascular calcification in patients on peritoneal dialysis and hemodialysis, also demonstrated the usefulness of measurements of the levels of messenger RNA of defensin 3 (DEFA3) in leukocytes circulating mononuclear cells, as an accurate marker of early granulopoiesis with expansion of immature LDGs LDG CD14-, CD16-, CD15+3. However, in the latter, defensin 3 messenger RNA levels are not associated with greater bone deterioration.

Limitations

Our study's greatest limitation is that bone deterioration was not evaluated with the gold standard, DXA, but by the presence of radiological osteopenia and/or fragility fractures. Thus, the result was a categorical variable, with less capacity to show a gradation of effects.

In addition, the small sample size due to the exclusion criteria, although it is appropriate to determine early effects and not interference due to other processes that alter immunity, may limit the real quantification of the influence of other variables.

Conclusions

The most important contribution of this study is the demonstration of the usefulness of measurements of the circulating density of immature LDG CD14-, CD16-, CD15+ in estimating bone deterioration regardless of the patient's age and sex. This makes circulating immature LDG CD14-, CD16-, CD15+ LDG an early marker of bone disorders in kidney patients that could allow a proactive attitude to be taken both in diagnosis and in decision-making to attenuate its progression.

Our study is in sync with current knowledge that bone deterioration is greater in elderly people, females, with a high Charlson Index, and in those with less muscle mass. On the other hand, we realize that it may open new avenues to deepen research into the pathogenic mechanisms of CKD-MBD in earlier stages of CKD, with a larger sample of patients and using DXA for an adequate validation of these findings.

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Delphi Consensus on Therapeutic Strategies and Health Prevention of hypovitaminosis D

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Summary

Background: The high prevalence of hypovitaminosis D in Spain is considered a genuine epidemic with crucial health implications due to the multiple functions that vitamin D exerts both at the skeletal and extraskeletal levels. In order for people with insufficiency or deficiency in vitamin D to reach the most adequate serum levels, they must receive vitamin D supplements. This study was carried out to evaluate whether, in routine clinical practice, hypovitaminosis D management was done in accordance with international recommendations established by scientific societies.

Methods: Two rounds of a Delphi questionnaire were carried out among a panel formed by experts who regularly prescribe vitamin D.

Results: In general, the physicians on the panel recognized the high prevalence of hypovitaminosis D in Spain, the need for screening in the different risk groups and the benefits of supplementation in patients with insufficient or deficient vitamin D. However, no consensus was reached on some of the statements related to vitamin D quantification methods or recommendations for managing hypovitaminosis D.

Conclusions: The lack of agreement for some of the items revealed the need to carry out training actions aimed at providing adequate and updated knowledge about the scientific evidence and recommendations for the clinical practice of vitamin D supplementation.

Key words: Delphi consensus, vitamin D supplementation, vitamin D, hypovitaminosis D, skeletal and extraskeletal actions.

Introduction

Vitamin D is an essential hormone for skeletal metabolism, since it regulates the absorption of calcium and phosphorus at the intestinal level and bone remodeling[1,2]. In addition, some studies suggest that vitamin D performs other multiple functions at the extraskeletal level, acting as a protector against diseases such as cancer, inflammatory and autoimmune diseases, diabetes and cardiovascular diseases[1-4].

The main source of vitamin D is synthesis at the skin level by the action of ultraviolet B rays (UVB) on its precursor[2], giving rise to cholecalciferol or vitamin D3. Another less important source of cholecalciferol is found in food, mainly fish, eggs and dairy products. Regardless of its origin, cholecalciferol must be hydroxylated in the liver, becoming 25-hydroxyvitamin D3 [25(OH)D] or calcifediol and, subsequently in a highly regulated manner, in the kidney to give rise to the active metabolite, 1, 25-dihydroxyvitamin D3 [1,25(OH)2D] or calcitriol[1].

Serum levels of 25(OH)D offer the best biomarker to assess vitamin D levels, since its plasma concentration and half-life are higher than those of 1,25(OH)2D[2]. However, there is no clear consensus on the optimal levels of 25(OH)D in serum[3].

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Recent observational studies have revealed a high prevalence of hypovitaminosis D worldwide[5], associated with an increased risk of skeletal and extraskeletal diseases, due to the multiple functions of vitamin D.

In Spain, the prevalence of vitamin D deficiency is at least 80% among people over 65 years of age and up to 40% in the population under 65[6], despite the high degree of sunshine available, which should facilitate synthesis of vitamin D. Therefore, hypovitaminosis D has become a recognized epidemic with important health implications, so that a large portion of the population could benefit from vitamin D supplements.

In order to determine if hypovitaminosis D is diagnosed, treated and prevented in accordance with international recommendations and scientific evidence, current clinical practice of vitamin D supplementation has been analyzed based on the knowledge of those physicians who regularly prescribe supplementation.

Material and methods

The data presented in this study were obtained using the Delphi method[7]. To do so, our scientific committee prepared a questionnaire that was completed by a panel of experts made up of doctors from different specialties and geographical areas, those who regularly prescribe of calcifediol and/or cholecalciferol. After two rounds of circulation of the survey, the scientific committee met to collect, analyze and discuss the results.

Preparation of the questionnaire

Based on the current knowledge of hypovitaminosis D and its clinical consequences, as well as therapeutic and prevention strategies and diagnostic methods, the multidisciplinary scientific committee identified a total of 73 variables related to hypovitaminosis D and divided into 4 thematic blocks:

1. Vitamin D and general health (21 items)
2. Evaluation of vitamin D deficiency (26 items)
3. Treatment with vitamin D according to the patient's profile (19 items)
4. Differences between supplements (7 items)

Expert panel

The project was directed by a scientific committee of 10 vitamin D experts from different areas of specialization: endocrinology, rheumatology, nephrology, gynecology, internal medicine, primary care, dermatology, digestive and geriatrics.

In all, 180 specialists were invited to participate in the study, having fulfilled the following criteria: a minimum of 5 years of clinical experience, experience prescribing vitamin D on a regular basis, belonging to centers that serve heavily populated areas and, in the case of primary care, belonging to centers located in areas with high population density.

A first group of participants in the panel (40%) were chosen directly by the authors of the study taking into account the inclusion criteria. The remaining 60% was completed with the invitation to experts, through the delegates of the study sponsor, always respecting the established inclusion criteria.

Analysis of results

The participants prepared the questionnaire using an online platform. As in all Delphi questionnaires, the survey consisted of a series of statements. Respondents expressed their level of agreement with the each statement based on a numerical 1-to-9 scale (≤3, disagree; 4-6, doubtful; ≥7, agreement).

The median of the scores and the percentage of positioning were analyzed. Consensus was reached when less than a third of the respondents positioned themselves outside the region of three points that contained the median. Otherwise, when these respondents showed conflicting opinions (equivalent positioning in the extreme sectors of the scale) or when there was a greater dispersion of opinions (equivalent distribution of positioning in the three sectors of the scale), it was considered that there was no there was consensus due to polarization or indeterminacy, respectively. Items that did not reach consensus in the first round were kept in the second circulation round of the survey, the results of which were analyzed as described above.

To formalize the questionnaire, they were assigned a period of 26 days for the first round and 11 days for the second.

The data provided by the participants were subject to a confidentiality clause and were only used for statistical purposes with no dissemination by any channels.

Results

In our study, 146 experts participated out of the 180 invited (81%) in the first circulation round of the Delphi survey with the following distribution by specialties: nephrology 9, rheumatology 27, geriatrics 10, endocrinology 23, family and community medicine 39, gynecology and obstetrics 9, internal medicine 9, digestive system 9, pediatrics 1, dermatology 9 and urology 1. Of the initial 146 experts, 125 participated in the second round (85.6% participation compared to the first round). The 21 experts who withdrew in the second round did so due to lack of availability or compatibility with other professional activities. This panel of experts included representatives from different specialties and geographical areas as shown in the figure 1.

In the first round, 73 statements were analyzed, of which 47 (64.4%) reached consensus in agreement. The remaining 26 statements, 25 undetermined and one with polarized opinions, went on to the second round of circulation of the survey. In this phase, a new level of agreement was reached, reducing to 16 (21.9%) the non-consensual statements (figure 2), of which 14 remained indeterminate and 2 with polarized opinions. Therefore, after completing the second round, the level of consensus was obtained in agreement on 57 (78.1%) of the 73 statements in the survey (figure 2).

Block 1, *Vitamin D and health in general*, reached the greatest consensus. The respondents agreed with 19 (90.5%) of the 21 statements that made up this block (table 1), 16 in the first round and 3 in the second, while the remaining 2 (9.5%) were indeterminate. by dispersion or non-positioning of the experts.

In block 2, *Evaluation of hypovitaminosis D*, consensus was obtained on a total of 19 statements, 16 in the first round and 3 in the second, which corresponds to 73.1% of the 26 proposals (table 2). Of the 7 statements that did not reach consensus, 5 (19.2%) were indeterminate and 2 (7.7%) showed polarization in the position of the respondents at the end of the second round.

Block 3, *Treatment with vitamin D according to the patient's profile*, is the one that obtained a lower degree of consensus. The percentage of agreement was 68.4%,

Figure 1. Characteristics of the panel of physicians who participated in the study. (A) Distribution of doctors according to their specialty

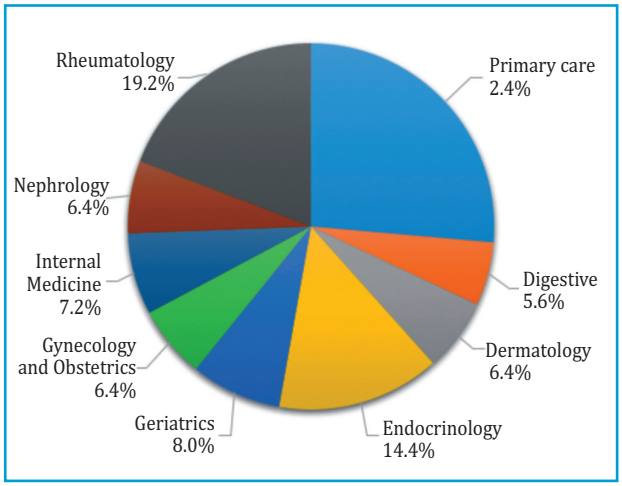


Figure 1. Characteristics of the panel of physicians who participated in the study. (B) Distribution of doctors according to the Autonomous Community in which they practice their profession

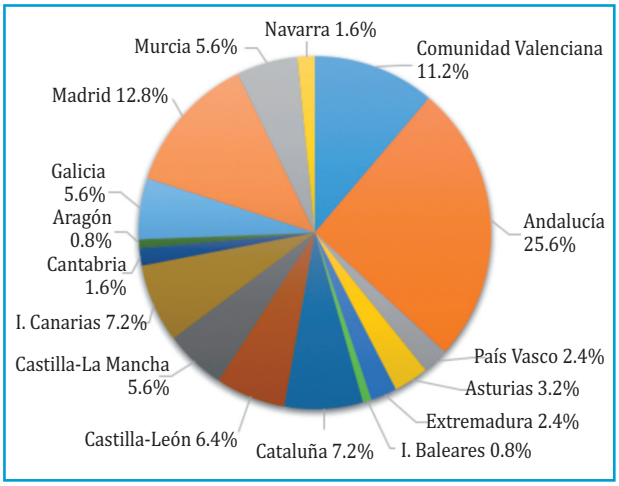
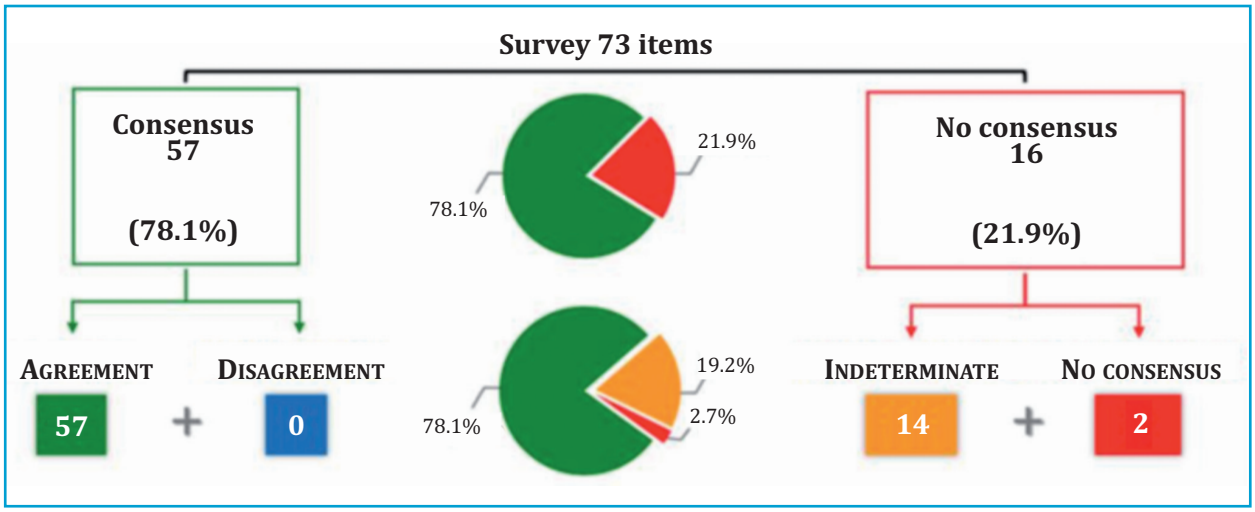


Figure 2. Delphi results



corresponding to 13 consensual statements, 10 in the first round and three in the second, of the 19 proposals (table 3). The remaining 6 statements remained indeterminate, giving rise to a 31.6% non-consensus due to dispersion of opinions. None of the non-consensual assertions of this block presented polarization in the results.

In block 4, *Differences between supplements*, consensus was obtained in 6 of the 7 proposed statements (table 4), all in the first round, which corresponds to a general percentage of consensus of 85.7%. The statement that did not obtain consensus was left as indeterminate.

Discussion

This Delphi survey reached consensus in 78.1% of the statements (figure 2) aimed at assessing knowledge about vitamin D and about the diagnosis, treatment and prevention of hypovitaminosis D.

In relation to vitamin D and health in general, there was agreement that hypovitaminosis D affects all population groups in Spain. The experts surveyed recognize its high prevalence in our country[6], and that its severity depends on environmental factors, such as time of day, season of the year[8] and geographical latitude, and of individual factors, such as skin pigmentation, diet, the use of sunscreens and clothing worn, since all of these factors condition cutaneous synthesis of vitamin D3. As the experts point out, diet is insufficient to satisfy the daily needs of vitamin D and this is due to the fact that there are few natural foods with a high content of this vitamin[2]. In addition, the reduction in sun exposure due to changes in lifestyle has been detrimental to the cutaneous synthesis of vitamin D. Given this situation, and as recognized by experts, an increase in the hours of effective sun exposure should be considered. and safe, taking into account the already known carcinogenic risks associated with it, so that a balance is achieved between sun exposure, diet and vitamin D supplementation, as measures for the prevention of hypovitaminosis D. According to the Spanish Research Society Bone and Mineral Metabolism (SEIOMM), 15 minutes of daily sun exposure on the arms and face are recommended, between the months of March and October, for the Caucasian population, with a protection factor between 15 and 30, bearing in mind radiation intensity and lati-

tude. In the elderly population and patients with osteoporosis, 30 minutes of daily sun exposure are recommended[9].

Due to individual variations in vitamin D supplementation, establishing the appropriate dosage for each patient is required. To this end, the experts agreed that, in addition to serum levels of 25(OH)D, the Body Mass Index (BMI) must be taken into account (considering the relationship between BMI and concentrations of 25(OH)D)[10], the degree of habitual sun exposure of the patient and the use of certain drugs that can alter the absorption and catabolism of vitamin D[11]. There was also agreement that supplementation can be established on a weekly, fortnightly or monthly basis without affecting its efficacy[12,13], and that it should be 800-1,000 IU/day in people over 65 years of age, to reach plasma concentrations enough of 25(OH)D[9].

Regarding the clinical consequences of hypovitaminosis D, the experts surveyed recognized that the risk of osteoporosis[1] and fractures[14] increases in the skeletal tissue, in addition to being associated with rickets[2] and osteomalacia[2], while the risk of cardiovascular diseases increases in the extraskeletal system[2,15] and the appearance of some types of cancer, especially breast, prostate and colorectal[3,16]. Despite an observed tendency towards agreement, however, there was no consensus that hypovitaminosis D is associated with an increased risk of type 2 diabetes (DM2) and autoimmune diseases. Most of the experts who disagreed acknowledged that certain studies had shown an association between hypovitaminosis D and DM2[17] or autoimmune diseases[18], but not a direct causal relationship. This suggests that the participants interpreted the statements as intending to establish a relationship of cause-effect between hypovitaminosis D and DM2 or autoimmune diseases, which could explain the lack of consensus. In fact, the real contribution of low levels of vitamin D as a cause of DM2 or autoimmune diseases is controversial, especially considering the discrepancies between observations and clinical trials of intervention. These discrepancies also exist in studies of the benefits of vitamin D at the extraskeletal level in general. This is because, in many of these intervention studies, the participants had normal serum 25(OH)D levels at baseline, so it would be difficult to observe vitamin D supplementation benefit. This is the case from the study by Pittas et al.[19] on the benefits of vitamin D in the prevention of DM2, and from the VITAL[20] study on the effects of vitamin D supplementation in the prevention of cancer and cardiovascular diseases, both with negative results. From a pathophysiological point of view, vitamin D supplementation may not provide any protection if there is no evidence of hypovitaminosis D[21,22], so intervention studies should be performed in patients with vitamin D deficiency as recommended by different authors[21,22].

As for assessing hypovitaminosis D, the experts recognized that the levels of 25(OH)D are the best biomarker of vitamin D status[1,2,11], since they reflect both the dietary contribution and that of sun exposure and the supplementation. There was also agreement that serum concentrations of 25(OH)D below 30 ng/mL indicate vitamin D insufficiency, while values below 10 ng/mL indicate severe deficiency[13].

However, the lack of consensus in the disagreement in considering insufficiency when 25(OH)D concentrations are less than 20 ng/mL shows that the definitions of insufficiency (<30 ng/mL) and deficiency (<20 ng/mL /mL) of vitamin D are not so clear[3]. There was also variability of opinion regarding safe concentrations of 25(OH)D. Although until recently it was considered that concentrations of 25(OH)D less than 150 ng/mL did not present any risk of toxicity[11,13], currently it is recommended to maintain serum levels between 30-50 ng/mL[6]. This is due to the observations that serum 25(OH)D values greater than 50 ng/mL are associated with an increased risk of cardiovascular mortality[23], although two studies were published in 2017 that cast doubt on these results[24,25]. In the first of them, upon standardizing serum 25(OH)D values from a previous study, no higher mortality was found[24], and in the second, which was the first and only meta-analysis that has used standardized values of 25(OH)D and individual data, no higher mortality was observed with serum values above 50 ng/mL[25].

On the other hand, once the recommended levels of 25(OH)D have been reached, patients must continue with a maintenance dose so that hypovitaminosis D does not reappear, since the causes of the insufficiency remain.

Regarding the screening of vitamin D deficiency, scientific societies such as the National Institute for Health and Care Excellence (NICE), the United States Preventive Services Task Force (USPSTF), the Endocrine Society and the Spanish Society of Endocrinology, have positioned themselves against universal screening[6,11,26,27], probably because there is no evidence that it is cost-effective. In this sense, many experts recognized that screening should only be carried out in patients with pathologies associated with hypovitaminosis D and in risk groups such as institutionalized elderly, as established by the recommendations. However, some of those surveyed believed that screening should be universal from the age of 18, a position that is possibly due to the high prevalence of hypovitaminosis D. Despite these discrepancies, there was agreement that measuring 25(OH)D levels was required in older people at risk of falls, in patients with osteoporosis with or without osteoporotic fracture, fragility fractures, chronic kidney disease, liver disorders or intestinal disease and in patients treated with drugs that can interact with vitamin D[6,12]. The experts also recognized that parathyroid hormone is a valid marker of vitamin D deficiency since there is an association between vitamin D deficiency and secondary hyperparathyroidism[2,3].

In relation to the methods for determining 25(OH)D and despite the differences observed between them[1,11], there was no consensus in disagreement that all the methods were similar or that most of them overestimated the levels of 25(OH)D. (OH)D, which suggests that many of the respondents had not considered or were unaware of the importance of the method for determining 25(OH) vitamin D. However, they did recognize the importance of using the same method in all measurements of follow-up of vitamin D supplementation, which should be carried out in the days prior to the next dose, every 3-4 months from the start of treatment until reaching adequate levels of 25(OH)D[6], and then at intervals every 6 -12 months. There was also agreement that these techniques need to be standardized. This can be done by implementing the reference materials for the National Institute of Standards and

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Table 1. Level of agreement reached in block 1: Vitamin D and health in general

Variable	Round	Mean	Median	Range	% outside median	Result
1. Hypovitaminosis D in Spain affects all population groups	1	7.29	8	2	18.49	Agreement
2. The time of day conditions the cutaneous synthesis of vitamin D due to the greater or lesser inclination of the solar radiation	1	7.42	8	2	17.81	Agreement
3. The season of the year determines the cutaneous synthesis of vitamin D due to the greater or lesser inclination of solar radiation	1	7.92	8	1	8.9	Agreement
4. Geographical latitude conditions the cutaneous synthesis of vitamin D due to the greater or lesser inclination of solar radiation	1	7.82	8	2	12.33	Agreement
5. Skin pigmentation conditions skin synthesis of vitamin D due to melanin content	1	7.52	8	2	21.23	Agreement
6. The use of sunscreens with a high protection factor conditions the cutaneous synthesis of vitamin D due to the blocking of UVB rays on the skin	1	7.68	8	2	18.49	Agreement
7. The way of dressing conditions the cutaneous synthesis of vitamin D because it can reduce skin exposure to the sun	1	7.77	8	1	13.01	Agreement
8. The diet followed by most people is insufficient to meet daily vitamin D needs	1	7.28	8	2	23.97	Agreement
9. Increasing the hours of effective sun exposure has been shown to be useful in preventing vitamin D deficiency	2	6.64	7	2	29.6	Agreement
10. Vitamin D supplementation has been shown to be useful in the prevention of hypovitaminosis D	1	8.32	9	1	4.11	Agreement
11. For the maintenance of bone health in people over 65 years of age doses between 800-1,000 IU/day of vitamin D are necessary	1	8.02	8	1	8.9	Agreement
12. In addition to serum levels of vitamin D, when calculating the dose of vitamin D to administer, we must take into account both BMI and sun exposure	1	6.99	7	3	29.45	Agreement
13. As vitamin D is fat-soluble, we can administer it in weekly, fortnightly or monthly doses	1	8.21	9	1	8.9	Agreement
14. Certain drugs interact with vitamin D reducing its absorption	1	7.68	8	2	19.86	Agreement
15. Insufficient vitamin D is associated with an increased risk of osteoporosis because it is essential for proper bone metabolism	1	8.35	9	1	5.48	Agreement
16. Insufficient vitamin D is associated with an increased risk of failure in the treatment of osteoporosis, since it determines a greater probability of fractures and less bone mass gain even when receiving effective anti-catabolic/anti-resorptive treatment	1	8.14	8	1	6.85	Agreement
17. Deficiency rickets and osteomalacia are associated with a severe vitamin D deficit	1	8.44	9	1	4.11	Agreement
18. Hypovitaminosis D is associated with an increased risk of type 2 diabetes	2	6.44	7	2	36	Indeterminate
19. Hypovitaminosis D is associated with an increased risk of cardiovascular diseases	2	6.75	7	2	31.2	Agreement
20. Hypovitaminosis D is associated with an increased risk of autoimmune diseases	2	6.71	7	2	34.4	Indeterminate
21. Hypovitaminosis D has been associated with the appearance of some types of cancer, especially breast, prostate and colorectal cancer	2	6.63	7	2	31.2	Agreement

Technology measurement of 25(OH)D[11]. In general, most hospitals use immunoassays to measure serum concentrations of 25(OH)D, although these methods are not standardized and overestimate these concentrations[28] due to cross-reactivity with other inactive metabolites of vitamin D, such as 24-25 (OH)D and the epimer C3. In contrast, liquid chromatography with tandem mass spectrometry (LS-MS/MS), which is the reference method, does not present the problem of overestimation of 25(OH)D, since it allows independent analysis of each one of the metabolites of vitamin D[28], which translates into an increase in the percentage of hypovitaminosis D[29]. However, this method is not applicable to clinical routine because it is more complex, time-consuming and expensive than immunoassays. In addition, the values that define vitamin D insufficiency and deficiency are based on the results of immunoassays, therefore, despite the fact that there was no agreement among the experts, these methods are acceptable to determine the concentration of 25(OH)D in clinical practice. There was also no agreement on establishing that 25(OH)D monitoring should be done in winter or early spring, when vitamin D synthesis is more deficient. In general, monitoring should be performed in all patients at risk of hypovitaminosis D, regardless of the time of year. However, primary care centers with limited access to 25(OH)D measurement and who cannot request it without justification, could choose to measure 25(OH)D concentrations in winter or early spring, which is the time when the patient is most likely to have hypovitaminosis D.

Regarding treatment with vitamin D, although some experts contemplated its interruption in summer if it was accompanied by a diet rich in vitamin D and provided that hypovitaminosis D was not serious and there were no diseases that perpetuated it, withdrawal of supplementation is not recommended in summer. In this sense, as with the monitoring of 25(OH)D, it must be taken into account that the difference between the concentrations of 25(OH)D in summer and winter is small and that there are many factors that, together with variations in sun exposure, they can intervene in hypovitaminosis D.

Although the experts recognized that supplementation should be performed only after confirming hypovitaminosis D, even in patients over 65 years of age, there was a dispersion of opinions on the fact of prescribing vitamin D supplements to the institutionalized elderly without determining serum levels of 25(OH)D. In this sense, the ideal is to know the serum levels of 25(OH)D to adjust the dose. However, the prevalence of hypovitaminosis D in this population group is 87%[30] so, if there is no access to the determination of 25(OH)D levels, supplementation with safe doses, such as those doses between 1,000 and 2,000 IU daily of vitamin D, which are recommended by the International Osteoporosis Foundation (IOF) for this population[13], is probably the situation with the most effective cost-benefit, especially at the level of prevention of fractures and loss of muscle strength.

On the other hand, there was no agreement that vitamin D supplementation in all people over 65 years of age is cost-effective. Some experts commented in this regard that in order to know this data, cost-effectiveness studies similar to those carried out by the NICE guidelines in the United Kingdom should be carried out in Spain, in which vitamin D supplementation is directly recommended in people over 65 years of age, pregnant women and infants and children under 4 years of age[27].

According to experts, vitamin D supplementation is necessary in all patients with vitamin D insufficiency or deficiency. In this sense, there was agreement that knowing the sun exposure habits of patients would be useful to identify those at risk of hypovitaminosis D. There was also agreement that patients receiving treatment with corticosteroids or drugs that increase the catabolism of vitamin D require supplementation, which implies that they recognized that these patients are at risk of hypovitaminosis D[6,13]. In addition, it must be taken into account that certain diseases interfere with the synthesis and bioavailability of vitamin D, so there was agreement that patients with intestinal malabsorption, chronic kidney disease, liver disease or obesity required doses of vitamin supplementation highest D[13]. This need was not agreed upon for patients with photosensitivity, in which case the most advisable thing would be to establish the dose of vitamin D based on the serum levels of 25(OH)D and not prescribe higher doses, as some experts contemplate, because the sun exposure of these patients is less or less effective due to the use of sun protection creams.

In addition, the experts recognized the importance of maintaining adequate levels of 25(OH)D in patients with osteoporosis, since it can reduce the risk of both hip and non-vertebral fractures[13]. However, there was no agreement that fracture reduction by vitamin D supplementation was dose dependent. This result is not surprising considering the discrepancies between different studies. Thus, while the meta-analysis by Bischoff-Ferrari et al shows that daily doses of 800 IU or higher are more beneficial for reducing fractures in patients over 65 years of age[31], the study by Bolland et al finds no evidence that supplements of vitamin D reduce fractures[32], although it should be noted that the latter has many limitations[22].

There was also no consensus, despite the orientation towards agreement, in considering that, in patients with DM2, vitamin D supplements contribute to better glycemic control. Although most of the experts who disagreed thought that there were no conclusive studies in this regard, it should be noted that it has been shown that vitamin D supplements contribute to better glycemic control[33], inducing a significant improvement when 25(OH)D levels are less than 20 ng/mL, although this does not occur when they are above 20 ng/mL.

Regarding the differences between supplements, the experts recognized that calcifediol is more potent than cholecalciferol, therefore lower doses are required, it increases 25(OH)D concentrations more quickly and is more effective in maintaining them above 30 ng/mL6,[34]. There was also agreement that calcifediol is the drug of choice in patients with deficient hepatic hydroxylation due to liver disease or advanced age (>70 years) as it does not require hepatic hydroxylation[34,35], and in patients with intestinal disease because it is better absorbed than the other metabolites[34].

Recently, the Spanish Medicines Agency has published an informative note on the appearance of hypercalcaemia due to cholecalciferol overdose in children and calcifediol in adults[36], although the most recent studies on the use of calcifediol have not described any toxicity associated with this drug[12,37].

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Table 2. Level of agreement reached in block 2: Assessment of hypovitaminosis D

Variable	Round	Mean	Median	Range	% outside median	Result
22. Screening for hypovitaminosis D should be applied to the entire population over 18 years	2	3.78	3	3	44	Indeterminate
23. The measurement of 25(OH)D is the best indicator to know the status of vitamin D	1	7.93	8	2	10.27	Agreement
24. The determination of 25(OH)D reflects the total vitamin D obtained both from intake and from sun exposure and pharmacological treatments	1	7.3	8	3	25.34	Agreement
25. Vitamin D insufficiency is understood as a level of 25(OH)D less than 30 ng/mL	1	6.99	8	3	26.03	Agreement
26. Vitamin D insufficiency is understood as a level of 25(OH)D less than 20 ng/mL	2	5.6	7	7	44.8	No consensus
27. Severe vitamin D deficiency is understood as a level of 25(OH)D less than 10 ng/mL	1	8.05	9	1	8.22	Agreement
28. Serum levels of 25(OH)D must be maintained below 50 ng/mL due to possible increased risk of total mortality and cardiovascular and other side effects	2	5.03	5	4	75.2	No consensus
29. It is essential to carry out periodic controls of serum levels due to individual variability in vitamin D supplements	2	6.74	7	1	24	Agreement
30. All vitamin D quantification methods are similar	2	4.03	4	2	48.8	Indeterminate
31. Immunoassay methods, despite the lack of standardization and interference with other metabolites, are clinically acceptable to assess the concentration of calcidiol	2	6.1	6	2	40.8	Indeterminate
32. As the half-life of calcidiol is 18-21 days, it is important that the blood draw for monitoring of 25(OH)D be performed in the days prior to the next intake	2	7.13	7	2	30.4	Agreement
33. For the monitoring of 25(OH)D, it is important that the determination is made in winter or early spring, which are the seasons in which the synthesis of vitamin D is most deficient	2	6.22	7	2	40.8	Indeterminate
34. Most of the laboratory techniques used overestimate the levels of 25(OH)D by also quantifying inactive metabolites	2	5.22	5	0	24.8	Indeterminate
35. It is important to monitor vitamin D always using the same determination method	1	7.9	8	2	7.53	Agreement
36. Clinical laboratories should be integrated into programs of standardized vitamin D measurement	1	8.08	8	1	6.16	Agreement
37. The results of studies that do not have standardized measurements of 25(OH)D	1	7.86	8	2	10.96	Agreement
38. Vitamin D levels should be determined (calcidiol) in cases of chronic kidney, liver and intestinal disease	1	8.34	9	1	2.74	Agreement
39. Vitamin D levels should be determined in cases of osteoporosis without osteoporotic fracture	1	8.35	9	1	3.42	Agreement
40. Vitamin D levels should be determined in all patients with fragility fractures	1	8.5	9	1	2.05	Agreement
41. Vitamin D levels should be determined in all elderly patients at risk of falls	1	8.07	8	1	8.9	Agreement
42. Vitamin D levels should be determined in all patients treated with drugs that can interact with vitamin D: anticonvulsants, glucocorticoids, antiretrovirals, antifungals and absorption modifiers of lipids (cholestyramine, orlistatin, etc.)	1	8.27	9	1	5.48	Agreement
43. In patients with vitamin D deficiency who start supplementation, serum concentrations of 25(OH)D should be determined every 3-4 months until adequate levels are reached	1	7.36	8	2	22.6	Agreement
44. After reaching adequate levels of vitamin D after supplementation, annual analysis is recommended	1	7.52	8	2	17.81	Agreement
45. Parathyroid hormone can be considered a marker of vitamin D insufficiency due to increased levels from 25(OH)D levels below 31 ng/mL	1	6.83	7	2	32.19	Agreement
46. Patients with vitamin D insufficiency have secondary hyperparathyroidism	2	7.12	7	2	25.6	Agreement
47. In the case of secondary hyperparathyroidism, the levels of parathyroid hormone decrease after correction of vitamin D insufficiency	1	7.68	8	2	16.44	Agreement

Table 3. Level of agreement reached in block 3: Treatment with vitamin D according to the patient's profile

Variable	Round	Mean	Median	Range	% outside median	Result
48. Vitamin D levels should always be determined before administer supplements	1	7.01	8	3	30.14	Agreement
49. Sun exposure habits should be determined in the clinical history to identify patients at risk of vitamin D deficiency	1	7.26	8	2	24.66	Agreement
50. Treatment with vitamin D supplements should be interrupted in summer if the patient increases their sun exposure	2	4.9	5	4	67.2	Indeterminate
51. Vitamin D supplementation is necessary in all patients with hypovitaminosis D because diet and sun exposure do not cover daily needs	1	7.26	8	2	21.23	Agreement
52. In patients receiving treatment for osteoporosis, adequate levels of vitamin D must be guaranteed	1	8.49	9	1	3.42	Agreement
53. Treatment of vitamin D insufficiency can decrease the risk of hip fractures	1	7.84	8	2	11.64	Agreement
54. Treatment of vitamin D insufficiency can decrease the risk of non-vertebral fractures	1	7.65	8	2	15.07	Agreement
55. When there is evidence of hypovitaminosis D, vitamin D supplements offer dose-dependent protection against fractures	2	6.81	7	2	33.6	Indeterminate
56. In patients with type 2 diabetes, vitamin D supplements contribute to better glycemic control	2	6.46	7	2	38.4	Indeterminate
57. Vitamin D supplementation in all people over 65 years of age is cost-effective	2	6.84	7	3	33.6	Indeterminate
58. People over the age of 65 should take vitamin D supplements only in case of hypovitaminosis D	2	7.33	8	1	18.4	Agreement
59. In the institutionalized elderly, vitamin D supplements should be prescribed without the need for prior determination of its levels	2	5.62	7	4	49.6	Indeterminate
60. Patients who present photosensitivity require higher doses of vitamin D than usual	2	6.28	7	3	46.4	Indeterminate
61. Patients with intestinal malabsorption require doses of vitamin D higher than usual	1	7.33	8	2	20.55	Agreement
62. Obese patients need higher doses of vitamin D than usual due to its lower bio-availability	2	7.7	8	2	17.6	Agreement
63. Patients with chronic kidney disease (CKD) require higher doses of vitamin D than usual	2	7.1	8	1	24	Agreement
64. Patients with liver disease require higher doses of vitamin D than usual	2	6.66	7	2	32.8	Agreement
65. Patients under treatment with drugs that increase the catabolism of vitamin D should receive supplementation	1	7.19	8	3	26.71	Agreement
66. Patients receiving corticosteroid treatment should receive vitamin D supplements because corticosteroids can cause resistance to this vitamin	1	7.14	8	3	30.14	Agreement

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Table 4. Level of agreement reached in block 4: Differences between supplements

Variable	Round	Mean	Median	Range	% outside median	Result
67. Calcifediol has been shown to be more effective than cholecalciferol in maintaining serum levels of 25(OH)D >30 ng/mL	1	7.32	8	3	25.34	Agreement
68. Calcifediol has been shown to be more potent than cholecalciferol by thus, fewer doses are needed to maintain serum 25(OH)D levels >30 ng/mL	1	7.57	8	2	17.12	Agreement
69. Because calcifediol is more powerful than cholecalciferol, it has a higher risk of hypercalcaemia	2	4.73	5	2	42.4	Indeterminate
70. Calcifediol increases 25(OH)D concentrations more rapidly than vitamin D3	1	7.47	8	2	21.23	Agreement
71. Calcifediol is recommended instead of cholecalciferol in patients with liver disease as they do not need hepatic hydroxylation	1	7.63	8	2	19.86	Agreement
72. Calcifediol is recommended instead of cholecalciferol in patients older than 70 years due to deficient hepatic hydroxylation	1	7.05	7	2	29.45	Agreement
73. Calcifediol is chosen in cases of intestinal disease because its absorption is better than that of other metabolites	1	7.14	7	2	26.03	Agreement

To sum up, the data from this study show that there is a consensus on the high hypovitaminosis D prevalence in Spain and the need to prescribe vitamin D supplements in patients with insufficiency and deficiency of this vitamin. However, the lack of consensus for some items reveals inadequate knowledge about vitamin D among the experts surveyed, especially regarding the recommendations for evaluating and treating this vitamin deficiency. Therefore, training sessions are required to provide adequate current knowledge to those who regularly prescribe vitamin D.

Conflict of interests: Dr. Javier Aguilar del Rey has received fees as a consultant, speaker, editor and grants for attending conferences from Amgen, FAES Pharma, Gebro, Italdrug, LACER and Lilly.
Dr. Esteban Jódar works as a consultant for Amgen, AstraZeneca, FAES Pharma, GSK, Helios-Fresenius, Italdrug, Lilly, MSD, Mundipharma, Novo Nordisk, Shire, UCB. He is a clinical investigator for Amgen, Boehringer, AstraZeneca, Faes, GSK, Janssen, Lilly, MSD, Novo Nordisk, Pfizer, Sanofi, Shire, UCB and speaker for Amgen, Asofarma, Astellas, AstraZeneca, Bayer, Boehringer, BMS, FAES Pharma, Lilly, MSD, Mundipharma, Novartis, Novo Nordisk, UCB and Theramax.
Dr. Fátima Brañas has received fees from MSD MISP funds; she has acted as a speaker at symposiums organized on behalf of MSD, ViiV Healthcare, Amgen, Fresenius and Janssen. She has developed various materials for MSD and is a member of the scientific committee of ViiV Healthcare and FAES Pharma.
Dr. Carlos Gómez has received grants and personal fees as a consultant and training courses from Amgen, Kiowa-Kirin, Italfarmaco, FAES, UCB and Gebro.
Dr. Jorge Malouf-Sierra is a speaker for Angelini, FAES, Gebro and Italdrug.
Dr. Rafael Sánchez has received research funds from Astellas and personal fees from Seid and LACER Laboratories.

FAES PHARMA laboratories and the technical secretary of Luzán collaborated in this study. It complies with all the precepts of the Declaration of Helsinki on clinical studies. FAES PHARMA has not intervened in the choice of questions, in the results analysis or in the writing of the article, which have been the sole responsibility of the listed authors.

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Usefulness of the trabecular bone score in adult subjects with osteogenesis imperfecta

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Summary

Objective: To analyze the usefulness of the trabecular bone score (TBS) in adults with osteogenesis imperfecta (OI) and its relationship with clinical, anthropometric and densitometric variables, especially with the presence of fractures and the severity of the disease.

Material and methods: Cross-sectional study conducted in 31 adult patients with OI (age 40.5±15.2 years, 68% women, 87% type I). The clinical characteristics of the patients (fractures, type of OI, BMI and treatment), bone mineral density (BMD) (using DXA), assessing the presence of densitometric osteoporosis, and TBS values (TBS iNsight), estimating the presence of degraded microarchitecture (values <1.230). The results were compared between the different OI types (I and III-IV) and with those of a control group of healthy subjects.

Results: Most of the patients (29/31, 94%) had a history of fractures and 29% received antiosteoporotic treatment. 61% had densitometric osteoporosis and 19% had degraded microarchitecture. No differences were observed in the TBS values according to OI severity (OI type I vs. III-IV: 1,297 vs. 1,339, p=n.s.); no patient with OI type III-IV had TBS <1230. TBS values were related to age (r=-0.6, p<0.01), lumbar BMD (r=0.4, p=0.03) and BMI (r=-0.5 , p=0.01). Patients with OI had lower values of TBS and BMD than the control group in all locations analyzed.

Conclusion: TBS is not very sensitive in assessing bone quality in OI, since none of the patients with severe OI had a degraded microarchitecture and this was only observed in 19% of patients with OI despite presenting a high prevalence of fractures.

Key words: osteogenesis imperfecta, trabecular bone score, TBS.

Introduction

Osteogenesis imperfecta (OI) is a congenital disease that comprises a heterogeneous group of clinical and genetic disorders of connective tissue, mainly caused by mutations in the *COL1A1* and *COL1A2* genes of type I collagen. It has been estimated that the incidence of OI is approximately 1:10-20.000[1-4] and the clinical manifestations can vary from almost asymptomatic forms to very severe cases. The main characteristic of this entity is bone fragility, due to a decrease in bone mass, cortical thickness and an alteration in the trabecular architecture which, together with defects in the bone matrix, affect its quality and resistance, and lead to to a marked increase in the risk of fracture, evident from childhood[1-3,5]. Classically, OI was considered an autosomal dominant genetic disorder and patients were classified into four subtypes based on clinical severity (classification by Sillence et al.[6]: type I being the mildest, followed by types IV, III and type II, the most severe that confers perinatal mortality). Over the years new genes and pathogenic variants have been identified, adding new groups to the classification (OI type V - type XX)[1,2,4,5]. However, this classification is no longer practical and some authors prefer to classify patients according to the degree of clinical involvement of the OI (mild, moderate, severe, lethal), including the genetic defect they present[4].

Although the diagnosis of certainty is obtained with the genetic study, in routine clinical practice, this can be done based on clinical and radiological findings and family history[1,3,7]. Dual-energy X-ray absorptiometry (DXA), the gold standard technique for the diagnosis

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and monitoring of osteoporosis (OP), is also of some use in patients with OI. However, by providing information on bone mineral density (BMD), which is not necessarily affected in this entity, and not on bone quality, a key aspect in OI, its results must be evaluated with caution, since has shown a clear relationship between BMD values by DXA and the severity of OI[7,8]. Therefore, new diagnostic methods applicable in routine clinical practice are necessary to assess other aspects of bone quality in this process. It has been suggested that the trabecular bone score (TBS), a parameter obtained from the measurement of a gray scale of the bone texture of the 2D DXA image of the lumbar spine, could be useful in this assessment[9]. In fact, TBS values have a good correlation with bone microstructure parameters obtained by high-resolution peripheral computed tomography (HRpQCT)[10] and have been related to the development of fractures in the population independently of BMD values[9], for what has been indicated that TBS could be a good method to assess other determining parameters of bone quality, especially those related to its microstructure.

The objective of this study is to analyze the usefulness of TBS in adult subjects with OI and its relationship with clinical, anthropometric and densitometric variables, especially with the presence of fractures, as well as to compare TBS values with those of a control group of healthy individuals.

Material and methods

A cross-sectional study has been carried out in adult patients with OI who follow control and treatment in a specialized consultation of bone metabolic disease of our hospital's rheumatology service. The study protocol followed the standards of the Declaration of Helsinki and was approved by the hospital's ethics committee. The included patients signed the informed consent.

A total of 31 adult patients diagnosed with OI (21 women/10 men) have been included (after genetic study and/or compatible family history). The clinical characteristics (including weight, height and calculation of the body mass index [BMI]), presence of fractures, type of OI and previous treatments carried out were analyzed. In all patients, BMD in the lumbar spine and proximal femur was analyzed by DXA (Lunar Prodigy equipment, General Electric Medical Systems, WI, USA) to assess the presence of densitometric OP (defined by T-score values ≤−2.5 SD [in subjects ≥50 years] or Z-score <−2 SD [in subjects <50 years]), osteopenia (T-score >-2.4 and ≤−1 SD) or normal BMD (T-score > -1 SD)[11]. TBS was calculated using TBS iNsight software (version 3.0.2.0) (Medimaps group, Geneva, Switzerland) on lumbar spine DXA images; a TBS value <1.230 was considered microarchitecture degraded, between 1.230-1.310 microarchitecture partially degraded and TBS >1.310 normal[12]. The results of the TBS were compared with those of a control group of healthy subjects of similar age, gender, and BMI (n=28, 71.4% women, 39 years old on average [21-60]), from the same geographical area and who collaborated in the TBS-SEIOMM study to obtain normal TBS values in our population.

Statistic analysis

Statistical analyzes were performed using the SPSS program (version 27) (IBM Corp., NY, USA). Quantitative variables are described by mean and standard deviation (S.D.) of the mean and qualitative variables by frequency and percentages. The differences between means of the continuous variables have been analyzed using the t-test and the non-parametric Wilconxon-Mann-Whitney test, and the differences between proportions using the chi-square test or the Fisher test. To assess association between variables, the Pearson correlation coefficient was used. Results with a value of $p<0.05$ have been considered significant.

Results

A total of 31 patients (67,7% women) with a mean age of 40,5±15,2 years (range 19-70) diagnosed with OI were included. Most patients had type I OI (n=27, 87.1%), two patients had type IV OI (6.5%) and two patients had type III OI (6.5%). 29/31 (93,5%) patients had a history of fragility fractures, being multiple fractures in most cases, and only two patients with OI type I had not presented fractures. At the time of assessment, 21/31 subjects were receiving (n=9) or had received (n=12) anti-osteoporotic treatment for a mean of 65±50 months, most with bisphosphonates (oral n=15, intravenous n=11), 3 patients with teriparatide, 2 with selective estrogen receptor modulators and 1 with denosumab. The main characteristics of the patients are summarized in table 1.

When TBS values were analyzed, the mean was 1.302±0.175 [0.737-1.510]; 6/31 patients (19%) had a degraded microarchitecture, 26% a partially degraded microarchitecture, and more than half (55%) had normal TBS values; of the 6 patients with degraded microarchitecture (<1230), all of them were >40 years old, 50% were women and half also had osteoporosis, all had type I OI and 5/6 patients had a history of fractures, multiple in all cases. The sensitivity of TBS in the evaluation of patients with OI and fractures was only 17%.

Regarding BMD, 61% of patients had densitometric OP, 36% had osteopenia, and only one patient had normal BMD. There were no significant differences in terms of TBS or BMD values depending on whether the patients were receiving active anti-osteoporotic treatment at the time of assessment.

When TBS values were analyzed according to the severity of the disease (OI type I vs. type III-IV), no significant differences were found between both groups of patients (table 1 and figure 1A), nor in relation to BMD values in the proximal femur (neck and total), age or BMI; however, patients with OI type III-IV presented lower BMD values at the lumbar level and a greater number of fractures (table 1). On the other hand, when these same parameters were compared according to the number of fractures (OI patients with ≤10 vs. >10 fractures), there were no significant differences between both subgroups. It is noteworthy that no patient with OI type III or IV presented degraded microarchitecture, however, all of them presented osteoporosis in the BMD (table 1). However, the TBS values were significantly lower than those of the control group, as were the BMD values in all locations analyzed (table 2 and figure 1B).

TBS values were positively related to lumbar BMD ($r=0.4$, $p=0.03$), and negatively related to age ($r=-0.6$, $p<0.01$) and BMI ($r=-0.5$, $p=0.01$) (figure 2).

Table 1. Clinical characteristics of patients with osteogenesis imperfecta

	All n=31	OI type I n=27	OI type III-IV n=4	p
Age (years) (mean ± S.D., [range])	40.5±15.2 [19-70]	41.2±15.7 [19-70]	35.8±11.2 [25-49]	0.550
Sex (W [n, %] / M [n, %])	21 (68%)/10 (32%)	19 (70%)/8 (30%)	2 (50%)/2 (50%)	0.416
OI type I/III/IV (n, %)	27 (87.1%)/ 1 (3.2%)/3 (9.7%)	-	-	-
Patients with fractures (n, %)	29 (93.5%)	25 (92.6%)	4 (100%)	0.574
Number of fractures (mean ± S.D., [range])	11.1±12.2 [0-50]	8.6±9.4 [0-37]	28.3±16.7 [10-50]	0.012*
Current anti-osteoporotic treatment (n, %)	9 (29%)	8 (29.6%)	1 (25%)	0.849
Previous anti-osteoporotic treatment (n, %)	12 (38.7%)	9 (33.3%)	3 (75%)	0.096
Weight (kg) (mean ± SD)	59.5±11.5	60.97±10.7	49.28±13.25	0.094
Height (m) (mean ± S.D.)	1.53±0.14	1.56±0.1	1.32±0.14	0.005*
BMI (kg/m^2)(mean ± SD)	25.5±4.1	25.1±4.2	28.0±2.4	0.107
DMO lumbar (g/cm^2)	0.868±0.153	0.890±0.150	0.716±0.062	0.019*
DMO cuello femoral (g/cm^2)	0.762±0.135	0.770±0.141	0.693±0.016	0.467
DMO fémur total (g/cm^2)	0.826±0.163	0.819±0.163	0.887±0.190	0.744
T-score in the lumbar spine (mean ± S.D.)	-2.73±1.36	-2.52±1.35	-4.05±0.37	0.030*
T-score in the femoral neck (mean ± S.D.)	-2.11±1.08	-2.04±1.12	-2.7±0.36	0.278
T-score in total femur (mean ± S.D.)	-1.68±1.43	-1.73±1.41	-1.30±1.92	0.856
Z-score in the lumbar spine (mean ± S.D.)	-2.22±1.31	-2.04±1.30	-3.45±0.31	0.052
Z-score in the femoral neck (mean ± S.D.)	-1.56±0.97	-1.50±1.02	-2.00±0.30	0.215
Z-score in total femur (mean ± S.D.)	-1.25±1.32	-1.33±1.30	-0.67±1.62	0.743
TBS (mean ± S.D.)	1.302±0.175	1.297±0.183	1.339±0.117	0.932
Densitometric osteoporosis (n, %)	19/31 (61.3%)	15/27 (55.6%)	4/4 (100%)	0.089
Patients with degraded TBS (n, %)	6/31 (19.4%)	6/27 (22.2%)	0/4 (0%)	0.377

S.D.: standard deviation; W: women; M: men; OI: osteogenesis imperfecta; BMI: body mass index. *: statistically significant result. Degraded microarchitecture was considered TBS values <1.230.

Discussion

Our study results suggest that TBS may not be a useful tool to assess bone strength in patients with OI. Thus, most patients with OI had TBS values in the normal range and only 19% of them, despite having a high incidence of fractures, had degraded microarchitecture values. Likewise, no differences were observed in the TBS values in relation to the severity of the disease (OI type I vs. type III-IV) and no patient with severe disease had degraded microarchitecture, suggesting a low sensitivity of this parameter in the estimation of bone quality in this disease. To date, there are hardly any studies that analyze the usefulness of TBS in OI. Kocijan et al.[7] examined the values of TBS in a cohort of 30 adult patients (>18 years) with OI, and as in our study, they found no differences in relation to the severity of the disease (comparing individuals with OI type III-IV vs. type I). Although, they also observed differences when they compared the values with those of a control group of healthy subjects. In said study, it was indicated that TBS could be a useful tool, especially to estimate a severe deterioration of bone microstructure in OI when the values are low. However, despite having presented multiple fractures, only 19% of our patients with OI had low TBS values (degraded microarchitecture), and in our study this parameter did not differentiate patients with greater disease severity, which suggests that it is an insensitive tool to assess bone quality in OI.

It should be mentioned that the TBS values were especially related to the age of the individual. In fact, all patients with OI and low TBS values (degraded microarchitecture) were over 40 years old, with a mean age of 57 years, the age at which there is usually a progressive decrease in TBS values in the general population. Therefore, it should be remembered that there are other factors that must be taken into account when assessing TBS, such as age and BMI[3]. In this sense, both in healthy subjects and in individuals with OP, lumbar TBS, like BMD, decreases with age[14], at the same time as, and contrary to BMD, there is a negative correlation with BMI[15]; findings, as indicated, also observed in our cohort of patients with OI (figure 2).

Figure 1. (1A) TBS values in patients with osteogenesis imperfecta type I (mild) and type III-IV (severe-moderate). (1B) TBS values in patients with osteogenesis imperfecta and in the control group

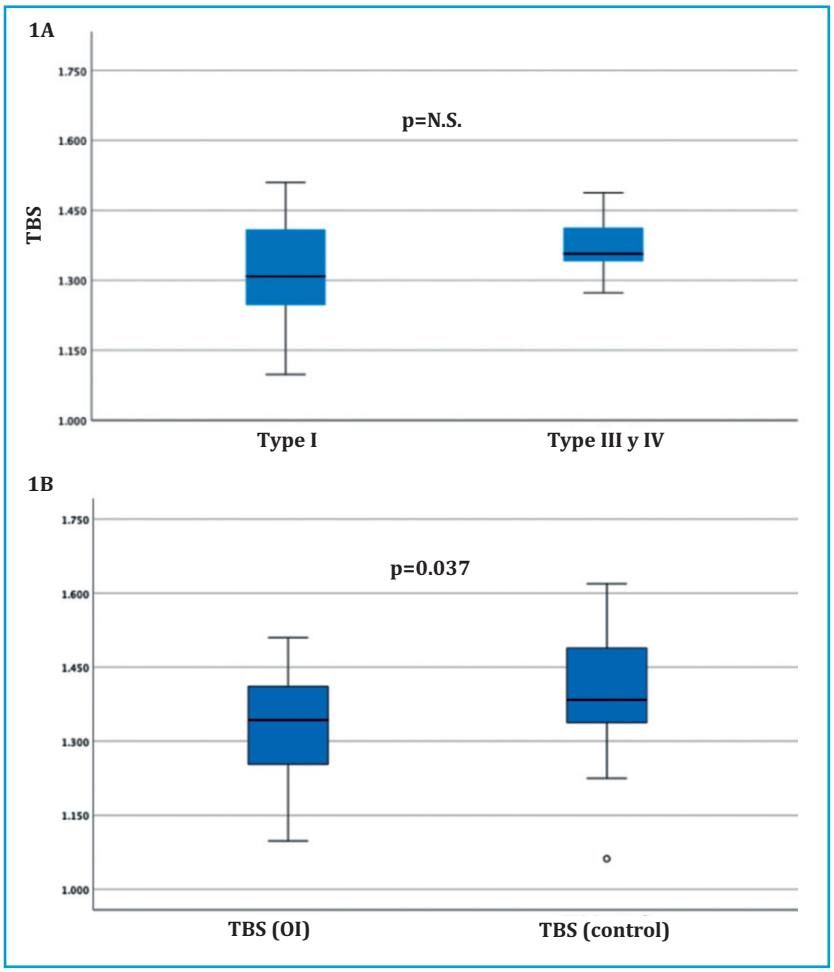


Table 2. TBS and BMD values in patients with osteogenesis imperfecta and in the control group

	Patients with OI	Control group	p
TBS	1.297 ± 0.180	1.399 ± 0.119	0.037
Lumbar BMD (g/cm^2)	0.887 ± 0.149	1.122 ± 0.172	<0.01
Femoral neck BMD (g/cm^2)	0.775 ± 0.135	0.969 ± 0.129	<0.01
Total femur BMD (g/cm^2)	0.844 ± 0.161	0.986 ± 0.124	<0.01
Lumbar T-score	-2.55 ± 1.32	-0.49 ± 1.39	<0.01
Femoral neck T-score	-1.98 ± 1.06	-0.19 ± 0.90	<0.01
Total femur T-score	-1.50 ± 1.41	-0.24 ± 0.86	<0.01
Lumbar Z-score	-2.09 ± 1.29	-0.07 ± 1.17	<0.01
Femoral neck Z-score	-1.46 ± 0.93	-0.20 ± 0.83	<0.01
Total femur Z-score	-1.11 ± 1.28	-0.02 ± 0.81	<0.01

TBS: trabecular bone score; BMD: bone mineral density; OI: osteogenesis imperfecta.
All variables are expressed as mean ± S.D. (standard deviation).

Another aspect to highlight is that 61% of our patients had a densitometric OP, and only one patient had a normal BMD, with BMD values being lower than those of the control group in all locations analyzed (lumbar spine and proximal femur). Again, these results coincide with those reported in some previous studies[16], and indicate that, although with limitations and pending the availability of better instruments in the future, the quantification of BMD continues to be, to date, the tool available in the most effective routine clinical practice to estimate the risk of fracture in these patients[1,3,5,7,16,17], despite not being able to assess the alterations in bone quality that patients with OI present.

Other techniques, such as HrpQCT, could be especially useful in assessing bone strength and quality in this entity by allowing BMD to be quantified three-dimensionally and evaluating the structure of trabecular and cortical bone in peripheral regions. Thus, in patients with OI, it has been indicated that HRpQCT, by analyzing microstructural parameters, would allow a better assessment of the severity of bone involvement, especially when compared to other techniques, such as BMD and TBS[7]. In this sense, patients with more severe forms of the disease (OI types III and IV) usually present worse structural parameters, with a greater decrease in the thickness and number of trabeculae and greater space between them[7,18]. Although, as previously indicated, it has been suggested that TBS could also provide information on bone microstructure parameters[9,10], in patients with OI the correlation between structural parameters assessed by HRpQCT and TBS is very low[7], indicating the need to assess the usefulness of this tool in these patients.

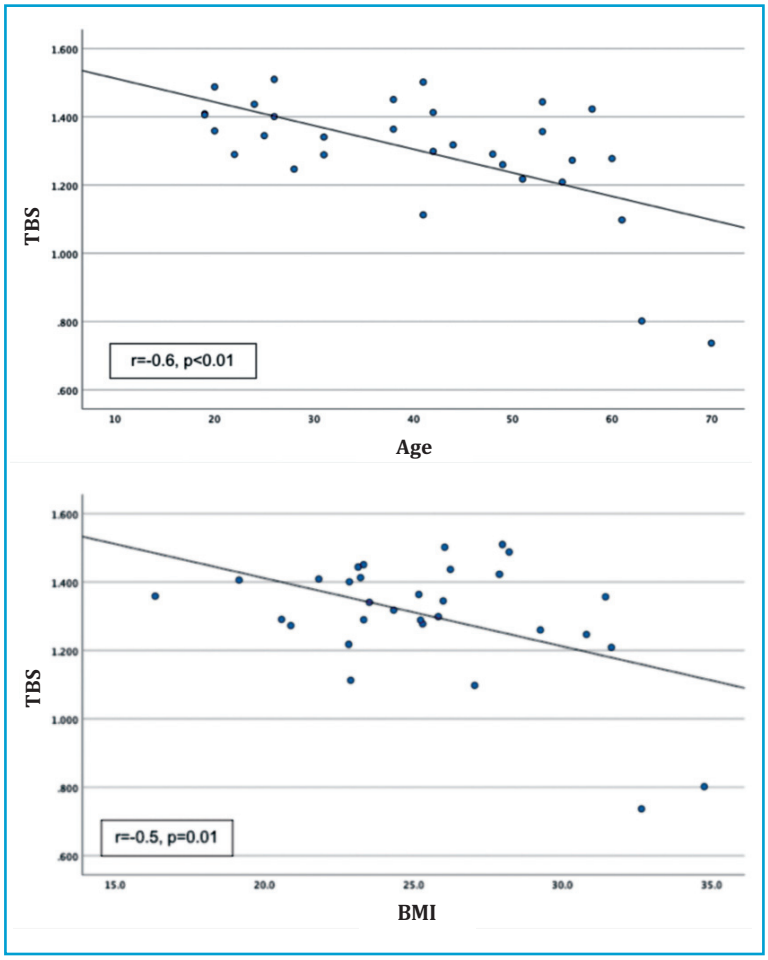
This study has several limitations, such as: the small number of patients with severe OI (types III and IV), an intrinsic limitation to the characteristics of the disease, since it deals with the less frequent types of OI. Or, the possible effect on TBS values that the antiosteoporotic treatment

followed by some of the patients included in the study may have had, a limitation also associated with this type of disease in which treatment, especially with bisphosphonates, is frequent. when there are multiple fractures, a fact present in most of our patients.

In conclusion, our study shows a low sensitivity of TBS in the assessment of bone fragility in OI, since a low percentage of patients with OI presented low values (degraded microarchitecture) of TBS, despite having suffered multiple fractures and no patient with severe OI (types III and IV) presented a degraded microarchitecture. In this study, TBS did not provide advantages in determining BMD. However, additional studies are recommended to confirm these results and include a larger number of patients with this disease, in which new tools are needed to assess bone quality and strength.

Conflict of interests: The authors declare no conflict of interest.

Figure 2. Correlation between TBS values and age and BMI



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Postoperative hypocalcaemia predictors after total thyroidectomy

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Summary

Introduction and objective: Given the increasing number of thyroid surgeries, the incidence of post-surgical hypoparathyroidism is on the rise. The frequency of hypocalcaemia due to hypoparathyroidism after total thyroidectomy is highly variable according to the literature (0.3-68%). The primary objective of this study is to analyze the biochemical, surgical and demographic factors related to an increased risk of hypocalcaemia.

Methodology: This retrospective study encompasses 297 patients who underwent total thyroidectomy over a period of 8 years in a tertiary hospital. Demographic, clinical and biochemical data, both preoperative, intraoperative and postoperative, and their relationship with postoperative hypocalcaemia are analyzed.

Results: The rate of total hypocalcaemia was 40.2%, being transient in 26.1%.
Statistically significant variables were age (p=0.04), Graves' disease (p=0.04), carcinoma confirmed by pathology (p=0.04), two-stage thyroidectomy (p=0.00), the number of transplanted parathyroids (p=0.00) and pre- and post-operative PTH (p=0.03 and p=0.00) and the PTH gradient (p=0.00).

Conclusions: This study demonstrates that there are a series of risk factors intrinsic to the patient and to the surgical procedure capable of predicting the risk of hypocalcaemia after total thyroidectomy. Possibly, the optimization of the surgical technique could prevent the appearance of hypocalcaemia after total thyroidectomy in some cases, while in others, the identification of these factors post-op could allow early detection and effective treatment of these patients. In the present study, age, Graves' disease, and parathyroid autotransplantation were associated with postoperative hypocalcemia. Thyroid carcinoma and two-stage thyroidectomy were protective factors.

Key words: total thyroidectomy, hypocalcaemia, hypoparathyroidism, risk factors.

Introduction

Total thyroidectomy is one of the most frequent cervical surgeries, with a growing incidence in recent decades due to the increase in diagnoses of thyroid disease[1-3]. One of the thyroid surgery complications is hypocalcaemia due to iatrogenic hypoparathyroidism[1,2,4-11]. This hypo-function may be due to direct mechanical or thermal damage, inadvertent devascularization or removal of the parathyroid glands, post-surgical edema or hemorrhagic complications[1-4,8,9,12]. Both the direct damage, as well as the edema and devascularization, can be reversed over time. This explains why hypoparathyroidism is usually transient in most cases[3,8,12].

According to the latest data provided by the Spanish Thyroid Cancer Association (AECAT), 75% of cases of hypoparathyroidism in Spain occur as a result of total thyroidectomy, affecting between 10,200 and 17,300 patients. This is the most frequent complication after a total thyroidectomy.

Its frequency varies greatly in the literature, from 0.3% to 68%[2,7,10-15]. It is difficult to interpret and compare the results of the various studies due to the lack of international agreement. Recently, the SEORL-CCC, together with the SEEN, have reached a consensus where they provide more specific definitions, and also recommendations to reduce hypoparathyroidism[16].

Given its high incidence, studying the predictive factors that help identify those patients with a high risk of postoperative hypocalcaemia assumes considerable importance. The main objective of this study is to analyze the biochemical, surgical and demographic factors related to an increased risk of immediate postoperative hypocalcaemia.

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Material and methods

This retrospective study of 297 patients who underwent total thyroidectomy was carried out from January 2011 to December 2018 in a tertiary care hospital.

All patients who underwent total thyroidectomy were included, both in one and in two stages, and regardless of the reason for the indication. All were referred from the Endocrinology Service of the center itself after a complete study. In each of them, the usual protocol was performed by the ENT service: complete history, cervical palpation and laryngoscopy to assess the mobility of the vocal cords.

In this study, post-op hypocalcaemia was defined as serum calcium <8.5 mg/dL and/or appearance of symptoms typical of hypocalcaemia. Hypoparathyroidism is defined as the presence of hypocalcaemia with low or inadequately normal PTH levels. This is deemed permanent if this situation lasts more than 12 months.

Preoperative, intraoperative, and post-op demographic, clinical and biochemical data were collected from each patient through medical records. Data on the surgical reports were collected, including the number of surgeries performed (1 or 2 times), the number of observed and spared parathyroid glands and also the autotransplanted parathyroid glands, the presence of intrathoracic thyroid extension, and the association of other surgical procedures with the thyroidectomy, such as lymph node dissections.

Among the biochemical parameters collected, the following stand out: PTH, albumin-corrected calcium and preoperative vitamin D; postoperative phosphorus, albumin-corrected postoperative calcium at 6 and 24 hours, and postoperative PTH at 24 hours after total thyroidectomy. Finally, in terms of factors related to the patient and their disease, we include age, gender, Graves' disease or carcinoma confirmed by pathological anatomy.

The autoanalyzer used to determine PTH levels was the Roche-Hitachi Cobas® 6000 Series system. The normal range for this test is between 15 and 65 pg/mL. Spectrophotometry was used to determine both serum calcium and vitamin D.

Data analysis was carried out with R software version 3.6.2. All variables were subjected to a normality test. The Student's t test compared continuous parametric variables, the Mann-Whitney U test for non-parametric continuous variables and the $\chi 2$ test for proportions, considering a value of $p<0.05$ as significant. The data are presented in percentages and averages with their respective standard deviations and ranges. For the analysis of the effect size based on differences between groups, Cohen's d and the Odds Ratio (OR) were used. To simplify the graphic presentation of the data, tables and histograms have been used. A table is shown with the risk factors analyzed (table 1).

Results

This study included 297 patients who underwent total thyroidectomy, the vast majority being women (81.5%), with a mean age of 54.2±14.2 years [17-90]. Forty-four patients underwent a two-stage total thyroidectomy, and in addition, 40 of them underwent cervical dissection. More than half of the procedures were performed for benign disease (64%), and of these, 32% with preoperative thyroid hyperfunction (52 with thyrotoxicosis and 43 with sub-clinical hyperthyroidism). Of the patients included in the present sample, 16 had a documented radiological image of intrathoracic goiter. Of those with diagnostic confirmation of cancer (33%), 92 cases corresponded to the differentiated subtype and 6 cases to medullary carcinoma.

Statistical analysis showed significant differences between hypocalcaemia and age (p=0.04), Graves' disease (p=0.04), carcinoma confirmed by pathology (p=0.04), thyroidectomy in two times (p=0.00), the number of transplanted parathyroids (p=0.00), preoperative and postoperative PTH at 24h (p=0.03 and p=0.00) and the PTH gradient (p=0.00). In contrast, no significant relationship was shown with gender (p=0.22), preoperative calcium level (p=0.54), preoperative vitamin D (p=0.24), goiter with intrathoracic extension (p=0.61), cervical lymph node dissection (p=0.33) or the number of parathyroid glands observed during surgery (p=0.99) (table 2).

A total of 187 complications related to thyroid surgery were recorded in 154 patients. With great difference, the most frequent complication was hypoparathyroidism with 65%.

40.2% of the patients who underwent total thyroidectomy developed hypocalcaemia in the postoperative period (figure 1). Of the patients with hypocalcaemia, 43 recovered their calcemia levels in the first 3 months, 22 in the following 3 months, and finally 13 after the sixth month of follow-up. Therefore, the total number of patients with transient hypocalcemia was 78 cases (26.1%) and the remaining patients who did not recover after 12 months of follow-up were classified as permanent hypocalcemia with a total of 42 cases (14.1%).) (figure 2). No case of hypocalcaemia could be attributed to hungry bone syndrome.

Discussion

According to the latest data provided by the Spanish Thyroid Cancer Association (AECAT), 75% of cases of hypoparathyroidism in Spain occur as a result of total thyroidectomy, affecting between 10,200 and 17,300 patients. This is the most frequent complication after a total thyroidectomy.

No factor alone predicts post-total thyroidectomy hypocalcemia accurately. Rather, it is about several factors that interact with each other, with a high probability of jointly predicting hypocalcaemia. For this reason, there is a great discrepancy in the literature. Some authors propose as possible risk factors the fact of completing a total

Table 1. Risk factors analyzed in relation to hypocalcaemia

Biochemical factors	-Preoperative CA -Preoperative PTH -Preoperative vitamin D -Postoperative PTH -PTH gradient (% decrease)
Intraoperative factors	-Thyroidectomy in 1 or 2 stages -Lymph node emptying -Number of parathyroid glands observed -Number of autotransplanted parathyroids
Factors related to the patient and disease	-Age -Gender -Overactive thyroid -Goiter with intra-thoracic extension -Definitive AP diagnosis

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Table 2. Summary of the statistical analysis of the factors in relation to immediate hypocalcaemia

	Normocalcaemia	Hypocalcaemia	P-value	Magnitude of the effect *
Age (years)	55.3	52.7	rl (p = 0.04)	r = 0.116 IC (0.002 – 0.227) p = 0.04
Graves disease	13 (40.6%)	19 (59.4%)	χ^2 (p = 0.04)	OR = 2.23 IC (1.05 – 4.70) p = 0.03
AP Diagnosis Thyroid Cancer	66 (61.7%)	32 (38.3%)	χ^2 (p = 0.04)	OR = 0.58 IC (0.35 – 0.96) p = 0.03
2-stage total thyroidectomy	39 (84.8%)	7 (15.2%)	χ^2 (p = 0,00)	OR = 0.22 IC (0.09 – 0.50) p = 0.00
Parathyroid autotransplantation	14 (31.1%)	31 (68.9%)	χ^2 (p = 0.00)	OR = 4.95 IC (2.37 – 10.32) p = 0.00
Preoperative PTH (pg/ml)	55.2	39.1	t (p = 0.03)	Cohen's d: 0.89 IC (0.01 – 1.77)
Postoperative PTH (pg/ml)	37.2	8.7	u (p = 0,00)	Cohen's d: 2.59 IC (2.19 – 2.99)
PTH gradient (%)	31.2	73.1	u (p = 0.00)	Cohen's d: -1.57 IC (-2.52 – -0.63)
Female gender	139 (57.4%)	103 (42.6%)	χ^2 (p = 0.22)	
Preoperative calcium	9.4	9.5	u (p = 0.54)	
Preoperative vitamin D	28.2	23.3	t (p = 0.24)	
Intrathoracic extension	8 (50%)	8 (50%)	χ^2 (p = 0.61)	
Lymph node dissection	27 (67.5%)	13 (32.5%)	χ^2 (p = 0.33)	
Parathyroid observed	2.5	2.5	u (p = 0.99)	

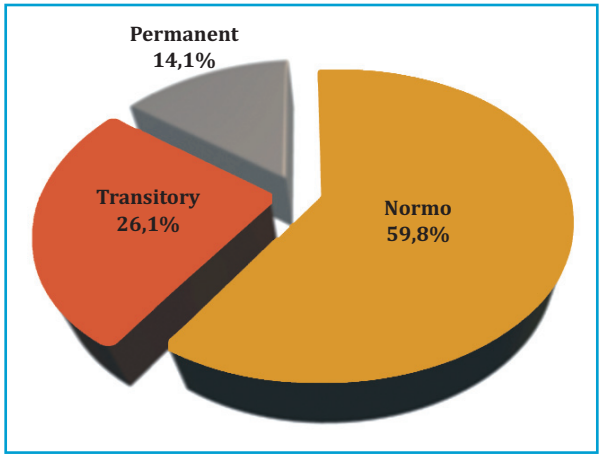
* OR calculated on the rate of overall postoperative hypocalcaemia.
lr: linear regression; r: Pearson's correlation coefficient; t: T-Student test; u: Mann-Whitney U test; χ^2: Chi^2 test.

thyroidectomy in a patient previously operated on a hemithyroidectomy, due to the distortion of the anatomy and the difficult recognition of the parathyroids[14,17].

The prevalence of transient hypocalcaemia, that is, when it lasts less than 6-12 months, ranges between 10% and 40%. The permanent one, present beyond 6-12 months, varies from 0.12% to 16.2%, according to the literature[1,3,4,14,18]. Diez et al. published in 2019 a prevalence of hypoparathyroidism at discharge after total thyroidectomy of 48%, of which 52.5% recovered in the first 3-6 months[12]. In the present study, the total hypocalcaemia rate was 40.2%, with 54.2% recovering parathyroid function in the first 6 months. Both the transitory (26.1%) and the permanent (14.1%) conform to what is described in the international literature. Thus, studying the predictive factors that help identify those patients with a high risk of post-op hypoparathyroidism is very importance.

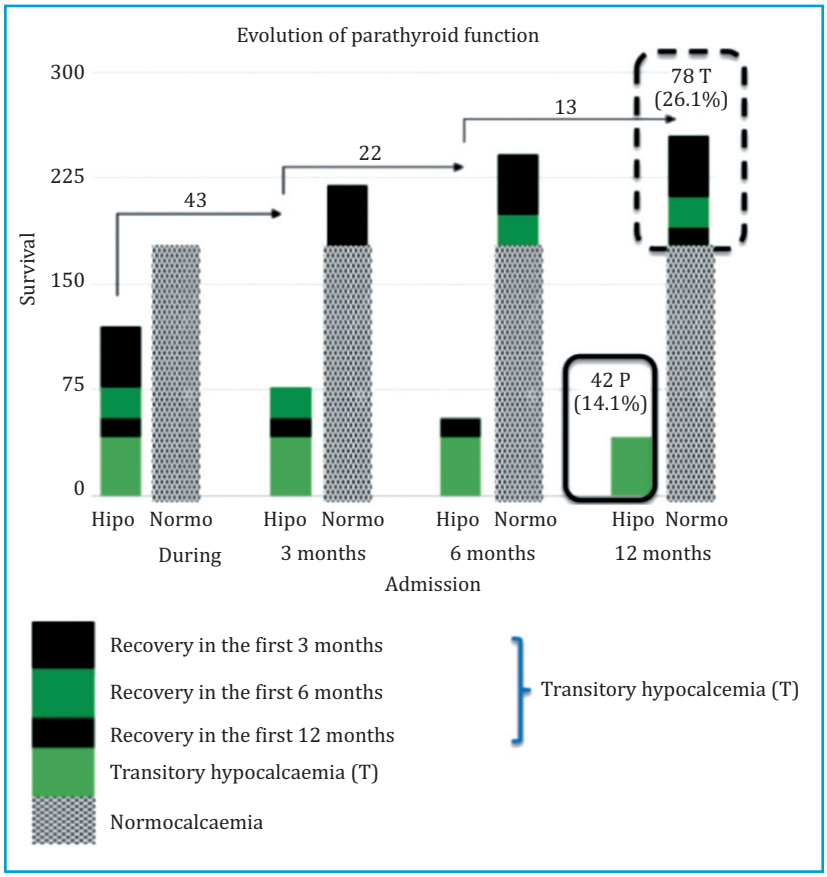
The average PTH at 24 hours was 24.6±17.9 pg/mL [3-78]. Patients with postoperative hypocalcaemia had lower mean PTH levels at 24h (8.7 pg/mL) compared to normocalcemic patients, who obtained a mean of 37.2 pg/mL (p=0.00). PTH has been extensively studied as a predictor of hypocalcaemia in the literature. Some studies propose the gradient or percentage of decrease in the PTH level from the preoperative to the postoperative period, while others propose a single intra-operative or postoperative PTH level some time after surgery. In the present study, the largest effect size is presented by postoperative PTH (Cohen's d: 2.59), with the additional advantage of costing less, since it does not require preoperative PTH.

Figure 1. Rates of permanent (14.1%) and transient (26.1%) hypocalcaemia in the sample



Regarding total hemi-thyroidectomy in a second stage, in the present study the relationship found is protective, so that those patients operated on in a single stage present a higher probability (4.55 times greater) of transient hypocalcaemia (p=0.00), coinciding with the study by Díez[12]. There is no significant relationship bet-

Figure 2. Evolution of parathyroid function in 1 year of follow-up



54.2% of patients with hypocalcaemia recovered parathyroid function in the first 6 months.

ween total thyroidectomy in a second surgical time and permanent hypocalcaemia (p=0.29). This fact could be explained by the reversible parathyroid edema and devascularization caused by the surgery, allowing a second surgical time for recovery.

On the other hand, surgery for thyroid cancer[17,19,20] or for Graves' disease[17,20] and associated cervical dissection[14] have also been proposed as possible risk factors. In contrast, other studies have not found a significant association[11]. In the study by Díez et al., the presence of lymph node metastases was a negative predictor of parathyroid recovery in patients with thyroid cancer[21].

In our study, a significant protective relationship with pathology-confirmed carcinoma is found, probably because cases suspected of malignancy are handled by a more experienced surgical team. As for Graves' disease, there is a significant direct relationship, probably due to fibrosis due to thyroiditis, with these patients presenting 2.23 times more risk of hypocalcaemia. On the other hand, there is no significant relationship with lymph node drainage.

It is reasonable to think that the fact of preserving at least one parathyroid gland could maintain normal function, and even if it decreased, it could be a transient hypocalcaemia[22,23]. In various studies, however, including the present study, it has not been possible to demonstrate significantly that the observation and preservation of more than two parathyroids prevents hypocalcaemia[14,24].

In some studies, autotransplantation of removed glands could reduce the incidence of hypocalcaemia[11]. However, in the present study this association is inverse, so that in those patients who have autotransplanted one or more glands, they had a higher risk of hypocalcaemia at discharge (4.95 times more), but this relationship was not significant in terms of permanent hypocalcaemia. This concurs with other studies showing that parathyroid autotransplantation does not guarantee recovery of parathyroid function[7,12,18,24].

Conclusions

Given the increasing number of thyroid surgeries worldwide, hypocalcaemia is assuming increasing importance and increasing the burden of disease in the population. A more precise understanding of the risk factors would help to better predict the risk of postoperative hypocalcaemia.

The factors directly related to postoperative hypocalcaemia were age, Graves' disease, number of transplanted parathyroids PTH preoperative and postoperative at 24 h and the PTH gradient; meanwhile he carcinoma confirmed by pathology and two-stage thyroidectomy were inversely related.

Conflict of interests: The authors declare no conflict of interest.

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