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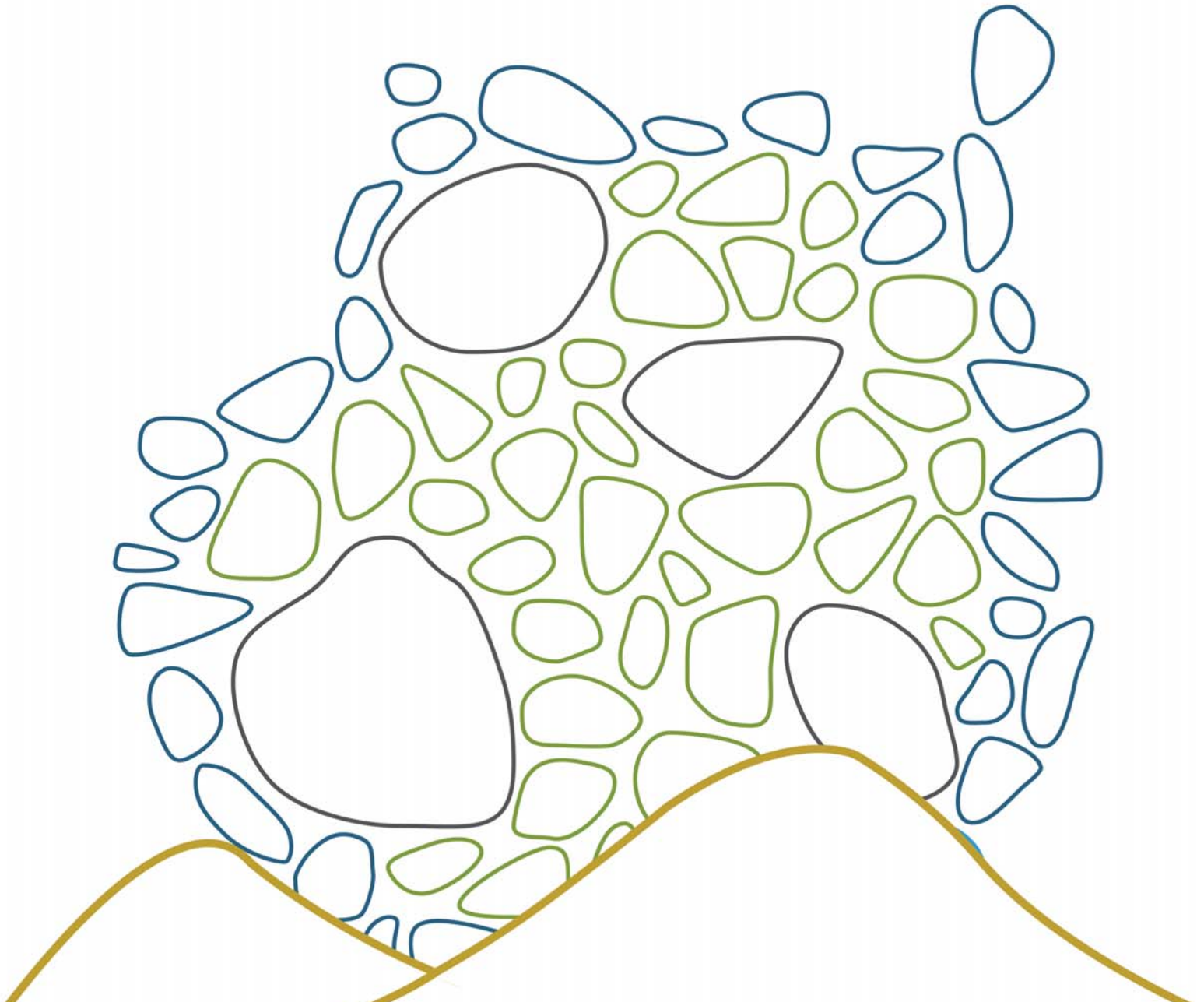

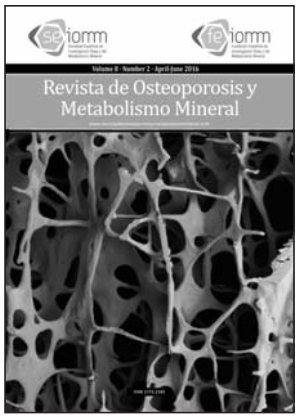

Our cover
Osteoporotic trabecular bone

Autor:
Courtesy of Professor Alan Boyde. London. United Kingdom

Velázquez, 94 (1ª planta)
28006 Madrid (Spain)
Telf: +34-625 680 737
Fax: +34-917 817 020
e-mail: seiomm@seiomm.org
http://www.seiomm.org

Editing
Ibáñez & Plaza Asociados, S. L.
EDITORIAL TÉCNICA Y COMUNICACIÓN
Avda. Reina Victoria, 47 (6º D)
28003 Madrid (Spain)
Telf. +34-915 538 297
e-mail: correo@ibanezyplaza.com
http://www.ibanezyplaza.com

Graphic design
Concha García García

English translation
David Shea

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Submit originals:
romm@ibanezyplaza.com

SUMMARY

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Revista de Osteoporosis y Metabolismo Mineral has recently been acepted for coverage in the Emerging Sources Citation Index, wich is the new edition of the Web of Science that was launched in november 2015. This means that any articles published in the journal will be indexed in the Web of Science at the time of publication.

Indexed in: Scielo, Web of Sciences, IBECS, SIIC Data Bases, embase, Redalyc, Emerging Sources Citation Index, Open J-Gate, DOAJ, Free Medical Journal, Google Academic, Medes, Electronic Journals Library AZB, e-revistas, WorldCat, Latindex, EBSCOhost, MedicLatina, Dialnet, SafetyLit, Mosby's, Encare, Academic Keys.

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Vitamin D deficiency: are we identifying it properly?

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Martínez Díaz-Guerra G[1], Aramendi Ramos M[2]

1 Servicio de Endocrinología y Nutrición - Hospital Universitario 12 de Octubre - Universidad Complutense de Madrid (España)

2 Unidad de Hormonas y Marcadores Tumorales - Laboratorio de Bioquímica - Hospital Universitario 12 de Octubre - Madrid (España)

e-mail: guillermo.martinez@salud.madrid.org

Subclinical deficiency of vitamin D or vitamin D deficiency is prevalent throughout the world, and there is great variability depending on the geographic region, genetic factors and lifestyle considerations.

Moreover, researchers now believe that serum 25-hydroxyvitamin D (25OHD) levels are the best indicator of vitamin D, although there are methodological issues that limit comparability between studies and how to establish deficiency cutoffs.

There are several criteria to establish the optimal level of 25OHD, which include the degree of maximal suppression of PTH, the intestinal absorption of mediated calcium $1,25(OH)_2$ vitamin D or reduction of fractures. Regarding the former, several studies have analyzed 25OHD concentration required for maximum suppression of PTH and offer variable results. This has led some researchers and scientific entities to recommend 25OHD levels above 20 ng/ml (Institute of Medicine, IOM) while others advise over 30 ng/ml (Endocrine Society, International Osteoporosis Foundation). The application variable for these recommendations has generated considerable confusion in clinical practice.

The article by López-Ramiro et al.[1] investigates 25OHD levels and PTH serum in 4,063 patients with a mean age of 60.6 years (70% women), and analyzed by ROC curves 25OHD value that allows us to predict optimal sensitivity and specificity to a value of high PTH (>70 pg/ml). In both cases automated methods used well-standardized electrochemiluminescence, especially in the case of 25OHD, wherein the method is validated against reference technique (liquid chromatography/mass spectrometry tandem, LC-MS/MS). The study's most important conclusion is that, to avoid inducing secondary hyperparathyroidism, 25OHD levels should be higher than 24 ng/ml. Strikingly, less than half of patients with low 25OHD levels according to the cutoff point used by the authors presented secondary hyperparathyroidism. This was even lower (24%) in the group of younger patients (18-40 years). Although the patients' clinical characteristics are not detailed in the study (concomitant diseases, or if they received treatment with vitamin D, etc), these data are relevant in our view. They confirm what other authors have already noted: 1) although the general recommendation is that the desirable range for 25OHD is 20-40 ng/ml, somewhat lower levels (15-20 ng/ml) may be sufficient in some cases, and 2) 25OHD determination should be reserved for patients who are considered at risk of deficiency because of their age, lifestyle or concomitant[2] diseases.

Moreover, the authors point out the importance of using a method quantification 25OHD sufficiently standardized. In 2010, the vitamin D Standardization Program (VDSP) proposed establishing LC-MS/MS (liquid chromatography/tandem mass) as the reference method. The various 25OHD tests must be referenced and made available to the approved reference manufacturers using this technique[3].

Although commercial methods exist that meet this requirement, there is still variability in determining 25OHD, whereby one patient can be considered deficient or not, depending on the method used. This high variability may be attributed to several factors[4]:

1) Concentration of vitamin-D binding protein (DBP). Levels of 25OHD may be low when there is a decrease in DBP concentrations.

2) The immunoassay used (competitive towards binding protein, capture Ac).

3) Forms detected vitamin D: percentage of cross-reactivity to 25 OHD_2 and 25 OHD_3, and metabolites 24,25 $(OH)_2$ D_2 and 24,25 $(OH)_2$ D_3.

4) Detection of epimers 3-epi-25 and 3-epi OHD_3 25-OHD_2.

5) Determining complexity due to the lipophilicity of the molecule, with nonspecific interference by the presence of other lipids.

In view of the methodological limitations that still exist and involving clinical implications has been proposed that concentrations of free 25OHD or free $1.25(OH)_2D$ could be a better marker of vitamin D status[5]. In fact, it is accepted that free (or bioavailable) concentrations of other hormones such as T4 or testosterone are more relevant from a physiological point of view.

Finally, the extensive information and published literature that indicate the benefits of vitamin D in bones and extra-osseous have led to an exponential increase in laboratory applications to quantify 25OHD levels. It has reached the point that in

some areas this has been become almost a routine determination. Clinically we should be cautious when interpreting 25OHD levels under the established ranges as desirable, especially in patients with no risk factors for vitamin D deficiency.

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López-Ramiro E[1], Rubert M[1], Mahillo I[2], de la Piedra C[1]
1 Laboratorio de Bioquímica Investigación
2 Departamento de Epidemiología
IIS Fundación Jiménez Díaz - Madrid (España)

Secondary hyperparathyroidism due to vitamin D deficiency

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Correspondence: Concha de la Piedra - Fundación Jiménez Díaz - Avda. Reyes Católicos, 2 - 28040 Madrid (Spain)
e-mail: cpiedra@fjd.es

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Summary

Introduction: Vitamin D is increasingly recognized as playing a significant role in combatting many diseases. One is the development of secondary hyperthyroidism due vitamin D deficiency. To date, laboratory quantification methods of serum vitamin D were not well standardized. It could not be established with certainty from which levels of vitamin D certains abnormalities take place, like an elevation of PTH. The present study was aimed at determining below what vitamin D levels we will find abnormally high levels of PTH, carrying out the vitamin D determination in the laboratory with a standardized, reliable technique.

Methods: This descriptive, retrospective study was conducted with patients over 18 years in which determinations were made simultaneously with PTH, 25 (OH) vitamin D (25OHD) and which also have normal values of calcium, glomerular filtration rate and phosphorus.

For determining vitamin D, standardized electrochemiluminescence method was used with gas chromatography-mass spectrometry method. Using the Stava version 11 statistical program, the 25OHD was calculated where PTH value was above 70 pg/ml with greater sensitivity and specificity.

Results: In all, 4,083 patients were included, of whom 2,858 were women (70%) and 1,225 (30%) males. The mean age of the study population was 60.60 years (standard deviation, 15.29). 74% of the population had a serum PTH under 70 pg/ml (normal values) and 26% had a serum PTH higher tan 70 ng/ml. By constructing the ROC curve levels of 25OHD, depending on PTH values below or above 70 pg/ml, the area under the curve was 0.5962 ($p<0.0001$). The cut having jointly account the sensitivity and specificity that determined vitamin D levels to predict PTH values above 70 pg/ml was 24 ng/ml. Of the patients with normal PTH, 71% presented normal vitamin D values, while patients with elevated PTH (Greater than 70 pg/ml), almost half had a vitamin D below 24 ng / ml, which increased as the PTH percentage was elevated.

Conclusions: The 25OHD value that presents better specificity and sensitivity to predict abnormally high PTH is 24 ng/ml, which is higher than the level reported in previous work, (about 18 ng/ml) value. The results of this study, carried out with an appropriately calibrated method, showed that 44.9% of patients with vitamin D values of less than 24 ng/ml PTH had abnormally high levels, with a normal value of calcium and phosphorus and normal renal function. This percentage is less in those individuals between 18 and 40 years (24%) and reaches 49% beyond 60 years. These patients could be treated with vitamin D to prevent possible secondary hyperparathyroidism due to vitamin deficiency. It is noteworthy that the method of determining vitamin D used must be properly standardized with respect to gas chromatography-tandem mass spectrometry method.

Key words: *secondary hyperparathyroidism, vitamin D deficiency, vitamin D standarization program, tandem-mass spectrometry, 25 (OH) vitamin D.*

Introduction

Vitamin D is involved in the metabolism of phosphorus and calcium. Vitamin D deficiency is associated with osteoporosis and osteomalacia in adults and rickets in children. Recent studies have also shown their role in autoimmune diseases, cancer, cardiovascular disease, etc.[1-4].

PTH is a hormone secreted by the parathyroid gland, involved in calcium metabolism, bone obtaining it if hypocalcemia, and increasing the production of 1,25(OH)2 vitamin D in the kidney, to promote absorption of calcium. Moreover, it also increases renal tubular reabsorption of calcium.

In the clinical laboratory, we often find PTH higher than the upper normal limit, but with normal creatinine or glomerular filtration, calcium and phosphorus and which therefore do not correspond to a primary or secondary hyperparathyroidism, data mystifying the clinician. In many of these cases, the increase in PTH is associated with vitamin D deficiency, made increasingly found in our population and that has not been paid much attention[5-10]. Vitamin D levels are measured through levels of metabolite 25(OH) vitamin D (25OHD), which express the status of this vitamin in patients[11]. Until recently, most 25OHD determinations were not adequately standardized and values varied greatly depending on the different methods[12].

Moreover, it is of great interest to know at what 25OHD levels an abnormal elevation of PTH occurs. This may help us rule out other likely causes of hyperparathyroidism[13].

Several studies show considerable variation between 25OHD results obtained in the laboratory by different methods: radioimmunoassay, electrochemiluminiscence, HPLC and tandem-mass spectrometry[9,10,14-16]. The variability between laboratories methods leads to a misallocation of patients[17], as well as a lack of standards when applying a health policy[18]. All this makes it difficult to establish normal vitamin D levels and beyond which it is likely that an abnormal increase in PTH occurs.

Because of this a program of international standardization (Vitamin D Standardization Program) has been launched in collaboration between the NIH-ODS (National Institutes of Health, Office of Dietary Supplements), Centers for Disease Control and Prevention (DCP) and the National Institute of Standards and Technology (NIST). This has led to the emergence of reference calibrators available to manufacturers so that the values obtained are the same for all methods[12,18]. Reference gauges are validated against liquid chromatography technique/tandem-mass, which is certainly the most accurate of those currently available.

The aim of this study is to determine the 25OHD value below which PTH abnormally increased. It is important to use a method of determining 25OHD properly standardized with respect to the gas-mass method, which is the gold standard, since most of the papers published so far in the literature are carried out with methods of determining 25OHD that are not properly standardized.

Materials and methods

A retrospective, cross-sectional, descriptive study was carried out at the Jimenez Diaz Foundation Hospital Biochemistry Laboratory (Madrid).

Consecutive blood tests were analyzed of the patients who had been asked for PTH, 25OHD, Ca and glomerular filtration rate (GFR) simultaneously during the period from May 2012 to November 2012 in the hospital.

Only those patients over 18 with FG greater than 60 and serum calcium between 8.4 mg/dl and 10.5 mg/dl simultaneously were included. Therefore, all patients with renal failure and abnormal calcium levels were excluded to rule out primary hyperparathyroidism or secondary to kidney failure.

A database was created containing all coded patients in which different variables were included in the study (age, sex, and analytical parameters) obtained manually through data from the clinical laboratory Hospital Foundation Jimenez Diaz.

This study was approved by the Ethics Committee of Health Research Institute of the FJD. As the data were drawn from a general Biochemistry Laboratory database, without using patients' names, informed consent was not required.

PTH determination was carried out in an automatic apparatus electrochemiluminescence ADVIA Centaur (SIEMENS). PTH levels greater than 70 pg/ml were considered abnormal. The reference range provided by the company was 14-70 pg/ml. As with the remaining second generation methods, it measures intact hormone 1-84, with the crosstalk of the truncated PTH at amino-terminal level. The sensitivity of the method is 5 pmol/ml and the coefficients of intra- and inter-assay variation are <7% and <10% respectively.

The 25OHD determination was carried out by electrochemiluminescence in an iSys autoanalyzer (IDS, UK), a method of determining properly standardized vitamin with respect to the gas-mass method. The sensitivity of the method is 4 ng/ml and the coefficients of variation intra- and inter-assay are <5% and <7%, respectively.

Using the statistical software Stava version 11, the 25OHD value was calculated for which PTH rose more than 70 pg/ml with a sensitivity and greater specificity, calculating the area under the ROC curve and checking that it was statistically significant.

A descriptive analysis of the sample by calculating the percentage of men and women, the mean and median age was also performed. The percentages were also calculated of patients with PTH above and below 70 pg/ml and the percentage of patients with 25OHD with different values (patients with vitamin D under 10, 10 to less than 24, 24 to less than 30 and patients with values greater than 30).

The differences between individuals with PTH higher and lower than 70 pg/ml were described using the Student t test, Mann-Whitney and Chi-square.

Results

A total of 9,225 patients were studied, of whom 5,142 were excluded for being under age, presenting kidney failure or abnormal calcium levels.

Therefore, only a total of 4,083 patients, 2,858 women (70%) and 1,225 (30%) men, all over 18 years old were included in the study.

The average age of the population was 60.60 years with a standard deviation of 15.29, and the median age was 62 years. The minimum age was 18 years and the maximum age of 100 years. 74% of the population had a PTH below or equal to 70 pg/ml (normal values) and 26% greater than 70 pg/ml. The demographics of the patients are shown in table 1.

Regarding 25OHD levels of our population, only 46.4% of the same showed that levels above 30 ng/ml metabolite, 20.9% had levels between 24 and 30 ng/ml, 30% from 10 to 23 ng/ml, and 2.7% levels ≤10 ng/ml (Figure 2).

The baseline description of the sample showed no clinically significant differences in terms of PTH values depending on sex (Table 2). However, if there is significant age difference between patients with normal and abnormal values of PTH (59.3±15.5 years vs 64.6±13.9 years, p<0.001), with older patients with abnormal PTH and also presenting 25OHD values significantly lower than in the group with normal PTH (Table 2).

With patient data we construct the ROC curve for 25OHD levels in terms of having PTH values below or above 70 pg/ml. An area below the curve of 0.5962 (p<0.0001) was obtained, which shows that there is a relationship between 25OHD and PTH (Figure 1).

When using vitamin D values for predicting PTH values above 70 pg/ml, the best cutoff point was 24 ng/ml (Figure 1) considering sensitivity and specificity jointly.

In our population, 32.7% of the sample were found to have vitamin D levels less than 24 ng/ml, which were 44.9% of PTH values greater than 70 pmol/ml. By dividing the patients into 3 age groups: 18 to 40 years between 40 and 60 years and older than 60, a remarkable fact was observed. In the 18 to 24 year group, of the patients with 25OHD less than 24 ng/ml, only 24% had values of PTH >70 pg/ml. Among patients 40-60 years, 33.7% of patients with low levels of 25OHD had elevated PTH value. Finally, in the group of older patients (beyond 60 years), 49% of the patient group with PTH >70 pg/ml and 25OHD <25 ng/ml was shown. Thus, as people reach an advanced age, the likelihood that a low 25OHD level produces a high figure of PTH is higher. There is not the same risk at all ages. It is also interesting to note that, in our work, the percentage of patients with 25OHD <24 ng/ml is 32.7%, and the percentages are similar in the different age ranges: 33.2%, 31.7% and 32%, respectively. That is, in our population we found that there is a higher percentage of patients with low 25OHD levels among those over 60 years.

Discussion

Our work shows an inverse association between serum 25OHD and PTH. Other authors have observed this correlation[19-21].

The value that maximizes 25OHD specificity and sensitivity, as to produce an abnormal increase in PTH is 24 ng/ml, greater than presented in previous work value, which was about 18 ng/ml[22].

Since this study corroborates the fact that a vitamin D deficiency may cause an abnormal elevation of PTH values, we consider it important, in clinical practice, to determine levels of 25OHD in those patients who present abnormally high PTH values without known cause, to rule out hyperparathyroidism secondary to vitamin D deficiency. This could be corrected by supplementing patients with the necessary vitamin D, thus avoiding the aggravation of diseases caused by elevated PTH, as is the case of osteoporosis[19].

The problem with studies until now is that they have used different methods of determining 25OHD which were not properly standardized, so there was a great variation between the results of 25(OH)D obtained. The levels of vitamin D considered normal were difficult to establish as were those from which it was likely that an abnormal increase in PTH occurred. The different laboratory techniques used to determine vitamin D are: radio-immunoassay, electrochemoluminiscence, HPLC or lipid chromatography tandem-mass spectrometry. Currently the most accurate technique is the liquid/tandem-mass chromatography, which undoubtedly is the most accurate of those available[11] and no validated reference calibrators against this technique. In this study we used an electrochemiluminescence in an IDS-iSYS (UK) autoanalyzer, properly standardized gas-mass relation, so the results are considered valid, and comparable to studies with other methods that are well calibrated.

In most studies on vitamin D to date, the method used is not discussed, nor do they specify whether the method is calibrated with respect to the gas-mass technique. Thus it is impossible to know whether the results of the values vitamin D found in the studies are correct and whether they could be extrapolated to the general population and applied to clinical practice once the clinician finds certain values in analysis of patients.

In a group of postmenopausal women, Capatina et al.[23] observed that 27.2% of patients with vitamin D deficiency had secondary hyperparathyroidism, a lower percentage than what we found, 44.9%. Laroche et al.[24] observed that 13% of patients with 25OHD <30 ng/mL had secondary hyperparathyroidism. However, Sadat Ali et al.[25] found in 200 patients (150 women and 50 men aged 18 to 69 years) all of whom presented vitamin D deficiency, determined by gas-mass, had secondary hyperparathyroidism.

As mentioned above, the results of this study, a properly calibrated method, show that 44.9% of patients with 25OHD levels <24 ng/mL had serum PTH values >70 pg/ml, no other proper reasons. This percentage is lower in the population between 18 and 40 years and more in the population >60 years. It seems, therefore, important that the clinician concerned with vitamin D to patients with 25OHD levels <24 ng/ml in order to avoid possible secondary hyperparathyroidism deficit of vitamin D. In order to establish a cut-off, it is important that 25OHD determination methods are properly standardized with respect to the gas-mass technique.

Table 1. Demographics and media and median values of PTH and 25 (0H) vitamin D in a population of 4,083 patients, 2,858 women and 1,225 men, all aged ≥18 years

	Mean	DS	P25	P75	Median
Age (years)	60.69	15.29	51	72	62
PTH (pg/ml)	57.36	38.11	35.50	71.05	50.30
Vit D (ng/ml)	30.70	14.52	21	37	29

Table 2. Comparison of mean age, calcium 25-OH vitamin D, PTH and number of women and men as having PTH values greater than 70 pg/ml, or less than or equal to 70 pg/ml in a population of 4,083 patients, 2,848 women and 1,225 men

	PTH ≤70		PTH >70		
Variable	**Mean**	**DS**	**Mean**	**DS**	**Value P**
Age (years)	59.3	15.5	64.6	12.9	<0.0001
Calcium (mg/dl)	9.57	3.80	9.52	4.10	0.7231
Vit D25 (ng/ml)	31.8	14.3	27.6	14.6	<0.0001
PTH (pg/ml)	42.5	15.3	99.8	50.0	<0.0001

	N	N
Women	2.123	735
Men	900	325

Competing interests: The authors declare no conflicts of interest.

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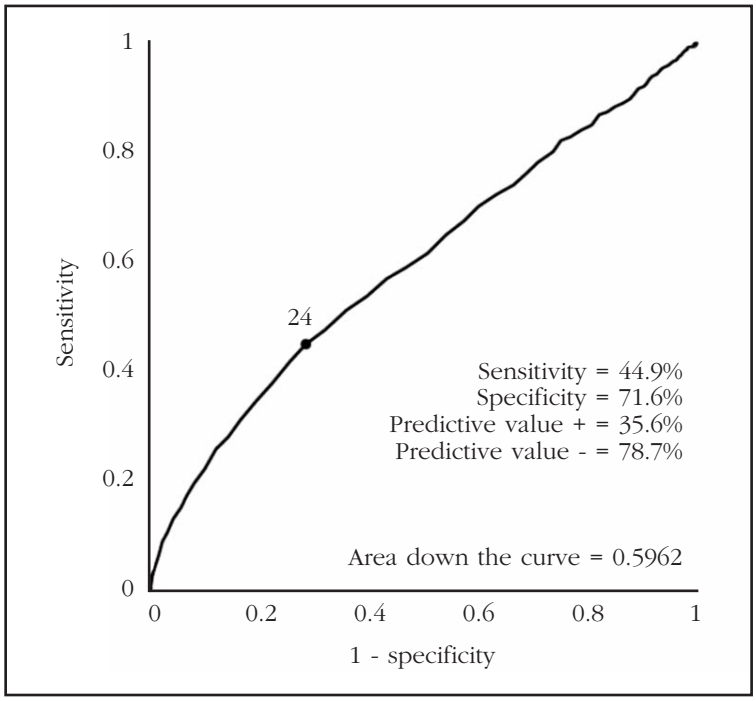
Figure 1. ROC curve obtained by relating the values of PTH and 25 (OH) vitamin D in a population of patients 4,083, 2,858 and 1,225 women men ≥18 years of age, with normal calcium and phosphorus and without renal failure. the number of patients with levels of 25 (OH) vitamin D greater than or equal to 24 ng/ml and less than 24 ng/ml is shown at the bottom, and more and less than or equal to 70 pg/ml PTH



Vit D (ng/ml)	PTH (pg/ml)	
	≤70	>70
<24	860	476
≥24	2,163	584

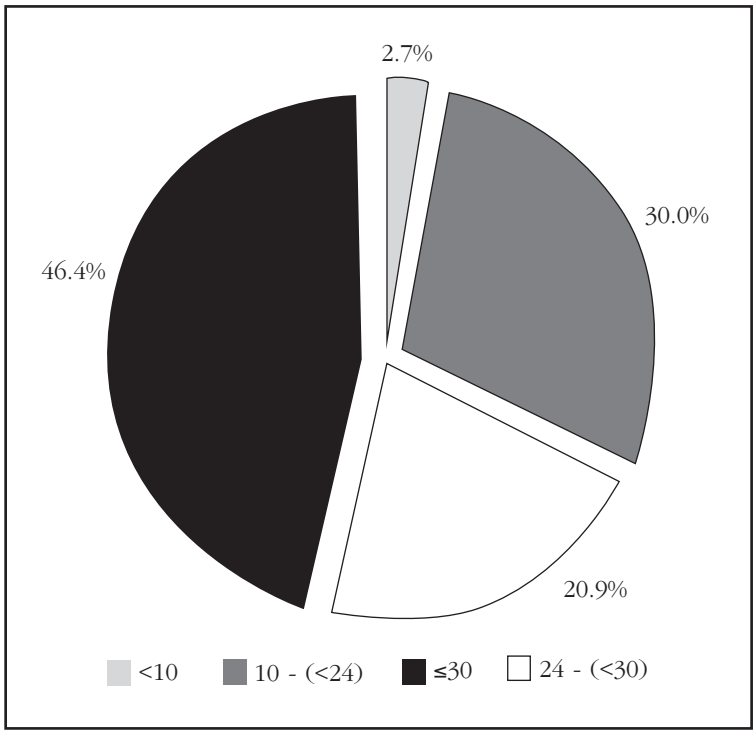


Figure 2. Groups of patients with 25 (OH) vitamin D under 10 ng/ml, between 10 and 24 ng/ml, between 24 and 30 ng/ml and greater than 30 ng/ml, a population of 4,083 patients, 2,858 women and 1,225 men, aged ≥18 years with normal calcium and phosphorus and without renal failure

Marco M[1], Giner E[2], Larraínzar R[3], Caeiro JR[4], Miguélez H[1]

1 Departamento de Ingeniería Mecánica - Universidad Carlos III - Madrid (España)

2 Universidad Politécnica de Valencia - Centro de Investigación en Ingeniería Mecánica CIIM - Departamento de Mecánica y de Materiales - Valencia (España)

3 Hospital Universitario Infanta Leonor - Servicio Cirugía Ortopédica y Traumatología - Madrid (España)

4 Complejo Hospitalario Universitario de Santiago de Compostela - Servicio de Cirugía Ortopédica y Traumatología - A Coruña (España)

Analysis of mechanical behavior variation in the proximal femur using X-FEM (Extended Finite Element Method)

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Correspondence: Miguel Marco Esteban - Universidad Carlos III de Madrid - Departamento de Ingeniería Mecánica - Avda. de la Universidad, 30 - 28911 Leganés - Madrid (Spain)
e-mail: mimarcoe@ing.uc3m.es

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Summary

Introduction: For years, the human femur has been extensively studied experimentally with *in vitro* analysis. Nowadays, with computer advances, it can also be analyzed numerically. Some authors report the usefulness of finite method in predicting the mechanical behavior of this bone. There are many possibilities using the synergy between the method finite element and experimental trials. In this paper, for example, we study how they affect different osteoporotic simulations involving femur fracture loads.

The aim of this study is to predict hip fracture, both the load to which this occurs as the propagation of the crack in the bone. By applying the finite element method to the field of bio-mechanics, simulation can be carried out to show the behavior under different bone load conditions.

Material and methods: Using DICOM images, CT scan of the proximal end of the right femur of a male has been obtained bone geometry. By a computer program they have been generated dependent mechanical properties of the BMD each voxel, and then used a finite code to apply different load configurations and study values bone fracture elements. The numerical model has been validated in the literature.

Results: Load breaking in lateral fall configuration is approximately half the load in the case of the normal position, which agrees with different experimental studies published.

In addition, we have studied various load conditions in everyday situations, where it was observed that the load fracture is minimal in mono-podal position. Osteoporotic conditions have also been simulated which confirmed that the load fracture has been reduced by decreasing mechanical properties.

Conclusions: By using the finite element method in conjunction with DICOM medical imaging, it is possible to study the biomechanics of the hip and obtain an estimate of bone failure. In addition, different load configurations can be applied and vary the mechanical properties of bone to simulate the mechanical behavior of low osteoporotic conditions.

Key words: *femur, hip fracture, CT scanner, finite elements.*

Introduction

According to recent information sources available in Spain[1], a total of 487,973 cases of hip fracture were reported between 1997 and 2008. There is a female predominance of 3 to 1[2] and the incidence increases with age (in 1997, it was 78.07 years, while in 2009 it increased to 80.46 years). Proximal femoral fractures (PFF) imply high health costs due to the long-term average stay (in 1997, the average was 16.05 days, while in 2008 the figure fell to 13.34 days) and the direct expenditure this entails. In 2008, the overall cost of hospitalizations in Spain's National Health System was 395.7 million euros. This was a 131% increase compared with 1997. Individually, the cost per patient rose from 4,909 euros in 1997 to 8,365 euros per patient in 2008. It should be noted that the fracture also carries an acutely high mortality rate of hospital care (4.71 to 5.85%) as well as a year later (25-33%)[3].

In this context, the need for predictive methods of fracture arises, both at individual and population-wide levels. The prediction of femoral fracture is a challenge in the world of biomechanics, with both doctors and engineers focusing on the study of crack propagation in human bones. For years there have been experimental analyses of human femurs from donors. Today, with technological advances, computers are more sophisticated and able to enhance this analysis, reducing experimental study costs. At the same time, we can better understand the processes of how cracking and fractures appear. With a finite element model, scientists can study the mechanical behavior of the femur under conditions of specific load and therefore assess the normal biomechanics of the hip and pathophysiological process fracture with a correlation of about 90%[4] despite a great variability in the choice of the mechanical properties that apply to the different numerical models studied to date[5,6].

The main objective of our study is to develop a PFF model using finite elements to analyze the different configurations required under normal and pathological load. As secondary objectives, we want to evaluate the morphology and minimum load configuration from which the dependent load conditions fracture is initiated and analyzed numerically how it affects osteoporosis breaking load bone, due to the reduced mechanical properties arising as a result of bone mass loss[7].

Material and method

Generation of model of finite elements

From computed tomography (CT) DICOM images of the right PFF of a young adult male without a known hip condition in the studied side, the macro-structure geometry of the bone was obtained. Medical images were obtained with a radiation dose and standard clinical exposure time enabling a resolution of 0.3 mm in the transverse plane and 0.7 mm in the longitudinal direction.

The femoral geometry was generated by image processing software and ScanIP finite element modeling (Simpleware, Exeter, UK). This allows us to select the range of Hounsfield unit (HU) scale necessary for proper display of CT images, applying volume/surface filters and generating an identical geometry to the specimen resulting in a numerical model with mechanical properties dependent on bone mineral density (BMD) supported in the following equations[8-11]:

$$\rho(g/cm^3) = 0.1259 + 1.15638 \cdot 10^{-3} \cdot HU$$

$$E(MPa) = 6850 \cdot \rho^{1.49}$$

Through these expressions, a relationship is established between the HU of each voxel, its density and Young's modulus (E). In this case, the model has 14 different materials in order to reproduce the maximum heterogeneity of real bone. Using these equations, the bone will present isotropic behavior, which does not really reflect reality, but, as has been shown in other studies, we can simulate the behavior of the femur globally through these expressions.

Loading conditions of the finite element model

The finite element analysis of the PFF generated is carried out using the Abaqus/Standard 6.12 program. (Dassault Systems, Providence, Rhode Island). The discretized mesh volume is formed by 198,764 second-order tetrahedral elements (C3D10 in Abaqus). The PFF area has a finer mesh of 2 mm while the rest of the model elements are 3 mm in size. The nodes of the bottom of the PFF are embedded (prevented from displacement in any direction). As shown in figure 1, it has been considered normal loading position that it assumes that the load vector has an angle of 8° adducted with the longitudinal axis of the hip in transversal plane[12]. lateral fall is considered one that is a load vector with a rotation of 20° in ante-version and 30° to the longitudinal axis as pivot point[13].

Validation of the numerical model

Results were validated by comparing values obtained in two different configurations: normal and lateral position with those obtained in similar conditions in experimental work in a human corpse[8]. To this end we assume that the initial bone break occurs when a critical deformation of 0.0061[14] is reached. The magnitude of the validation load is 470 N (75% of the individual's weight).

Loading boundary conditions and patterns of fracture in standing position and lateral fall

To analyze femoral fracture patterns in standing position and lateral fall, the Extended Finite Element Method (XFEM) implemented in Abaqus was used. With this method, the start of a crack is determined in the maximum deformation area. Then this field is spread depending on the stresses and strains surrounding it. The parameters of fracture toughness (density dependent) and the

critical energy for propagation in different modes are obtained in the literature, according to the following formulas[15,16]:

$$K(N\,m^{-1.5}) = 0.7413 \cdot 10^{6} \cdot \rho^{1.49}$$

$$G(J\,m^{-2}) = \frac{K^2(1-\upsilon^2)}{E}$$

$$G_{IIC}/G_{IC} = G_{IIIC}/G_{IC} = 0.33$$

Boundary conditions and breaking loads in normal locomotor activities

For analysis of stresses in various daily life activities, the fixed shaft is considered. Calculations are carried out according to the different angles at which the load is applied in the PFF, as reported by Bergmann[17]. In all, 9 different configurations are analyzed: monopodal support, climbing stairs, walking slowly down stairs, walking fast, regular walking, standing, bending knees and sitting.

Boundary conditions and loads of simulated fracture in osteoporosis

For stress analysis under simulated osteoporosis, the BMD model has been decreased and therefore a decrease of Young's modulus and overall femoral stiffness. BMD variation is carried out in different areas: in the PFF generally at the femoral neck, trochanteric area in the upper area of the shaft and in the middle of the shaft. The loading conditions are performed in the normal position of the PFF analyzing how this affects a loss of tissue stiffness, as that could be caused by osteoporosis, the breaking load of the PFF. Figure 2 the areas where BMD was varied and the boundary conditions corresponding to the loading position under study.

Results

Finite element model of the PFF

The finite element model of the PFF derived from DICOM medical images is shown in figure 3. The model was generated by ScanIP software; in figure 3b the femoral surface was observed, and in figure 3c, the heterogeneity model.

Validation of the numerical model

Table 1 shows the general properties assigned to the numerical model and the results in stiffness and breaking loads between the developed model and the comparator chosen. The mechanical properties assigned are virtually identical to those calculated in the experimental study that supports this article8. It is noted that both overall rigidity and fracture load in normal position are similar to the experimental test, therefore the numerical model may be considered validated. In figure 4 the strain field analysis obtained in this validation is shown.

Standing fracture patterns and lateral fall

Figure 5 shows the pattern of fracture start under the conditions described. It can be seen how the breaking load in lateral fall configuration is lower than in the normal position (around 50% lower, of 3,979 N to 1,890 N). In both cases the crack starts at the top of the femoral neck, although in the case of lateral crash onset is more posterolateral neck.

Boundary conditions and breaking loads in normal locomotor activities

Figure 6 shows the breaking load for each of the analyzed configurations discussed above. The load at which the critical strain is reached and at which fracture occurs is ordered from lowest to highest for easy viewing of data.

This clearly shows how the most critical configuration is corresponding to the lateral drop previously studied, followed by the normal position. The other positions have a higher breaking load, though not much variation as the lateral fall. In the configuration of the lateral fall, load value decreases considerably, assuming at least half load in the remaining cases.

Boundary conditions and simulated loading fracture in osteoporosis

Under the conditions described osteoporosis simulated by a percentile decrease of mechanical properties in different areas of the PFF weakening that occurs in bone structure by reducing the stiffness of this because of osteoporosis objective. Figure 7 shows the breaking load according to the decrease in BMD (has decreased to 50% of initial density).

This indicates how the most critical decline is related to the changed properties in the overall PFF, but also shows how the decreased BMD only in the neck area, the variation that the breaking load suffers is virtually identical to the general case. The trochanter area also proves be critical, although not as much as those already mentioned, while in areas of the diaphysis the reduction practically does not affect the breaking load, due to its distance from the neck area and the analyzed boundary conditions in particular. It shows how the BMD decrease is a great variation in the fracture load, reducing it by more than half for a 50% BMD decrease.

Discussion

In this study, a complete finite element model has been developed to predict the PFF failure and simulate the fracture that occurs depending on load conditions. The breaking load at different positions was also obtained for everyday life. These were compared to those of the lateral fall along with the effect of decreasing BMD and load necessary for breaking. Thus we have developed a computational model that allows experimental study of the proximal femur.

The method of developing the geometric structure from medical imaging has been used in other published studies[8-11]. Choosing a young adult patient is justified in order to obtain a proper numerical value transfer of bone density that represents a proximal femur under physiological conditions, and suitable from the biomechanical

Figure 1. Boundary conditions in normal position[12] and fall lateral[13]

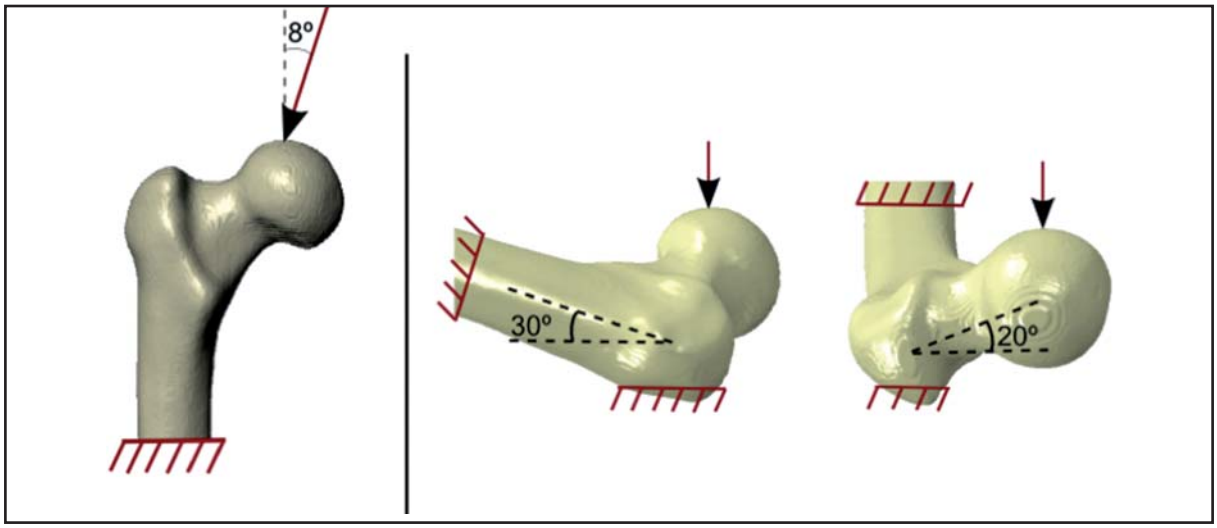
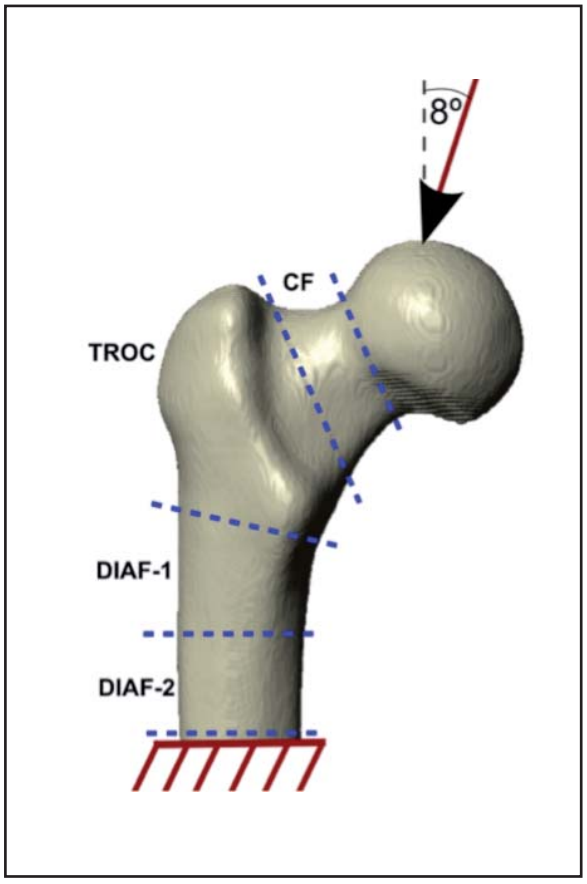


Figure 2. Areas of decreased mechanical properties



point of view. The choice of a patient of advanced age would seem a more clinically realistic hip fracture problem, but would not represent the standard physiological pattern of the proximal femur.

Having a geometric numerical model allows for changes in load conditions, intensity and vector, boundary conditions and mechanical strength properties. We believe that the 470 Newton load chosen for analyzing the different configurations is appropriate because it corresponds to the estimated value of a young adult who is the starting point of the model described. The vector load application in the standing position is admitted to several experimental studies, as is the vector application in lateral fall.

As in any numerical model, proper validation is required to ensure that that results achieved are close to reality. We believe that the proposed validation compared to experimental test results made by other authors, is appropriate because these mechanical stresses involve different human femurs and study their overall stiffness and breaking load in the normal position. The results Analysis of the results obtained it can be concluded that the model reasonably reproduces reality, and other results can be obtained by modifying boundary conditions, properties, etc. In addition, the maximum deformation occurs in the upper femoral neck. Several other studies reached this same conclusion[8,18].

The model shows that in both standing and lateral fall, the crack starts in the upper region of the femoral neck. As described in the literature, this actually shows that the bone better supports compression loads than those of traction. In the femoral neck in standing position this happens. In the lower area, compressive loads occur while in the upper region, they are related to traction. We believe that the similarity between the mathematical prediction and the one expected reinforces the validity of the methodology used. The femoral neck is not circular but oval, with the largest cortical thickness at the bottom rather than at the top. In our case, being a young adult, there is a big difference in the starting point between the normal position and lateral fall, because the difference between cortical thickness between the upper and lower areas is not very high. Probably if we had used images for an elderly patient, the starting fracture point in lateral fall would have been even more posterolateral in the femoral neck.

Figure 3. a) scanner employee at work; b) geometry obtained by ScanIP; c) finite element mesh and different materials

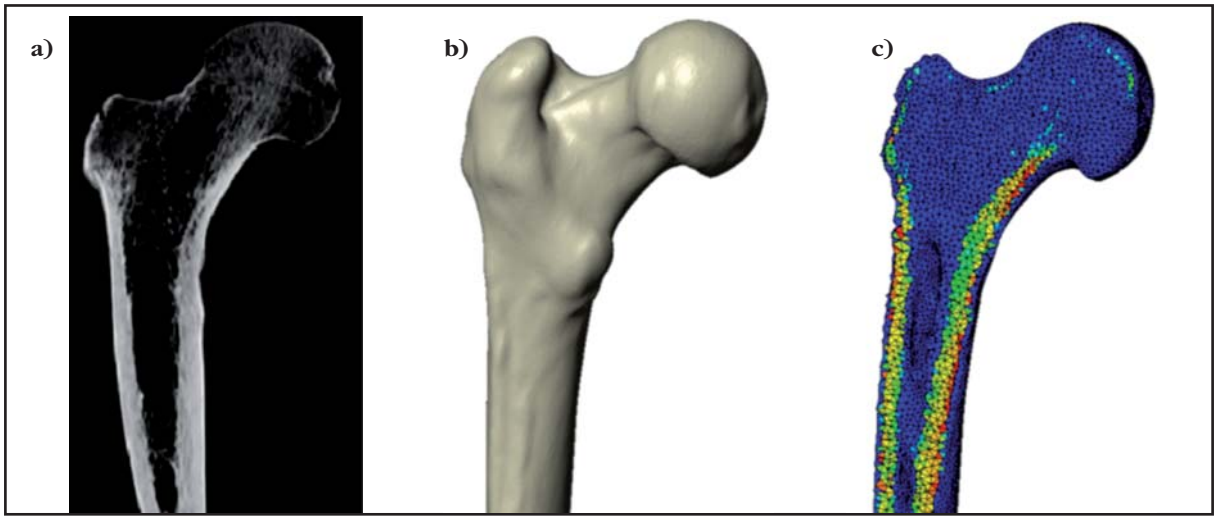


Table 1

	Current study (finite elements)	Specimen nº 1 Ali et al.[8] (experimental trial)
ρ_{mean} (g/cm³)	0.934	0.933
E_{mean} (MPa)	6187.4	6174.5
Overall stiffness (N/mm)	1,454	1,448
Fracture load in normal position (N)	3,979	4,555

The breaking loads for normal position and lateral fall within a usual range have been demonstrated experimentally in other studies[4,8,19,20]. The values for the breaking load fall on the side are 50% lower than in the normal position, consistent with experimental results[4,8]. Failure criteria considered are able to detect the area where the numerical model fracture starts. We also consider the presence of the crack and predict its spread by XFEM, although it is true that a complete femoral fracture due to problems of convergence in the solution is not achieved.

The breaking loads in the femur for different positions of everyday life whose load angles were obtained in the work of Bergmann et al.[17] had not been studied so far, and is a new source of information and study.

The results show how the femur is optimized for loads under physiological conditions; morphology and anisotropy make major stresses and strains converge towards alignment, allowing it greater load support. Instead, under an abnormal load, as is the case of a lateral falls, the charges are not aligned with the direction of the femoral support[21]. So we see how the fracture load for actions of everyday life is much higher than for non-physiological conditions, such as a lateral falls as there is logical to think that the femur is adapted to the loads of daily life.

From the macroscopic point of view, the 3 factors that most affected PFF strength are its geometry, bone mineral density and traumatic load fall. It is obvious that reducing the BMD of the model entails a lower breaking load to produce fracture, as indeed happens in reality. The ability to reduce the density globally or in specific areas opens a new line of research that may correlate the experimental work in which it is known that the trabecular bone provides much less bone strength in the PFF than the cortical bone.

Almost most clinical hip fractures occur in the trochanter area or neck. Interestingly, these two areas are the most sensitive to the simulated decrease in zonal BMD values. This means that the breaking loads are significantly lower and therefore there is a high clinical prevalence. These findings reinforce the validity of the model developed in predicting fracture.

We are aware of the limitations of the work; the model represents the analysis of a single patient and their particular conditions. This is especially interesting in individual predictions but we cannot infer age correspondence with other groups, morphotypes or gender. Furthermore, the

Figure 4. Deformation field validation under 470 N in normal position

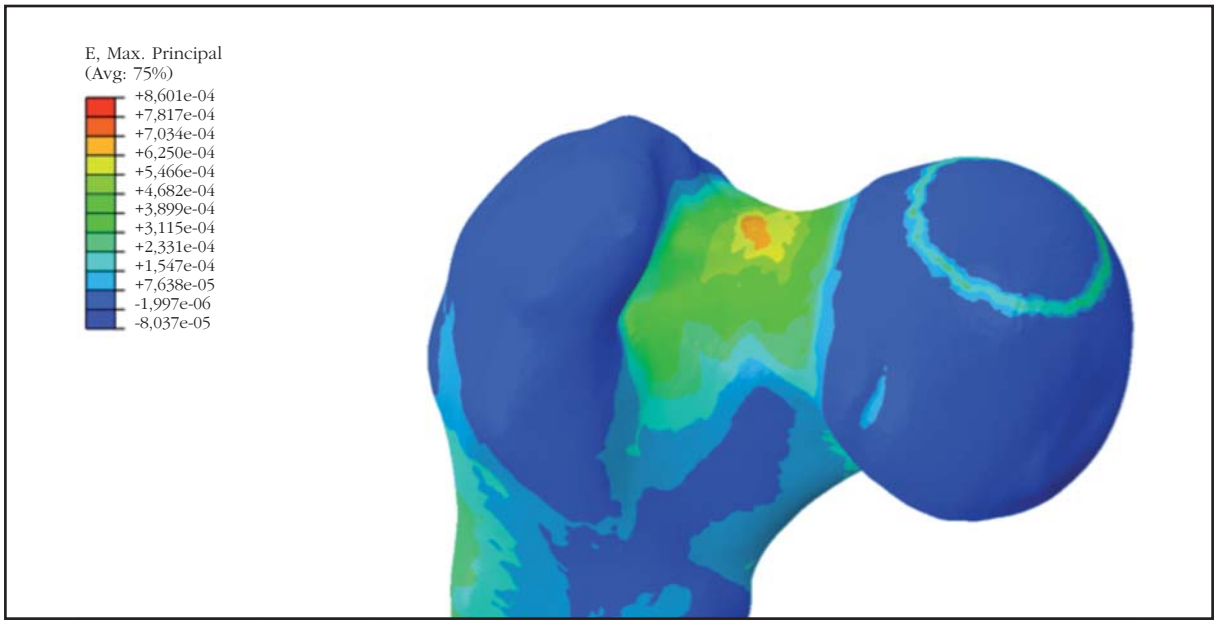
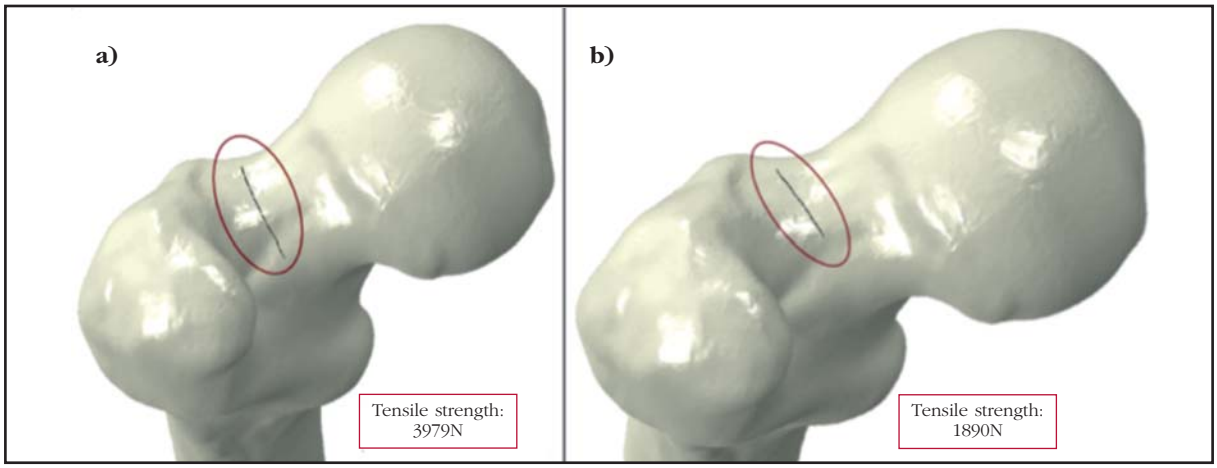


Figure 5. Patterns of fracture and breaking load: a) normal position; b) lateral fall



model does not provide any lateral fall of existing dampers in reality, such as static because soft tissue or due to dynamic osteo-tendinous reflection. Undoubtedly, adding these dampering factors to the numerical geometric pattern would correspond to a higher correlation with reality, but their absence does not invalidate the findings.

We believe that the proposed model represents the first step of our research group and has allowed us to define the procedure for the experimental and numerical study of the proximal end of the femur. In the future human femurs will be studied under normal conditions and compared to other senile groups, to analyze the influence of aging bone, both in the breaking load and its corresponding pattern.

Conclusions

• Using the finite element method and a computer program able to get the geometry and distribution of mechanical properties using a CT scan, we can predict failure with valid PFF results in different load configurations.

• By studying different loading configurations, we see how the most critical load corresponds to the lateral fall (50% lower than in the normal position). Other positions of daily life have a load greater than previous fracture. This is because the geometry of the femur has evolved to support common loads (normal position and the remaining positions of daily living) instead of lateral falls.

• By simulating conditions in different areas of osteoporosis, we observed how uniformly decreasing properties are most critical in terms of the breaking load. The next most critical area is that of the femoral neck, which shows that it is vital in the PFF structure. The area of the shaft has been the least influential in this study.

Competing interests: The authors declare no conflict of interest in connection with this work.

Figure 6. Tensile strengths in different load configurations

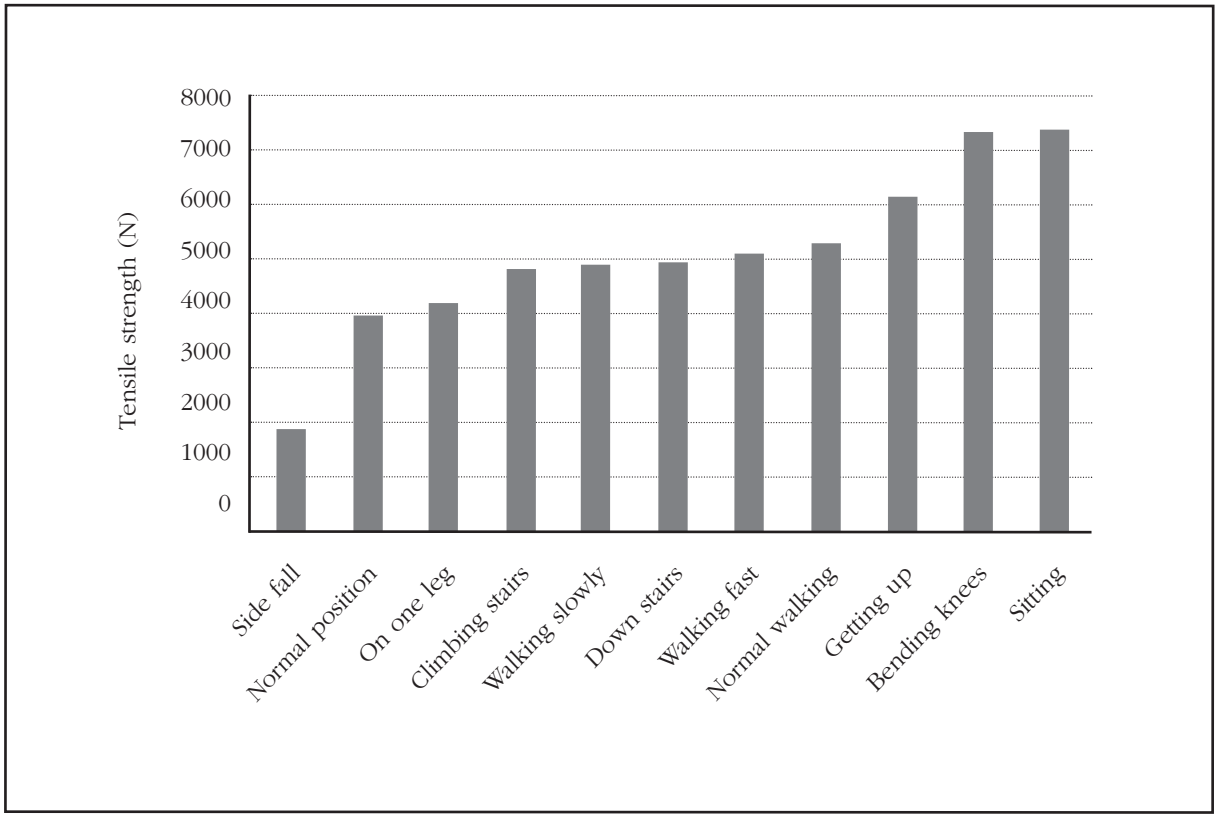
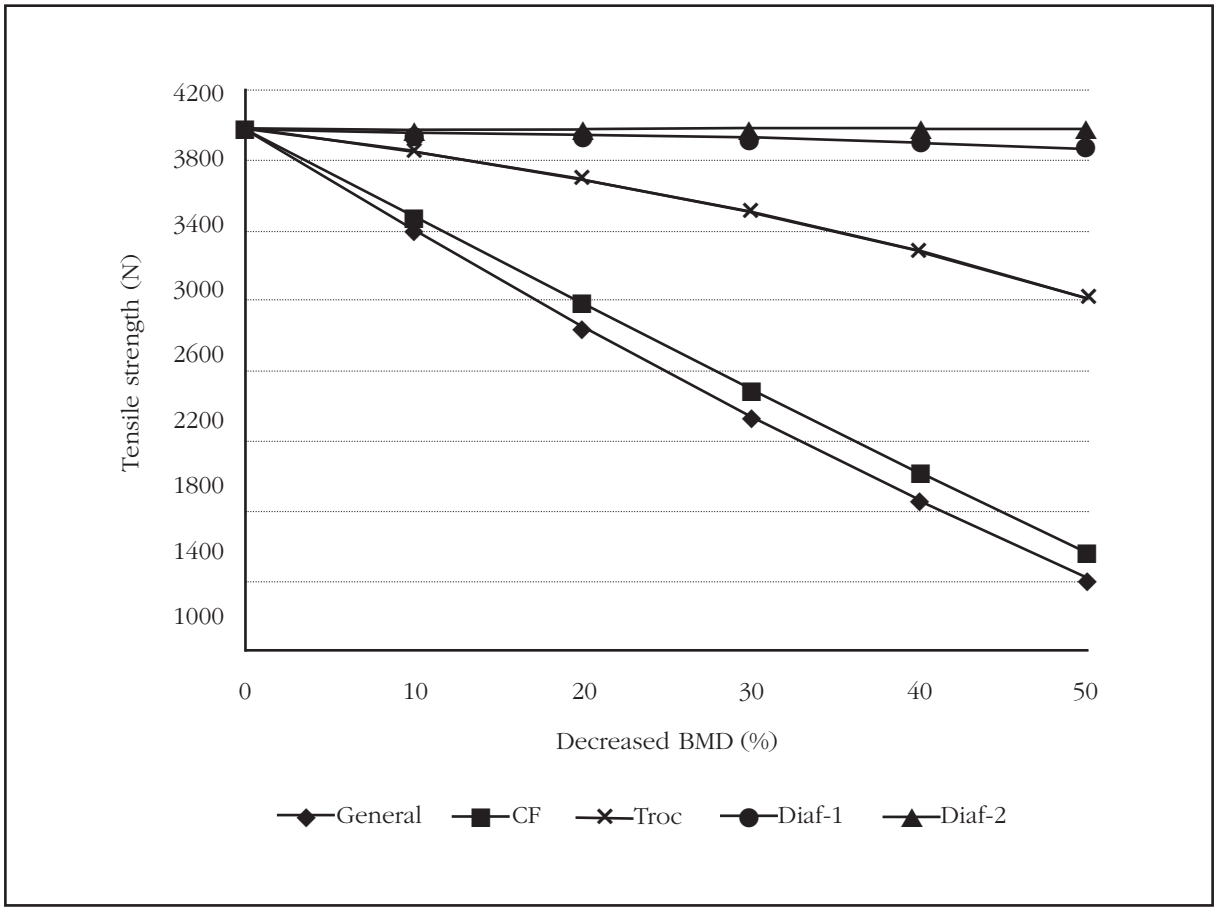


Figure 7. Tensile strength of the EPF in the femoral neck by decreasing BMD



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Torrubia B[1], Alonso I[1], López-Ramiro E[1], Mahillo I[2], De la Piedra C[1]
1 Laboratorio de Bioquímica
2 Servicio de Epidemiología
Fundación Jiménez Díaz - Madrid (España)

Comparison between two automated chemiluminescence immunoassays for quantifying 25 (OH) vitamin D

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Correspondence: Concha de la Piedra - Laboratorio de Bioquímica - Fundación Jiménez Díaz - Avda. Reyes Católicos, 2 - 28040 Madrid (Spain)
e-mail: cpiedra@fjd.es

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Summary

Introduction: Quantifying total blood 25 (OH) vitamin D is the most accurate marker of an individual's vitamin D status. The gold standard technique for measurement is liquid chromatography tandem mass spectrometry (LC-MS/MS), although currently clinical laboratories tend to use chemiluminescence techniques. The objective of this study was to compare 25 (OH) vitamin D concentrations obtained by two commercially-produced automated methods and study the correlation of these methods with the LC-MS/MS reference technique.

Material and methods: The 25(OH) vitamin D levels were quantified in 1,000 serum samples from the Jimenez Diaz Biochemistry Foundation Laboratory using 2 automated methods for chemiluminescence detection: ADVIA CENTAURO® (SIEMENS) and LUMIPULSE® G1200 (Fujirebio). Among all the samples tested, the 50 most discordant to each other were sent to be evaluated by LC-MS/MS reference technique.

Results: The results indicate that there is good correlation between the two methods: CCI=0.923 (0.914-0.932), with the G1200 LUMIPULSE® values 10% being higher than CENTAURO®. Regarding the 50 samples selected, we can see that there is a good correlation between the two immunoassays with LC-MS/MS, although both methods significantly underestimate 25 (OH) vitamin D results with respect to the gold standard.

Discussion: Although both techniques are suitable for use, it is worth considering whether the worldwide vitamin D deficiency epidemic is due to the analysis methodology used. This variability between immunoassays could be solved by standardizing the different commercial techniques in line with NIST-produced reference materials.

Key words: *25(OH) vitamin D, Fujirebio, SIEMENS, technical comparison, LC-MS/MS.*

Introduction

Vitamin D is a fat-soluble vitamin involved in calcium and phosphorus metabolism whose role is essential in bone formation and mineralization. Currently, its immunomodulatory actions, antiproliferative and cell differentiation stimulatory of associated pathologies such as cardiovascular diseases, diabetes and cancer have also been shown[1,2].

Quantification of 25 (OH) vitamin D total blood is the most accurate marker of vitamin D status in an individual, although its active metabolite is 1,25 $(OH)_2$ vitamin D[3,4].

The gold standard technique for measurement is liquid chromatography/tandem mass spectrometry (LC-MS/MS), but clinical laboratories routinely used chemiluminiscence immunoassays[5].

The main problems of these immunoassays are due to the hydrophobic nature of the analyte, the high concentration in which vitamin D binding protein (DBP) in serum is found, and the existence of cross reactions of multiple vitamin D with the metabolite antibodies used in the process. Therefore, proper immunoassay requires a pretreatment to inactivate DBP, a careful selection of antibody used and the standardization of the technique compared to the values returned by LC-MS/MS analyzer. Different commercial techniques for analysis of vitamin D differ in how to separate the binding protein, the percentage of crossed reactions with other metabolites of our analyte, as well as the specificity of the antibody used[4,6].

Currently, as a result of general knowledge on severe vitamin D deficiency in the world's population, it has become necessary to measure vitamin D levels in different populations, research cohorts and individual patient[7]. Several studies have shown considerable variation between the different analytical methods based on immunochemistry, liquid/UV and LC-MS/MS chromatography. It has been argued that a particular patient can be classified with levels of sufficiency or insufficiency of vitamin D depending on the laboratory where this analysis is carried out[7,8].

To solve this problem, the need for standardization of the levels of 25 (OH) vitamin D has been established by numerous scientific organizations. In 2011, the Office of Dietary Supplements (ODS) of the National Institutes of Health US (NIH) in collaboration with the National Institute of Standards and Technology (NIST) created the program standardization of vitamin D (VDSP). NIST has developed 4 based reference materials with different serum concentrations of vitamin D known order to standardize the various commercial techniques[7,9]. However, not all the current methods used to quantify 25(OH) vitamin D already calibrated against these accepted standards[10].

The aim of this study was to compare 25(OH) vitamin D concentrations obtained using two commercial automated methods and study the correlation of these methods with the LC-MS/MS technique.

Material and methods

We used 1,000 serum samples selected at random from those tested in FJD's clinical analysis laboratory. The samples were obtained from patients aged between 1 and 92 years (59±18, average ± SD) with 37% of women and 63% men. Levels of 25 (OH) vitamin D were quantified with ADVIA Centaur XP® (SIEMENS) and LUMIPULSE® G1200 (FUJIREBIO).

In all cases the samples were handled anonymously, so obtaining informed consent of patients was not required.

The analyzer LUMIPULSE® G1200 (FUJIREBIO) takes a noncompetitive sandwich-type immunoassay with chemiluminescent detection using two antibodies, a monoclonal sheep antibody that binds to 25(OH) vitamin D2 and D3, and a second monoclonal antibody that binds exclusively to the complex formed above. Separation of vitamin D binding protein is carried out by a chemical agent in 1st reaction.

According to the manufacturer's specifications, the technique has an intra-assay imprecision with a coefficient of variation (CV) ≤6% and a functional sensitivity of 3.491 ng/mL. Measurement interval is 4-150 ng/mL. Analytical specificity reflected by the percentage of cross-reactivity with other metabolites is 100% for 25(OH) vitamin D_3, 100.1% for 25(OH) vitamin D_2, and 19.9% for the epimer C3 25(OH) vitamin D_3.

The ADVIA Centaur XP® (SIEMENS) is a competitive immunoassay with chemiluminescent detection using an anti-fluorescein murine monoclonal antibody covalently coupled to paramagnetic particles (PMP), a murine monoclonal anti-25(OH) vitamin D marked with acridinium ester, and vitamin D analog with fluorescein. As separation medium binding protein release agent is used in buffered saline.

According to the manufacturer's specifications, the technique presents an intra-assay imprecision with a CV of 4.2% -11.9% and a functional sensitivity of 4.2 ng/mL. Measurement interval is 4.2 to 150 ng/mL. Analytical specificity reflected by the percentage of cross-reactivity with other metabolites is 97.4% for the 25(OH) vitamin D_3, from 106.2% for 25 (OH) vitamin D_2 and 1% for C3 epimer 25(OH) vitamin D_3.

Among all samples tested, the 50 discordant with each other were sent to be evaluated by LC-MS/MS method in the laboratory of Dr. Etienne Cavalier (Department of Clinical Chemistry, University of Liege, Belgium); in order to compare the two chemiluminescent immunoassays relative to the reference technique LC-MS/MS. The difference between these results 50 samples ranged between 14% and 133% (32±52%, mean ± SD) with respect to the average of the two values obtained. In all cases the percentage was higher than the coefficients of variation inter-analysis of the two methods: FUJIREBIO, 6%; SIEMENS, 11.9%. We analyzed the most discordant samples to ascertain whether they belonged to a particular group of patients. For example, pregnant women who have abnormal levels of vitamin D binding

protein. However, we note that these patients belonged mainly to Nephrology, Rheumatology and Endocrinology, an insignificant fact since these services are most demanded the determination of vitamin D. Moreover, the average age of these 50 patients was 63±16 years, with 34% male and 66% female, figures which were quite similar to the total group of 1,000 samples (age 59±18 years with 37% of women and 63% men). The most discordant samples were chosen together to analyze for gas-mass because our goal was twofold. First, to check its similarity to the technique of gas-mass and otherwise clarify which of the two techniques was closer to the reference technique. This second point could not clarify if we sent the samples whose results were similar. Regarding the choice of 50 samples as appropriate for the study was done because it was a sufficient amount to obtain statistically significant results samples. Due to the high cost of gas-mass determination it was not possible to send a larger number of samples.

Results

We assessed the degree of concordance of vitamin D measures provided by the two appliances: ADVIA Centaur XP® and LUMIPULSE G1200®. To do this, we calculated the intraclass correlation coefficients (ICC) with confidence intervals at 95%. The results indicate a good correlation between the two methods: CCI=0.923 (0.914 to 0.932). There are no significant differences in the ICC if the samples are divided into groups with vitamin D levels ≤20 ng/mL and> 20 ng/mL.

The regression line obtained from both trials was Y=1.221+1,035X, where Y corresponds to the values of LUMIPULSE G1200 and X to Centaur XP®. LUMIPULSE G1200 values were 10% higher than Centaur® (Figure 1).

With respect to 50 samples selected subgroup to analyze by LC-MS/MS was obtained with ICC=0.987 LUMIPULSE and CCI=0.938 with Centaur®. Although both are satisfactory, the intraclass correlation coefficient is the highest LUMIPULSE therefore measurements of this device is more like the exact ones (Figures 2 and 3).

Then with our subgroup of 50 selected samples, we carried out the Bland-Altman plots, where the X axis corresponds to the mean of each pair of observations and the Y axis differences between each pair of observations. In the graphs there are two horizontal solid lines. The gray solid line is drawn at zero height; if the measurements given by the apparatus were identical to the exact measurement points should be located right on this line. The solid blue line represents the mean of the differences. If this line is the line below the 0 value means that the device tends to lower measures the exact value, and if it is above the opposite.

As can be seen in figure 4, the mean difference between the analyzer LUMIPULSE® G1200 and the reference method LC-MS/MS is 20%; therefore this immunoassay underestimates values of 25 (OH) vitamin D by 20% compared to the gold standard. In figure 5, we see how in the case of the Centaur® the average of the differences is 42%, so the values which casts this technique are much lower than those of the reference method. Furthermore, the technique LUMIPULSE® G1200 is less spread in the results.

Discussion

Both methods have a good correlation between them, the values obtained in the Centaur® approximately 10% lower than those obtained by the LUMIPULSE® G1200.

Figure 1. Regression line between LUMIPULSE® G1200 (FUJIREBIO) and Centaur® (SIEMENS) using serum samples from 1,000 patients in the FJD

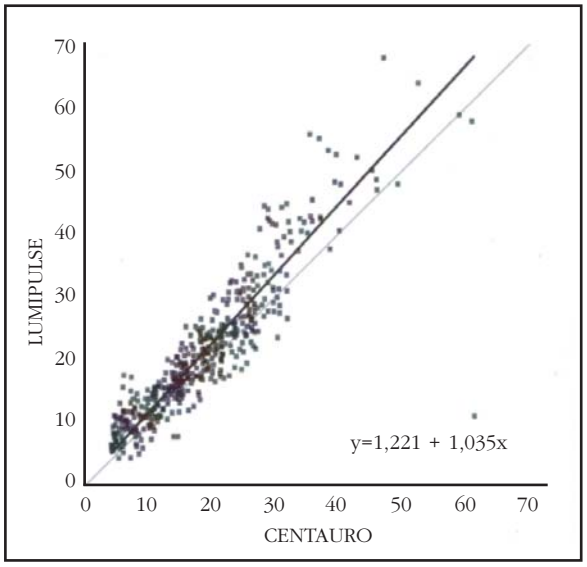
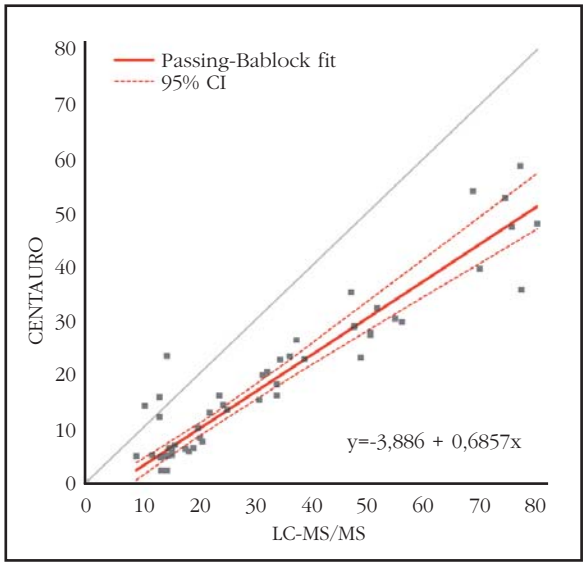


Figure 2. Regression line calculated by Passing-Bablok between LUMIPULSE® G1200 (FUJIREBIO) and LC-MS/MS using 50 selected samples (see Materials and methods)



The correlation of both immunoassays with the reference technique LC-MS/MS is good (although higher for LUMIPULSE that Centaur), which does not exclude that the two methods considerably underestimate the results of 25(OH) vitamin D with respect to gold standard. In this selection of samples, held by choosing those where the discrepancy between the two methods was higher, LUMIPULSE® G1200 underestimates the values by 20%, while yields Centaur® 25 (OH) vitamin D 42% lower than the reference method LC-MS/MS. This difference between the two immunoassays is given by the different technique used (competitive assay in SIEMENS and noncompetitive sandwich in FUJIREBIO), pretreatment of the sample to separate the 25 (OH) vitamin D and DBP selected antibodies.

Currently, according to studies, more than half of the world's population has insufficient levels or even frank deficiency of vitamin D[11]. This may be considered a global "epidemic", but should be questioned as to whether this state of widespread vitamin deficiency is largely influenced by the analysis methodology used in determining concentrations of 25(OH) vitamin D[8,11].

Figure 3. Regression line calculated by Passing-Bablok between Centaur® (SIEMENS) and LC-MS/MS using 50 selected samples (see Materials and methods)

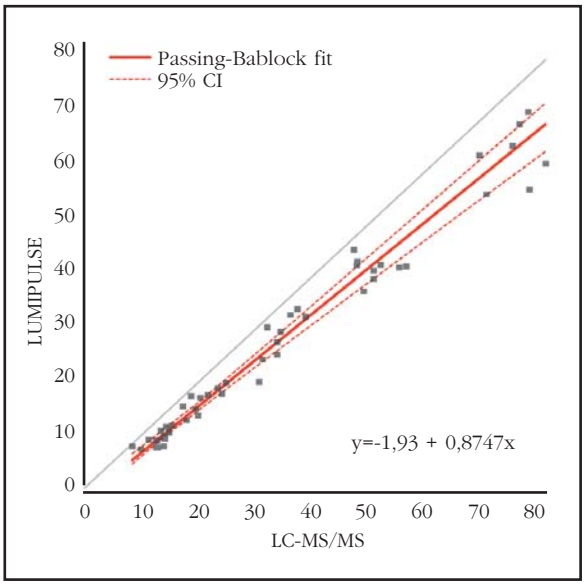


Figure 4. Bland-Altman Charts between LUMIPULSE® G1200 (FUJIREBIO) and LC-MS/MS using 50 selected samples (see Materials and methods)

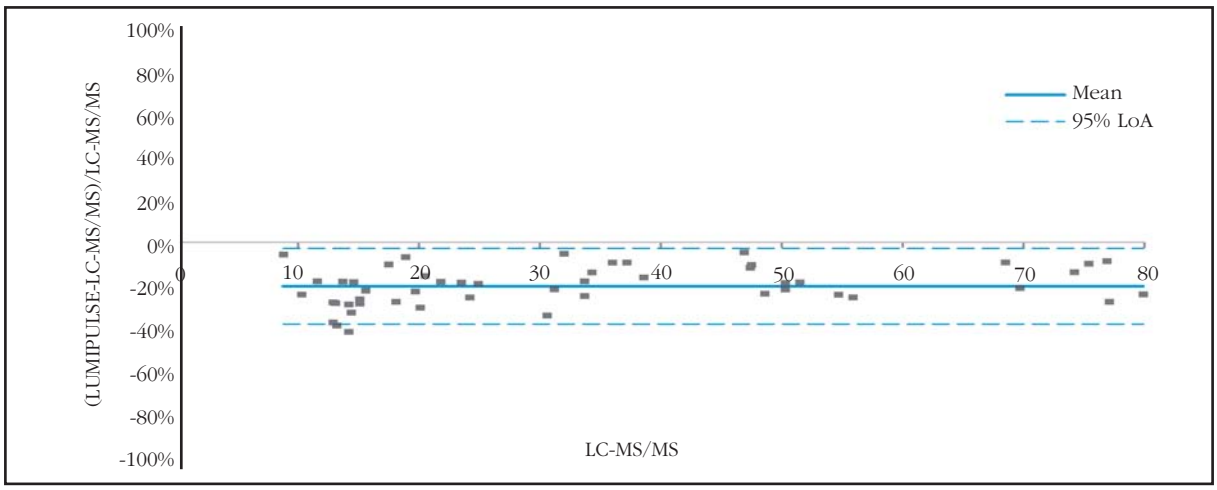
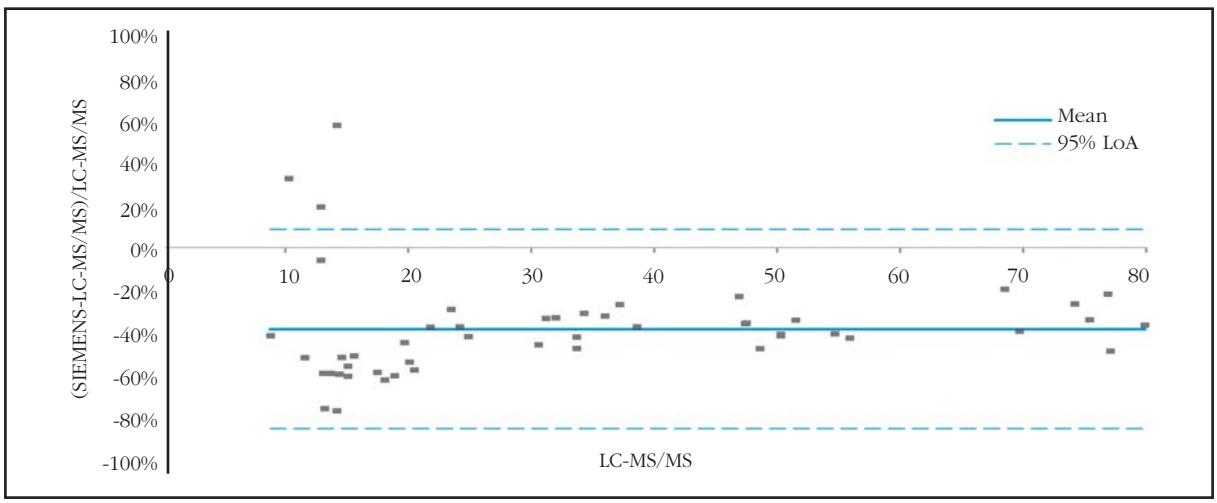


Figure 5. Bland-Altman Charts between Centaur® (SIEMENS) and LC-MS/MS using 50 selected samples (see Materials and methods)



This variability between immunoassays would be solved by standardizing different commercial techniques with reference materials for measuring 25(OH) vitamin D produced by the NIST[12].

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Competing interests: The authors declare no conflict of interest.

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Sainz-Aja Guerra JA[1], Alonso MA[2], Ferreño Blanco D[1], Pérez-Núñez MI[2], Ruiz Martínez E[1], García-Ibarbia C[3], Casado del Prado JA[1], Gutiérrez-Solana F[1], Riancho JA[3]

1 Departamento de Ciencia e Ingeniería del Terreno y de los Materiales - LADICIM - ETS de Ingenieros de Caminos, Canales y Puertos - Universidad de Cantabria - Santander (España)

2 Departamento de Traumatología y Cirugía Ortopédica - Hospital U.M. Valdecilla - Universidad de Cantabria - Santander (España)

3 Departamento de Medicina Interna - Hospital U.M. Valdecilla - Universidad de Cantabria - IDIVAL - Santander (España)

Study of the microstructure of femoral patients with hip osteoarthritis and hip fracture by microCT

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Correspondence: José A. Riancho - Departamento de Medicina Interna - Hospital Universitario Marqués de Valdecilla - Universidad de Cantabria - Avda. Valdecilla, s/n - 39008 Santander (Spain)
e-mail: rianchoj@unican.es

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Summary

Whereas bone mineral density (BMD) is characteristically low in osteoporosis, it has been postulated that in osteoarthritis BMD is increased. We aimed to check this concept by analyzing bone volumen and structure in the femoral heads of patients with hip fractures (n=10) and with hip osteoarthritis (n=9). Unexpectedly, the analysis of microstructural parameters by microCT did not reveal significant differences between both groups. In addition, we did not find a significant decline in the trabecular bone volume across the age range studied. These results suggest that the evolution of the trabecular bone of the femoral head is different from the age-related decrease of bone mass in other regions of the skeleton. Elucidating the mechanism involved could suggest new approaches to treat the bone loss associated with aging.

Key words: *hip fracture, osteoarthritis, microCT, microstructure.*

Introduction

Osteoporosis and arthrosis are very common disorders. Their manifestations include lower extremity involvement which is particularly significant. Thus, hip fractures and hip osteoarthritis are important because they may limit patients' quality of life. This often requires replacing the hip joint, a procedure that bears a significant burden on health services and takes up much orthopedic surgery and traumatology resources.

However, the pathogenesis of these processes is different. Osteoporotic fractures are the result of decreased bone strength, due to aging and other factors which cause a loss in structural mass and competence. Conversely, osteoarthritis reflects an alteration of all the joint components, and there is a destruction of articular cartilage, subchondral bone sclerosis and osteophyte formation, with varying degrees of inflammation [1-3]. The relationship between bone mineral density (BMD)and osteoarthritis has often been studied, with varied results. Several studies suggest that osteoporosis and osteoarthritis are accompanied by changes in bone mass in opposite directions, both locally and systemically [4-7]. These studies indicated that patients with osteoarthritis had a greater BMD than controls at multiple levels, yet that finding has not been confirmed universally. In other studies, it has been associated with osteoarthritis of the knee with an increased risk of vertebral fractures and non vertebra [8]. Alterations in gait and propensity to fall may play a role. Conversely, high BMD may favor arthrosis, perhaps by increasing the load to which articular cartilage is subjected in the presence of a denser subchondral bone [9]. However, this issue is also controversial. In fact, in some experimental models of osteoporosis, the development of arthrosis seems to accelerate [10,11]. To provide new information on this matter, we analyze the trabecular bone of the femoral head, comparing hip fracture sufferers with patients who present hip osteoarthritis.

Material and methods

Patients

In all, 20 patients were included with severe coxarthrosis or hip fracture (femoral neck) in which it was necessary to place a prosthesis. We excluded those with those diseases that cause osteoporosis or secondary osteoarthritis (inflammatory diseases, advanced kidney failure, cancer, paralysis, treatment with corticosteroids or immunosuppressants, dysplasia, etc.) and fractures related to high-energy trauma. Trabecular bone cylinders of the femoral head were removed using a 6 mm diameter trocar needle (Figure 1) and the ends (3 mm adjacent to cortical bone and the fracture or surgical section) were also removed. The cylinders were obtained regardless of the state of the articular cartilage, or anatomical orientation. In one patient, we were unable to extract a good cylinder, so he was not included in the analysis. Consequently, the fracture group consisted of 10 patients (5 men and 5 women), aged 87±5 years. The arthrosis group was made up of 9 patients (5 men and 4 women) of 66±5 years (Table 1). The bone samples were collected within a research project aimed at determining the differential pathogenetic mechanisms in fractures and osteoarthritis, approved by the Cantabria Ethics Committee on Clinical Research. Patients gave informed written consent.

MicroCT Analysis

The cylinders were fixed in buffered 4% formaldehyde prior to analysis. The storage period in formaldehyde was 2-4 weeks for 12 samples, and 18-30 months in the rest (similar in both patient groups). After rinsing them with water, the study was conducted in a Microtrac 1172 Skyscan-Bruker, with 360° rotation, 75 kV voltage, 100 μA intensity, 16x16 mm field of view, 8-micron pixel size and an aluminum filter of 0.5 mm. Coronal sections (2,000x2,000 pixels) of all cylinders, with a separation between them of 1 pixel was reconstructed. NRecon SkyScan program was used for reconstruction. For calculations of structural parameter, the sections with uniform threshold were dichotomized and SkyScan CTanalyzer program was used. The global threshold values was defined taking into account all the analyzed samples. Each cut in a region of interest (ROI), centered in the sample, 4 mm in diameter (thus avoiding analysis of the periphery that may contain irregularities due to the extraction process). For tridimensional analysis, two regions or volumes of interest (VOI) consisting of two non-overlapping sets of consecutive sections, of equal size along the bone cylinder (Figure 1) defined in each sample. The sample ends and objects smaller than 100 voxels were excluded to avoid artifacts.

Statistical analysis

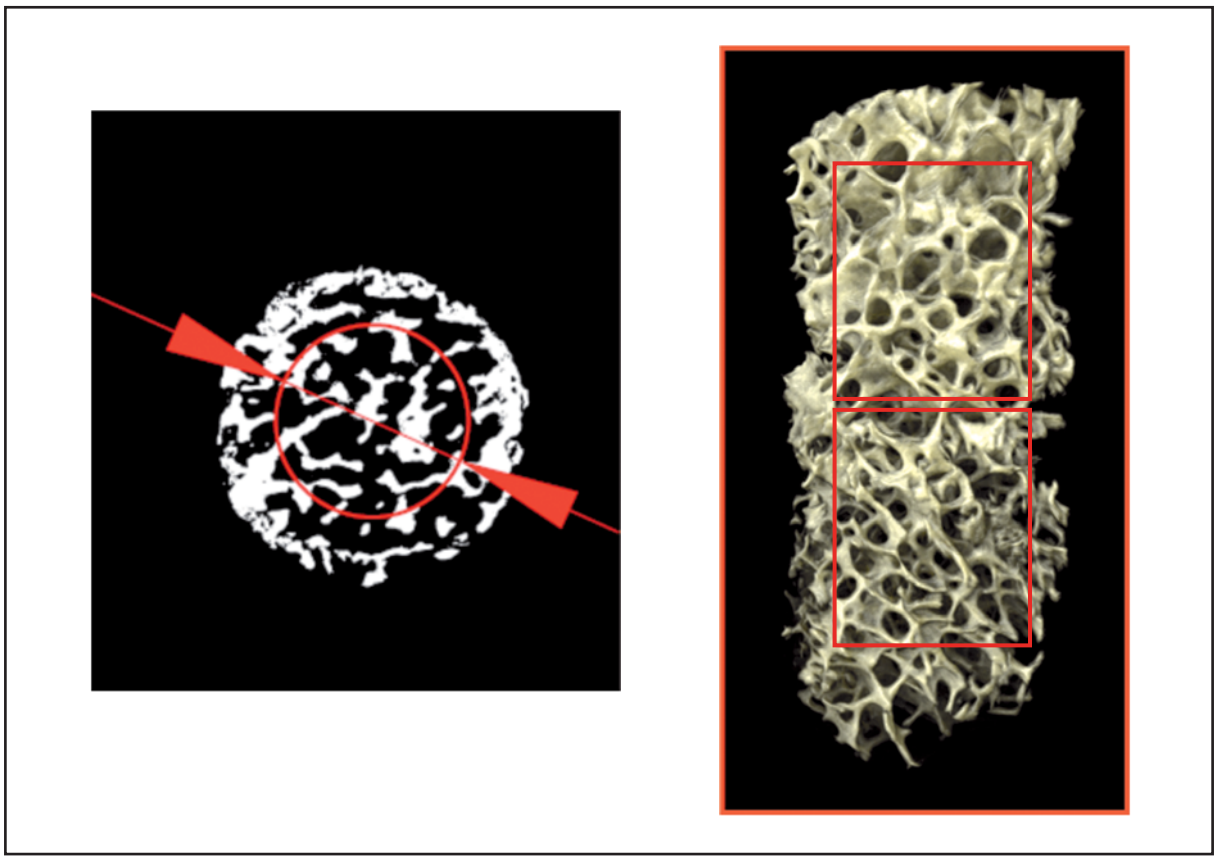
For each of the cylinders, the average value of the various parameters in the two regions studied (VOIs) as well as the variation among them was calculated. To analyze this variation for each parameter, the relative difference, as the resulting absolute value subtract 1 from the ratio of the value of each parameter in the two study regions (VOIs) was estimated. The Mann-Whitney test was used to analyze the differences between the average values of the two patient groups (fracture and osteoarthritis), as well as between men and women. The difference in standard deviations was analyzed by Levene's test.

Results

The average values of the main parameters analyzed are shown in table 2. In figure 2 the individual values of selected parameters are represented. As can be seen, no significant differences were found between fracture and osteoarthritis groups, or in terms of parameters (mean, median) in the central tendency, or as to the dispersion (variances).

The differences between the two regions analyzed were also similar in both groups of patients (Figure 3). In fact, the average variation of the trabecular bone volume between the two regions was analyzed as 0.232 in the fractures and 0.236 in osteoarthritis (p=0.9).

Figure 1. Cylinder sample and cross section. the region of interest identified in a horizontal section and the projection of the two regions or volumes of interest in the 3D reconstruction



In each of the cylinders, 1,000 sections were scanned and corresponding to the percentage of bone surface (B.Ar/T.Ar), equivalent to trabecular bone volume in three-dimensional analysis dimensional parameter analyzed. The minimum value of the identified parameter found in all sections could be a better predictor of the maximum voltage that the bone can support before suffering a fracture [12,13]. As shown in figure 4, there were no differences between patient groups.

No significant association between age and bone volume (Figure 5), or crude analysis even taken into consideration when sex and group membership (fracture or osteoarthritis). Within each of the patient groups, we also found a significant Tb.Sp correlation and other microstructural parameters with age or sex. However, given the number of subjects included, this subgroup analysis had limited power.

Discussion

In this study we observed that the trabecular bone of the femoral head is similar in patients with hip fractures and in patients with advanced hip osteoarthritis in the volume of bone and different microstructural parameters. This result may not support the idea that opposite changes occur in these processes in bone mass, and specifically in the trabecular bone volume. We need to take into account that the subchondral bone was not included in the region to analyze, as it is located just below the articular surface, in which local changes occur in the form of sclerosis that are not representative of the overall skeletal situation [2,14].

In any case, the relationship between osteoporosis and osteoarthritis is a subject of debate. Several epidemiological studies have suggested that bone mass is increased in arthrosis [6,7,9,15], but others have not confirmed increased BMD [5,16]. In fact, some experimental studies suggest that osteoporosis may be a facilitator of the progression of some forms of arthrosis factor [10,17,18]. On the other hand, some studies have found an increased risk of fractures in patients with arthrosis [8], although it is difficult to determine the influence of a possible increased susceptibility to falls in individuals with advanced osteoarthritis. The reason for these differences is unclear. In part, it may be because osteoarthritis is not a homogeneous disorder, but there are epidemiological and pathogenic differences, not only in terms of the affected joints, but also in the type of alteration. In particular, there are relevant differences between "atrophic" and "hypertrophic" defined phenotypes depending on the absence or presence of osteophytes respectively. In fact, in a cohort analysis of Rotterdam, researchers observed that patients with atrophic type hip arthrosis had a higher risk of osteoporotic fractures [19].

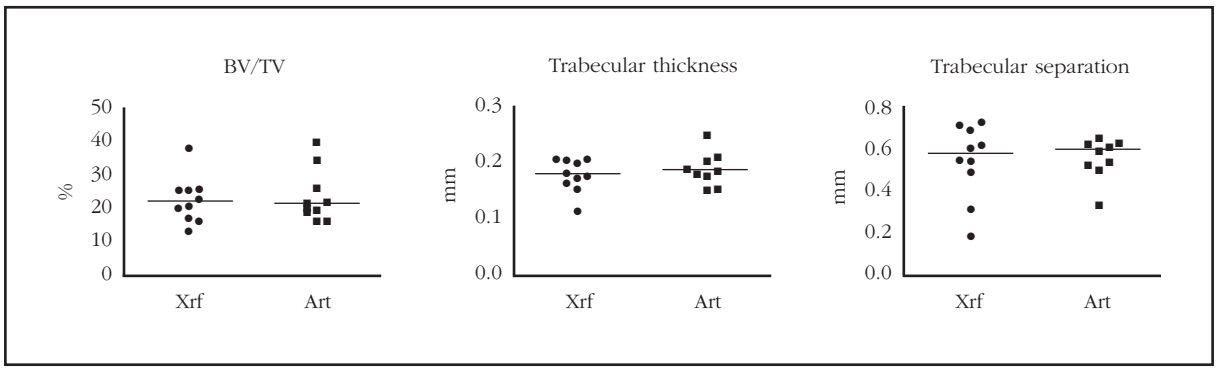
Table 1. Patient characteristics

Bone	Group	Age	Sex	Comorbidity
1	Fracture	88	women	Diabetes
2	Fracture	88	women	Diabetes, dementia
3	Fracture	83	men	
4	Fracture	97	women	
5	Osteoarthritis	70	women	
6	Osteoarthritis	67	men	
7	Osteoarthritis	59	men	
8	Osteoarthritis	60	men	
9	Fracture	84	women	
10	Fracture	89	men	
11	Fracture	80	men	Diabetes
12	Fracture	88	women	
13	Osteoarthritis	73	women	Ischemic heart disease
14	Fracture	90	men	Diabetes, dementia
15	Fracture	88	men	
16	Osteoarthritis	62	men	
17	Osteoarthritis	67	men	
18	Osteoarthritis	65	women	
19	Osteoarthritis	67	women	

Table 2. Main parameters in three-dimensional structural analysis

Parameter	Acronym	Units	Fracture (mean)	Osteoarthritis (mean)	Fracture (SD)	Osteoarthritis (SD)	p
Percentage bone volume	BV/TV	%	23.0	24.5	6.8	7.9	0.67
Index structural model	ISM		2.2	1.6	0.8	0.6	0.11
Trabecular thickness	Tb.Th	mm	0.18	0.19	0.03	0.03	0.39
Trabecular separation	Tb.Sp	mm	0.55	0.56	0.18	0.10	0.86
Total volume of pore space	Po.V(tot)	mm^3	26.2	28.5	12.7	9.1	0.65
Total porosity	Po(tot)	%	77	75	7	8	0.67
Connectivity	Conn		2243	3059	3115	5468	0.69
Connectivity density	Conn.Dn	$1/mm^3$	120	70	254	108	0.59
Degree of anisotropy	DA		1.66	1.66	0.33	0.23	0.95

Figure 2. Values of some structural parameters in patients with bone fractures (XRF) and osteoarthritis (Art). The horizontal lines indicate the median



There are also conflicting results regarding the local state of bone in patients with hip osteoarthritis and fractures. Thus, in some studies, increased bone mass (i.e., greater trabecular bone volume) has been identified in patients with coxartrosis [20,21], including a study of Montoya et al. Their design was similar to the present study, but samples were collected from the area of maximum load femoral head [22]. In some cases, the results may be influenced by a marked difference in age and sex distribution between groups [23], to exclude precisely the hip arthrosis group which had "osteoporosis" [20], having studied the femoral neck, instead of head, or for having studied a region very close to the subchondral plate [20]. Anyway, ours is not the only study which did not find any differences in trabecular bone between the two processes. Other authors published similar findings [24,25].

One limitation of our study is determined by variations in the orientation of the cylinders. This could theoretically increase the dispersion of results and therefore decrease the ability to find differences, as trabecular heads are not oriented uniformly in space. However, the absence of anisotropic differences of both groups suggests that this limitation may not play an important role. Likewise, the fact that two regions in each cylinder were analyzed independently and having obtained a similar correlation between them in both groups of patients, suggests that possible differences in the microstructure as a function of depth (after excluding the subchondral region) were not responsible for significant bias in the results. Another limitation of the study is that the cylinders were obtained at random, regardless of the state of cartilage. This could present a certain confusion, since it seems that the bone found beneath regions with damaged cartilage has a stronger trabecular structure than those located in areas under unharmed cartilage [26]. This may be the result of lower damping mechanical stresses in the areas lacking cartilage. The average age of patients in our two study groups was somewhat different, older in the fractured group. However, the age disparity highlighted even more the failure to find a lower bone volume in samples of patients with fracture. We excluded those fractures related to high-energy trauma (traffic accidents and falls from heights), so the hip fractures included could be considered due to fragility or osteoporosis. On the other hand, patients with osteoarthritis had no history of fragility fractures. We did not have a densitometry, so we could not completely exclude some degree of overlap between the groups.

Perhaps more surprising than the lack of differences between hip osteoarthritis and fractures is the absence of a relationship between the parameters analyzed and age, within the range studied. This is clearly contrary to the trend of BMD to decrease with age is generally observed in the skeleton. However, this result does not represent an isolated observation. In a study that included 37 individuals aged 40 to 90 years, Perilli et al. found no relationship between age and various structural parameters, including the volume of trabecular bone [13]. The reason for this peculiar behavior is unknown. It could be associated with biomechanical factors such as the persistent application of load on the femoral head when standing, with humoral factors dependent on the bone itself or other nearby tissues, including muscles, or the peculiar conditions of vascularization of the region. In any case, its clarification could improve our understanding of bone biology and perhaps open the way to new approaches for treating bone loss associated with aging.

Competing interests: The authors declare no conflict of interest in connection with this research study.

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Figure 3. Correlation of estimates of trabecular bone volume in the two regions studied in each individual fracture (left panel) or osteoarthritis (right panel)

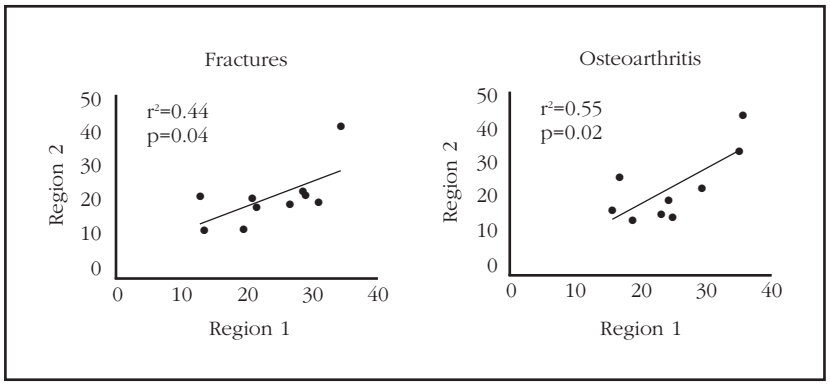


Figure 4. Minimum value of the percentage of bone surface in a cross-section found in each individual (two-dimensional analysis)

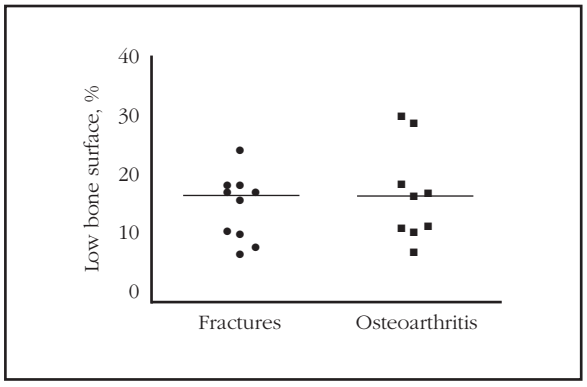
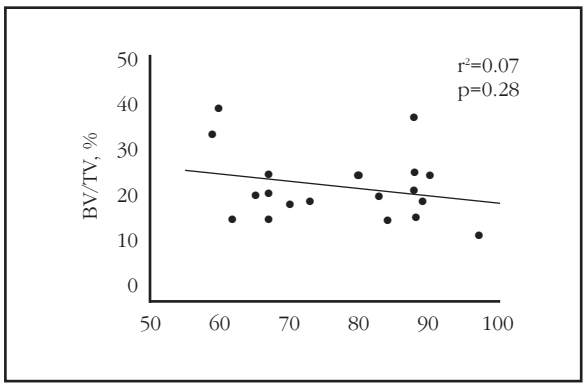


Figure 5. Association between trabecular bone volume (D analysis) and age



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Alonso G[1], Varsavsky M[2]
1 Servicio de Endocrinología - "Humane Especialidades Médicas" - Río Cuarto (Argentina)
2 Servicio de Endocrinología - Hospital Italiano de Buenos Aires - Buenos Aires (Argentina)

Osteomalacia in a young adult

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Correspondence: Mariela Varsavsky - Juan D. Perón 4190 - C1181ACH - Buenos Aires (Argentina)
e-mail: mariela.varsavsky@hospitalitaliano.org.ar

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Summary
Cases of hypophosphatemic osteomalacia respond to various causes, both genetic and acquired. Some variants of mesenchymal tumors produce inappropriate amounts of fibroblast growth factor 23 (FGF-23), a mediator which induces renal phosphate loss. The biochemical picture is characterized by hypophosphatemia, decreased tubular reabsorption of phosphates, low or inappropriately normal serum calcitriol and high or unusually normal levels of FGF-23 plasma. This paraneoplastic syndrome is called tumor-induced or oncogenic osteomalacia.
There are a limited series of published cases, although it has been increasingly accepted in recent years. Diagnosis may be complex given its low incidence, the difficulties in localizing the tumors and heterogeneity in histopathologic interpretation. Complete surgical removal has healed, but there may be recurrences whereas phosphorus and calcitriol oral supplements offer alternative medical treatment.

Key words: *hypophosphatemia, oncogenic osteomalacia, fibroblast growth factor 23, phosphaturic mesenchymal tumor..*

Introduction

Paraneoplastic syndromes are a set of remote effects of a tumor on different organs and systems. These are mediated by molecules with hormonal action, growth factors, cytokines, autoimmune mechanisms and other unknown factors. A rare paraneoplastic syndrome is caused by renal phosphate loss induced by inappropriate tumor secretion of fibroblast growth factor 23 (FGF-23). The condition is characterized by a generally severe hypophosphatemic osteomalacia, and termed tumor-induced osteomalacia (TIO) or oncogenic osteomalacia in the literature. In this report, a case that illustrates the diagnostic and therapeutic difficulties of this syndrome is described.

Clinical Case

A 41-year-old man at the first consultation presents a case history that started 9 years earlier with minor trauma fractures to hands and lower limbs, diffuse bone pain and progressive proximal muscle weakness. Various medical centers offered differing diagnostic possibilities, such as ankylosing spondylitis, metastatic prostate carcinoma, primary hyperparathyroidism and Paget's bone disease. He was treated in several cycles with pamidronate, zoledronate and intravenous ibandronate, oral calcium supplements, vitamin D and analgesics.

Response to this therapy was not effective, with progression of the clinical and radiological manifestations and important functional limitation. He presented no other relevant medical history or family history of metabolic bone diseases.

At the time of the first consultation, a review of previous studies showed several biochemical determinations with hypophosphatemia and high values of phosphaturia, parathyroid hormone (PTH) and total alkaline phosphatase (TAP). Remaining mineral metabolism parameters were normal. Plain radiographs showed lesions compatible with pseudo-fractures in metatarsals, metacarpals, pelvis and proximal end of the tibia and fibula. Bone scans showed multiple foci of increased uptake in long bones of the limbs, pelvis, vertebrae and costal arches. Bone densitometry showed low bone mass in the lumbar spine and femoral neck (T-score= -1.4 and -1.3, respectively). Two iliac crest biopsies were taken, directed by computed tomography (CT), without prior tetracycline marking, unspecific and insufficient for the diagnosis of Paget's bone disease.

Magnetic resonance imaging (MRI) was also carried out on the neck and lumbosacral spine, abdomen and pelvis CT scan, sestamibi parathyroid and upper endoscopy with biopsy of gastric and duodenal mucosa, all without significant findings. In Table 1 the initial results in our laboratory are summarized. No measurements could be carried out for calcitriol and serum fibroblast growth factor 23 (FGF-23).

Given this background, the diagnosis of hypophosphatemic osteomalacia was reconsidered. The patient began treatment with oral supplements of phosphorus salts (equivalent to 1,000 mg of elemental phosphorus) and calcitriol (0.50 mg daily). Clinical improvement and partial biochemical (Table 1) was observed. A positron emission tomography with fluoro-deoxyglucose (FDG-PET / CT), showing multiple hypermetabolic foci of bone location and an area of moderate uptake between the 2nd and 3rd right metatarsal (Figure 1) was requested.

MRI showed that location in a hypointense, heterogeneous tumor lesion, lobed edges 3 by approximately 2 cm. Then resection surgery of the lesion was performed. Pathological examination reported the presence of osteoclastoides and mesenchymal cells arranged in irregular morphology varied sheets, pseudocartilaginoso stroma and prominent vascularization with hemangiopericitoide focal pattern, initially interpreted as enchondroma and in a second review as chondromyxoid fibroma. Subsequent laboratory surgery showed persistence of biochemical abnormalities (Table 1). Further MRI tests detected the presence of residual tumor. We proceeded to a second intervention to remove the residual lesion. The piece is sent to another pathologist who reported variant phosphaturic mesenchymal tumor mixed connective tissue. Immunohistochemistry using immunoperoxidase technique detected an expression of FGF-23 in large areas of the tumor (Figure 2).

The patient experienced a gradual clinical improvement, with recovery of muscle strength and progressive resolution of bone pain. Post-op control after stopping oral calcitriol phosphorus supplements indicated laboratory parameters remained within normal limits (Table 1).

Discussion

Symptoms and signs of TIO are similar to familiar hypophosphatemic osteomalacia. The main clinical manifestations in adults are bone pain, proximal muscle weakness and fractures. The clinical picture may be confused with rheumatic, oncological, psychiatric and other diseases, leading to a varying delay in the correct diagnosis.

In our patient the study presented the first manifestations at 32 years of age, resulting in numerous visits to different specialists with several different diagnoses and treatments with bone anti-resorptive for years without clinical improvement. The biochemical diagnosis is based on the finding of hypophosphatemia, hyperphosphaturia, decreased tubular reabsorption of phosphate (TRP), low or inappropriately normal serum calcitriol and inappropriately normal or high levels of plasma FGF-23[1].

Some authors recommend the determination of the tubular threshold phosphate adjusted by glomerular filtration rate (TMP/GFR), as their values are independent of the level of plasma phosphorus and renal function, although this may vary by age and sex and has not been validated in widespread population studies[2]. Often secondary hyperparathyroidism is a physiological response to low levels of calcitriol. TSAP and the bone isoenzyme (BI) may be elevated due to increased osteoblast activity.

Tabla 1. Biochemical parameters of the patient before and after the various treatments

Parameter	VR	Initial	P/vit D	Post 1ª Cx	Post 2ª Cx
Phosphatemia	2.6-4.7 mg/dl	1.3	2.9	2.1	3.5
Phosphaturia	400-1,000 mg/24 h	661	848	722	560
TRP	85-95%	73	77	71	89
TMP/GFR	3.09-4.18 mg/dl	1.32	1.61	2.25	3.70
FAT	60-280 U/ml	669	445	482	156
FAO	31-95 U/l	112	101	115	77
PTHi	10-65 pg/ml	157	91	102	57
Calcaemia	8.6-10.2 mg/dl	9.3	8.8	9.5	9.1
Calciuria	80-250 mg/24 hs	40	60	73	172
25(OH)vitD	30-60 ng/ml	34	41	39	46
Creatinine	0.6-1.3 mg/dl	0.9	1.1	0.8	0.9

VR: reference values; P/vit D: oral supplements salts of phosphorus and calcitriol; Cx: surgery; TRP: tubular reabsorption of phosphates; TMP/GFR: tubular phosphorus threshold corrected by glomerular filtration; FAT: Total serum alkaline phosphatase; FAO: bone alkaline phosphatase isoenzyme; PTHi: intact parathyroid hormone molecule; 25 (OH) VITD 25-hydroxyvitamin D.

In the case presented here, the revision of previous analyses and our initial laboratory showed hypophosphatemia, inappropriately low TRP, high levels of TRP and TSAP. The determination of serum FGF-23 by ELISA can confirm the clinical diagnosis, although there are cases of TIO with normal serum FGF-23[3]. FGF-23 is normally expressed by osteocytes, regulates phosphorus metabolism and vitamin D by binding to Klotho-FGF[4] receptor complex and has been linked to the pathophysiology of several entities (Table 2). At renal level it reduces renal tubular reabsorption of phosphates by decreasing expression cotransporter sodium/phosphate type 2a and 2c (NaPi-2a 2c) and inhibiting the activity of the 1α-hydroxylase kidney. These mechanisms result in hypophosphatemia, hyperphosphaturia and low levels of calcitriol.

Osteomalacia linked to tumors is usually small, benign, slow-growing and often located in the extremities, both bone and soft tissue. In our case the lesion was located in soft tissues of the plantar of foot. Cases have also been reported in paranasal sinuses, nasopharynx, brain, ovary, and pelvic column[5-7].

Sometimes the tumor is not found, and some authors refer to this situation as similar to tumor-induced osteomalacia. This has been linked to sarcomas, prostate and breast carcinomas, multiple myeloma, chronic lymphocytic leukemia and small cell carcinomas[1]. Paraneoplastic syndrome is now recognized as tumor-induced osteomalacia associated with mesenchymal tumors generally described as benign giant cell tumors, ossifying fibromas, osteoblastomas, granulomas, hemangiopericytomas and other names.

Weidner et al. proposed the term phosphaturic mesenchymal tumors, subdividing them into categories: mixed connective tissues, simile-osteoblastoma tumors, tumors simile-non-ossifying fibroma, ossifying fibroma tumors and simile-metastatic tumors[8]. The first variant represents 75% of cases in the literature identified as PMT-MCT (phosphaturic mesenchymal tumor mixed connective tissue). They are characterized by osteoclast-like giant cells, myxoid stroma or chondromyxoid, low or absent mitotic activity, ossified areas and important vascularization, with vessels of different size and morphological pattern. While it is generally benign, tumors have also been reported malignant metastatic presentations[9].

In our case there were discrepancies in the interpretation of three pathologists, and it was finally characterized as PMT-MCT. As localization methods have been proposed sinus CT, total body MRI, FDG-PET/CT, scintigraphy with labeled octreotide labeled with ^{111}In and ^{99}mTc scintigraphy or ^{201}Th sestamibi.

In recent years, DOTANOC AG68-PET/CT and venous sampling dosage of FGF-23 have been included in areas where functional studies suggest suspicious injuries[10]. The FDG-PET/CT is a method of high sensitivity but low specificity, especially in patients who have many areas of pseudofractures, healing fractures or lytic areas[11].

In this case, the phosphaturic tumor was small, benign, slow-growing, was located by FDG-PET/CT and confirmed using MRI. The treatment of choice is complete surgical resection of the tumor with a wide margin, as described postsurgical recurrences[12]. When the intervention is successful the clinical and biochemical features are gradually resolved, although some of its manifestations may persist for several months. Metastasizing late recurrence is possible but rare, and pulmonary involvement has been the most commonly reported1. While particular surgery, or to an incomplete excision tumor recurrence, medical treatment is indicated with oral supplements of phosphorus salts (15-60 mg/kg per day of elemental phosphorus) and calcitriol (0.50 to 1.0 μg/day) in separate doses (4-6 times per day).

Our patient received supplements while the location and tumor excision was finalized, achieving a partial improvement of his condition. Variable results have been reported with octreotide, cinacalcet, radiofrequency ablation and intratumoral injection of ethanol.

Using monoclonal antibodies is a novel approach that disrupts the interaction of FGF-23 with its receptor[13]. The series of published cases highlight the main features of TIO: diagnostic delay, the difficult location of tumors, predominantly in the lower limbs, healing after complete removal and the possibility of relapse[14]. Less than 400 TIO cases have been reported, mostly in recent years, reflecting its low incidence and difficulties in identification.

The case presented illustrates the difficulty and delay in diagnosis, persistently incomplete resection of phosphaturic tumor and discrepancies in interpretation.

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Figure 1. FDG-PET/CT. Multiple bone hypermetabolic foci and an area of high uptake in soft tissues of the plant are observed right foot

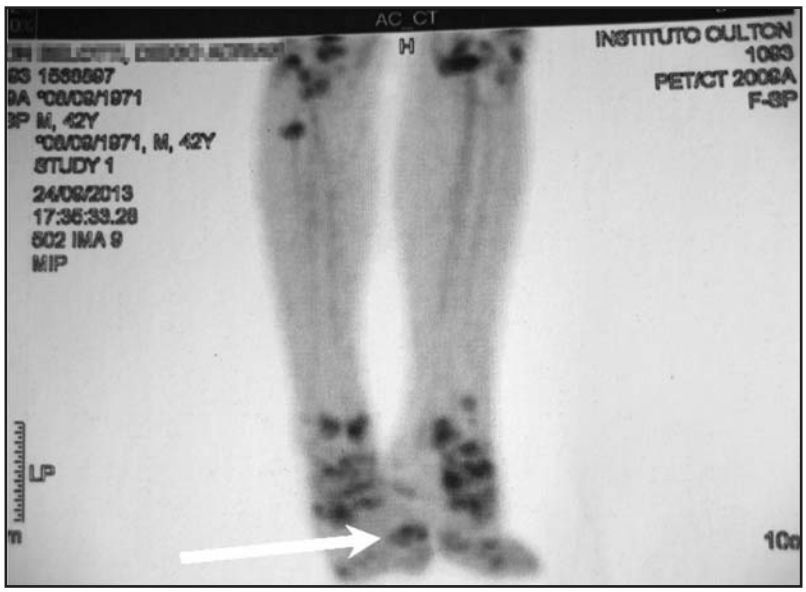

Figure 2. Histopathology of the surgical specimen: phosphaturic variant mesenchymal tumor mixed connective tissue. Hematoxylin-eosin staining, 200x field. Immunohistochemistry was performed with dial for FGF-23 by ELISA. Areas with positive dialing seen in red (arrows)

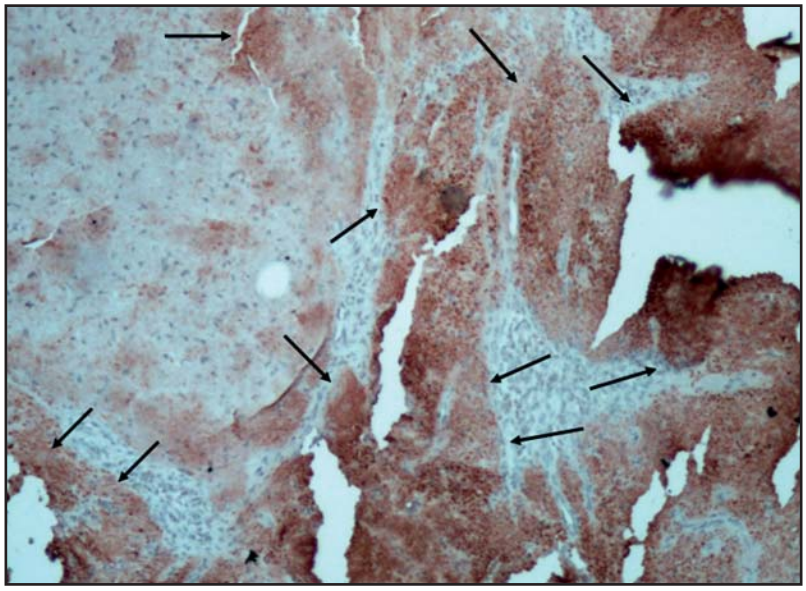

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Tabla 2. Hypophosphatemic disorders mediated by FGF-23

Pathology	Gene involved
Family X-linked hypophosphatemic rickets	PHEX
Family autosomal dominant hypophosphatemic rickets	FGF23
Type 1 family autosomal recessive hypophosphatemic rickets	DMP1
Type 2 family autosomal recessive hypophosphatemic rickets	ENPP1
Hypophosphatemic disorder with dental anomalies and ectopic calcification	FAM20C
Linear nevus sebaceous syndrome	___
Osteomalacia/rickets saccharate or iron polimaltosato	___
McCune-Albright syndrome/fibrous dysplasia	___
Tumor-induced osteomalacia	___

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López Méndez P[1,2], Gómez de Tejada Romero MJ[1,3], Sosa Henríquez M[1,4]

1 Universidad de Las Palmas de Gran Canaria - Instituto Universitario de Investigaciones Biomédicas y Sanitarias (IUIBMS) - Las Palmas de Gran Canaria (España)

2 Servicio de Neurología - Hospital Universitario Insular de Gran Canaria - Las Palmas de Gran Canaria (España)

3 Departamento de Medicina - Universidad de Sevilla - Sevilla (España)

4 Hospital Universitario Insular de Gran Canaria - Unidad Metabólica Ósea - Las Palmas de Gran Canaria (España)

Medical publications: science or business?

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Correspondence: Manuel Sosa Henríquez - c/Espronceda, 2 - 35005 Las Palmas de Gran Canaria (Spain)
e-mail: msosah@hotmail.com

Introduction

In recent years there has been an enormous increase in the number of scientific publications[1,2], to such an extent that it is now impossible for us to read even 1% of what is coming out in our area of expertise or into the fields which interest us. The proliferation of scientific periodicals in general and medical journals in particular may be attributed to many factors. Among these we would highlight the Internet and changes in the very raison d'etre of such publications. Here we will comment on some aspects that have led to this situation.

What is the raison d'etre of scientific publications?

1st reason. The theory, what we believe it should be

The scientific publication must complement scientific research[3]. The journal is the way to report our research findings to the scientific community, whether they are positive and pose a significant advance in the knowledge concerning an issue, or, on the contrary, they are negative results, whose importance is increasingly accepted, especially to validate the usefulness of meta-analyses. Scientific journals offer the most common way to transmit and document scientific progress, especially in the biomedical field, which is subjected to constant change[4]. Progress in the field of medicine generated by the large number of research groups requires the timely publication of their findings, while it is required to verify their quality and rigor, generally supported in the prestigious journals by anonymous peer review of scientific articles. We cannot, therefore, conceive research without the support of the scientific journal as in the maxim "Science does not exist until it is published".[5]

2nd reason. The crude reality

Far from this erudite reasoning, which proclaims that we publish altruistically to offer our findings to the scientific community, there is another version of the story. Probably our eagerness to enhance a CV, either individually (our own) or as part of a team (research group) is the real reason why so much is being published today. Our byline, in turn, will be the tool to secure other benefits. The first aim would be to attract the prestige and recognition of the scientific community as experts in that field. Other reasons involve recognition of publications as part of the point scale. In our country, for example, where public tests offer regional health service posts, scientific publications have a certain value. The same is true in the merit accreditation scales to secure a university tenured professorship, whether for tenure or full professorship, currently under the control of the National Agency for Quality Assessment and Accreditation (ANECA)[6]. Universities can also be granted a so-called six-year research award based largely on scientific publications. Once this is granted, the researchers can expect greater financial reward. In the allocation of associate professorships for teaching practical lessons and in the scales for awarding research grants, one prized aspect is having a line of solid research that is cre-

dited precisely with the publication of scientific papers. Therefore, the researcher, teacher or just the public candidate, not because they have obtained results that they want to report to the scientific community, but rather because they hope to bolster their CV for any of the above objectives. This is, of course, quite reasonable. There are many examples in the literature of items of dubious interest to the general scientific community whose usefulness and application do not go beyond the local scope itself[7-16].

A bit of history. How we got scientific articles a few decades ago?

The emergence of the Internet some 20 years ago drastically changed scientific literature. Until then, access to some items was limited, especially those published in less "normal" journals. First, we subscribed to certain journals, either personally or through scientific societies to which we belonged. Then we had access to those journals which our hospitals or universities libraries received. Librarians often possessed contacts with other libraries with which an exchange was established, usually fast, free, by which the items could be fax (that was faster) with quite variable quality. There was also Current Contents, a small weekly booklet which featured the covers of journals selected by the editor, including the author's address for correspondence. We could contact this author using a standard form which requested a reprint of the article. Sometimes they replied, perhaps to tell us that the reprints had run out. Finally, some pharmaceutical companies offered a service, similar to Current Contents, through which, and from such journal covers, the laboratories themselves would secure reprints or photocopies of higher quality than faxes. It is true that this service was as good as it was ephemeral.

After the advent of Internet, scientific journals adapted to new technologies as best they could. Some did so immediately, adapting their format to the Internet to offer PDF versions of articles, the chance to watch videos and articles in audio format for the deaf, among other features. In this sense, the *New England Journal of Medicine* changed quickly and exemplary, adapting to new technologies offered and even improving its content. Other journals took over. Today the whole process involving the publication of a scientific article is done via the Internet, from obtaining publishing journal standards, the referral of the article, usually by a computer search engine designed ad hoc, through sending to the reviewers, article correction and finally its publication, which is usually conducted in a previously unknown format, which is the publication "online" (advance online) available on the journal's website, format that can remain even several months after the printing on paper. Now classic journals coexist with the publication in PDF or HTML form on the journal web page. A step further has been the emergence of scientific journals published only online in PDF format and not printed on paper.

Access to online journals

Today, all journals published on paper have a Web page that offers the same content but in PDF format. There are also journals that have chosen to be present only digitally, on their website. Access to articles published in these journals is varied:

a) First, there are paid subscriptions, either personally or by an institution: university, research institute or hospital. With your own password you can access the journal's entire contents even as far back as 20 years ago in most cases. There are usually no restrictions and the only drawback of this method is obviously the cost factor.

b) To pay for them directly: if you do not have a subscription, items are sold as any other merchandise online, with prices generally very high. For this service, subscribers do not have to be identified as health care providers. You simply pay.

c) Time lapse period: some journals offered free content access once a certain time has transpired. This varies from one to another and the time lapse ranges between six months and one year. In other words, one would only have to pay for access to the latest articles.

d) Subsidized articles such as open, journal subscription.

It is not uncommon for most of the classic journals to offer one or two articles in open format in each issue, which are clearly marked with an online or free link. These articles are published in this way either because the author has decided to pay to be published in this format (as discussed below, the free articles are read and referenced by payment), or because the laboratory responsible for the drug will benefit from the results of the product will pay, or because the editors deemed it very important for the interest of the scientific community. Some journals, such as the *British Medical Journal*, allow access to "reserved" articles if the reader signs in. The period of validity thereof and the number of items that can be found in this way are limited.

e) Some scientific social networks web pages such as *Research Gate* or *Edu Academy* have reached agreements with publishers for authors of scientific articles to include publications in them and that could be accessed freely or through a personal request. This is what has been called "repository" and that is very much in relation to open access movement which we will refer later.

The open-access journals. Transmitting science or business?

In recent years we have seen a plethora of so-called open access journals (open access) that could be loosely translated as free access to the reader. All these share a common features: a) they tend to be published in English; b) they exist only in electronic form and therefore are not published or distributed on paper; c) you can access all of their content for free and complete from the time

of publication, with no cut off period; d) most of them are not been indexed nor have impact factor; and e) in all the article, authors must pay to publish their articles.

The number of these journals has grown exponentially in recent years, so that today only in the field of medicine this number is several hundred journals. As indicated above, most of them are not included in the *Journal of Citation Reports* and therefore have no impact factor, and are not available in *PubMed*. Usually they are obtained from databases that share the journals' open access format. A non-exhaustive list of these databases is shown in table 1.

The open access phenomenon began to develop in this century, and the bases justifying it were set out in the so-called Budapest Open Access Initiative of 2002[17], which was eventually ratified in Bethesda and Berlin[18,19]. The principle underlying this initiative is literally: By open access [to peer scientific literature], we mean its free availability on the public Internet, which allows any user to read, download, copy, distribute, print, search or add a link to the full text of such items, to track them for indexing, incorporate them as data into software, or use them for any other purpose that is legal, without financial, legal or technical barriers other than those inseparable in Internet access itself. The only constraint on reproduction and distribution, and the unique role of copyright (property rights) in this area, control should be to give authors over the integrity of their work and the right to be properly acknowledged and cited[17].

The price for access to scientific articles and publishing in the field of bone mineral metabolism

In the classic journals that exist in paper format and have been subsequently adapted to digital format, publishing articles is often free. The exception is the *Journal of Bone and Mineral Research*, which charges $50 US for reviewing expenses. This is non-refundable should the manuscript be rejected. Other traditional journals do not charge for publishing articles. They usually offer the possibility to pay to be published in open access format. They generally cover their expenses by a combination of subscription readers combined with the support from the scientific society of which the journal is the official publication. One of the incentives offered by some scientific associations is journal subscription, which leads to journal subscription costs being higher than registration as an associate. A list of these journals is shown in table 2, in order of highest impact factor. It shows subscription price only in its online version.

The question is how much they are willing to pay the authors for the service they receive. Probably, if we consider that most open access journals operate exclusively online, eliminating the costs of printing and distribution, and that the authors of the articles do not receive financial remuneration for their work, current prices could be considered by most to be too high. Perhaps open access means good business for publishers, but there are still doubts as to the benefits and drawbacks of this model for authors. Probably in a few years all scientific studies subsidized with public money shall be published in open access, but between publishers and research centers a fair price should be negotiated for publishing according to the product received[21]. Current controversy on this issue has yet to be resolved[22-31].

The only specific journal on bone mineral metabolism with impact factor is *Bone Research*. Its characteristics are shown in table 3.

The burden generated by open access journals. Seeking to disseminate science or generate income?

It is little wonder that a few days after publishing an article in a journal that is indexed and has impact factor, we receive email invitations to publish a similar article in one of these journals[20]. Nor is it unusual that the invitation to go even further and coordinate of a special issue. Of course the invitation is made "because we are a reference and renowned in the publishing field". So do not forget that the invitation clearly reflects the obligation to pay for this publication. This information does not appear in the letter of invitation and it is only discovered when we read in detail the "instructions for authors" that are often hidden behind several links which require patient accessing. Not surprising considering we finish these spam or unwanted, or in some cases we should turn to the editors begging they remove us from a distribution list, which is not always done.

The Journal of Osteoporosis and Mineral Metabolism (ROMM)

The Journal of Osteoporosis and Mineral Metabolism is the only journal in open format that is completely free, not only of specific journals in bone mineral metabolism but in the field of medicine. Reading its articles and using their website is completely free, it does not require a subscription and there is no expiration period.

It is also absolutely free for authors. The cost involved in maintaining the journal is covered by the Spanish Society of Bone and Mineral Metabolism Research (SEIOMM). ROMM is its official scientific journal. Being completely free makes it a unique journal in the field of medicine.

ROMM is included in more than 20 databases related to current open access1 (Table 3) and recently in others, which do not share this philosophy, such as *Web of Science and Emerging Source Citation index*. It is still not included in the *Journal of Citation Reports or PubMed*, and so they have not recognized the impact factor. A new application for inclusion, corrected some existing defects, was taken in June 2016, which we hope will provide good news to all who participate actively in it.

Table 1. Databases that collect articles published in open access format

Acronym	Name	URL (Web address)	It includes ROMM
DOAJ	*Digital Open Access Journals*	https://doaj.org/	YES
e-journals	*Electronic Journals*	http://www.e-journals.org/	YES
Redalyc	*Red de Revistas Científicas de América Latina y el Caribe, España y Portugal*	http://www.redalyc.org/	YES
SciELO	*Scientific Electronic Library On Line*	http://www.scielo.org/php/index.php?lang=es	YES
Dialnet	*Dialnet*	https://dialnet.unirioja.es/	YES
Free Medical Journals	*Free Medical Journals*	http://m.freemedicaljournals.com/	YES
Latindex	*Sistema Regional de Información en Línea para Revistas Científicas de América Latina, el Caribe, España y Portugal*	http://www.latindex.org/latindex	YES
IBECS	*Índice bibliográfico español en ciencias de la salud*	http://ibecs.isciii.es	YES
Academic Keys	*Academic Keys*	http://medicine.academickeys.com/res_main.php	YES
SafetyLit	*Safetylit*	http://www.safetylit.org/index.htm	YES
EZ3	*Electronic Journals Library*	http://rzblx1.uni-regensburg.de	YES
WorldCat	*WorldCat*	http://www.worldcat.org/	YES

Table 2. Journals of bone mineral metabolism in closed-access format or subscription

Journal	Impact factor	Price annual subscription, only online version per person in €	Price for publishing article in open format	Editorial
Journal of Bone and Mineral Research	6,832	515	3.000 $*	*Wiley*
Osteoporosis International	4,169	151,50	2.200 €	*Springer*
Bone	3,973	337	2.150 $	*Elsevier*
Calcified Tissue International	3,272	2.433**	2.200 €	*Springer*
Current Osteoporosis Report	2,728	1.156**	2.200 €	*Springer*
Journal of Bone and Mineral Metabolism	2,460	76,25	2.200 €	*Springer*
Bone and Joint Journal	1,961	150	3.000 $	*British Editorial Society*
Journal of Clinical Densitometry	2,644	201,15	3.000 $	*Elsevier*

* Price if you want to publish the article in open access. If you choose to leave it closed or limit access only to subscribers, the publication of the article is free but always check charges $50 for the same, regardless of whether or not it is accepted.
** Institutional subscription. We could not find the rate for personal subscription.

Table 3. Journal of Bone Mineral Metabolism in open format

Journal	Impact factor	Price for article	Editorial
Bone Research	3,549	3.975 $	*Nature*
Journal of Osteoporosis	No	800 $	*Hindawi*
Journal of Bone Reports & Recommendations	No	919 $	*Imed.pub*
BoneKey Reports	No	2.200 €	*Nature*
Journal of Osteoporosis & Physical Activity	No	1.019 $	*OMICS International*
Journal of Bone Metabolism	No	70 $ per page	*Korean Society for Bone and Mineral Research*
Bone Reports	No	1.500 $	*Elsevier*
Osteoporosis and Sarcopenia	No	1.500 $	*Elsevier*
International Journal of Osteoporosis and Metabolic Disorders	No	625 $	*Asian Network for Scientific Information*

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