Our cover: Masson Goldner’s trichrome of a patient with hyperparathyroidism.

Author: Dr. José Manuel Olmos Martínez. Marqués de Valdecilla University Hospital-Health Research Institute (IDIVAL), University of Cantabria. Santander (Spain).

Summary

Vol. 13 - Nº 1 - January-March 2021

EDITORIAL
Hypercalcemia and autoimmune diseases
Sosa Henríquez M, Gómez de Tejada Romero MJ

ORIGINALS
Hypercalcemia in patients with rheumatoid arthritis: a retrospective study
Cordoba A, García-Unzueta MT, Riancho-Zarrabeitia L, Corrales A, Martínez-Taboada V, Riancho JA

Association of biochemical parameters of bone metabolism with progression and/or development of new aortic calcifications

Search for variants of the LRP4 gene in women with high bone mass and in patients with Chiari type I malformation
Martínez-Gil N, Grinberg D, Balcells S

The cut-out phenomenon in intertrochanteric femur fracture: analysis using a finite element model
Arias-Blanco A, Marco M, Giner E, Miguélez MH, Caeiro-Rey JR, Larraínzar-Garrio R

REVIEW
Pathophysiology of osteoporosis in chronic inflammatory joint diseases
Castañaeda S, Garcés-Puentes MV, Bernad Pineda M

IMAGES IN OSTEOTOLOGY
Unusual case of bone proliferation: Nora’s lesion
Moreno-Ballesteros A, García-Gómez FJ, De la Riva-Pérez PA, Calvo-Morón MC

SPECIAL ARTICLE
Care profile of susceptible patients with osteoporosis by telemedicine visits in the post-COVID-19 era
Cancio JM, Capdevila-Reniu A, Casanova T, Cuadra L, Ivanov A, Llopis A, Santiago JM, Sopena E

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

Revista de Osteoporosis y Metabolismo Mineral has recently been accepted for coverage in the Emerging Sources Citation Index, which 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.
Editorial Committee

Teresita Bellido, PhD
Department of Anatomy and Cell Biology Department of Medicine. Division of Endocrinology Indiana University School of Medicine Roudebush Veterans Administration Medical Center Indianapolis. Indiana (United States)
e‐mail: tbellido@iupui.edu

Ernesto Canalis, PhD
Director, Center for Skeletal Research. Professor of Orthopedic Surgery and Medicine UConn Health. Farmington, CT (United States)
e‐mail: canalis@uchc.edu

Patricia Clark Peralta, MD, PhD
Head of the Clinical Epidemiology Unit. Hospital Infantil de México Federico Gómez-Faculty of Medicine UNAM. Mexico City (Mexico)
e‐mail: patriciaclark@prodigy.net.mx

Oswaldo Daniel Messina, MD, PhD
Director of Rheumatology. Cosme Argerich Hospital. Buenos Aires (Argentina). Medical Director. IRO. Center for Rheumatological and Osteological Research. Buenos Aires (Argentina). Associate Professor of Rheumatology and Director of the post graduate programme in Rheumatology. University of Buenos Aires (Argentina). Board member and member of the Committee of Scientific Advisors. International Osteoporosis Foundation (IOF)
e‐mail: drosvaldodanielmessina@gmail.com

Lilian I Plotkin, PhD
Department of Anatomy and Cell Biology and Indiana Center for Musculoskeletal Health Indiana University School of Medicine. Indianapolis, Indiana (United States)
e‐mail: lplotkin@iupui.edu

Manuel Naves Díaz, MD, PhD
Bone Metabolism Clinical Management Unit. Central University Hospital of Asturias (HUCA). Institute of Health Research of the Principality of Asturias (ISPA). REDINREN of ISCIII. Oviedo University. Oviedo (Spain)
e‐mail: mnaves.huca@gmail.com

Manuel Díaz Curiel, MD, PhD
e‐mail: mdcuriel@fjd.es

Adolfo Díez Pérez, MD, PhD
Hospital del Mar Institute of Medical Investigation (IMIM) and Internal Medicine Department. Hospital del Mar. Autonomous University of Barcelona. CIBER on Frailty and Healthy Aging (CIBERFES). Instituto Carlos III. Barcelona (Spain)
e‐mail: adiez@parcdesalutmar.cat

Jose Antonio Riancho, MD, PhD
Department of Medicine and Psychiatry, University of Cantabria. Service of Internal Medicine. Marqués de Valdecilla University Hospital. Valdecilla Research Institute (IDIVAL). Santander (Spain)
e‐mail: rianchoj@unican.es

Methodology, Data Study and Statistics: Pedro Saavedra Santana
University of Las Palmas de Gran Canaria. Department of Mathematics. Las Palmas de Gran Canaria (Spain)
e‐mail: pedro.saavedra@ulpgc.es

Manuel Sosa Henríquez, MD, PhD (Director)
University of Las Palmas de Gran Canaria. Research Institute in Biomedical and Health Sci Research Group in Osteoporosis and mineral metabolism. Bone metabolic Unit. Hospital University Insular. Las Palmas de Gran Canaria (Spain)
e‐mail: manuelsosa@ulpgc.es

María Jesús Gómez de Tejada Romero, MD, PhD (Editor)
Department of Medicine of the University of Sevilla. Sevilla (Spain). Research Group in Osteoporosis and mineral metabolism. Bone metabolic Unit. Hospital University Insular. Las Palmas de Gran Canaria (Spain)
e‐mail: mjgtr@us.es
Hypercalcemia and autoimmune diseases

DOI: http://dx.doi.org/10.4321/S1889-836X2021000100001

Sosa Henríquez M1,2, Gómez de Tejada Romero MJ1,3
1 Research Group in Osteoporosis and Mineral Metabolism. University of Las Palmas de Gran Canaria. Las Palmas de Gran Canaria (Spain)
2 Bone Metabolic Unit. Insular University Hospital. Las Palmas de Gran Canaria (Spain)
3 Department of Medicine. Sevilla University. Sevilla (Spain)

Hypercalcemia is a very common water-electrolyte imbalance found in daily clinical practice. It is defined as the presence of a serum calcium concentration greater than 2 standard deviations from the mean laboratory value, which is usually 10.6 mg/dL.

From the pathophysiological point of view, high levels of calcium in the blood increase the difference in electrical potential between cell membranes, which increases the depolarization threshold. Clinically, hypercalcemia may present a very wide spectrum that can range from a certain muscle weakness to depression and even coma and death, and this depends on several factors such as the severity of hypercalcemia, the speed of its onset and other circumstances specific to the patient, such as age, comorbidity and medication received. Therefore, it is not surprising that two patients with the same high serum calcium values present completely different symptoms.

The causes of hypercalcemia may vary considerably. In our environment, the most frequent is the existence of primary hyperparathyroidism, a very common endocrine disease that has an incidence in the United States with 230 cases per 100,000 inhabitants in women and 85 cases per 100,000 in men. Furthermore, rheumatoid arthritis is an autoimmune-based rheumatic disease, which is also very frequent. In Spain, it constitutes an estimated prevalence of 0.9% of the population. In a recent review of the comorbidity described in rheumatoid arthritis, depression appears as the most frequent condition, not always considered a priority, reaching figures that range from 15% to 29%; curiously, hypercalcemia is not among them.

Some years ago, several studies suggested that hypercalcemia could be a marker of the activity of rheumatoid arthritis. In the series by Oelzner et al., 30.1% of the patients who suffered rheumatoid arthritis presented hypercalcemia and these patients had higher ESR and CRP levels, as well as lower PTH and 1.25 dihydroxyvitamin D values. In another series, the same authors suggest that low levels of 1.25 dihydroxyvitamin D could cause osteoporosis associated with rheumatoid arthritis. However, other authors have described that the prevalence of hypercalcemia and its causes are similar in rheumatoid arthritis as in the general population. Thus, there is a controversy and data have been published that would support both points of view, that hypercalcemia is part of the clinical spectrum per se, perhaps as a marker of its activity, and also the opposite, one that suggests that the causes of hypercalcemia in patients with rheumatoid arthritis are the same as in the rest of the population.

Delving deeper into the study of this dilemma, in this issue of the Revista de Osteoporosis y Metabolismo Mineral, Córdoba et al. report a study carried out in 500 patients with rheumatoid arthritis, among which 24 patients of both sexes have hypercalcemia. In them, possible causes of hypercalcemia were found in several cases (9 patients with primary hyperparathyroidism, multiple myeloma, vitamin D intoxication, etc.) but in a third of them (8 of 24) no cause was found that justify it. Moreover, they could not establish a relationship between the activity of rheumatoid arthritis and hypercalcemia. Thus, the authors suggest that in the presence of hypercalcemia in a patient with rheumatoid arthritis a search for some other cause is required. This search may be unsuccessful in a high proportion of patients and, furthermore, hypercalcemia is not related to the activity of the disease.

In other words, the results of the Córdoba et al. study show data that coincide with those of previous studies in both directions, without being conclusive in any of them. So, there is no doubt that the often repeated phrase “further studies are required” is perfectly valid in this case.

Correspondence: Manuel Sosa Hernríquez (manuel.sosa@ulpgc.es)
Bibliography

Hypercalcemia in patients with rheumatoid arthritis: a retrospective study

DOI: http://dx.doi.org/10.4321/S1889-836X2021000100002

Cordoba A1, García-Unzueta MT2, Riancho-Zarribetia L3,4, Corrales A5, Martínez-Taboada V3, Riancho JA1
1 Internal Medicine Service. Marqués de Valdecilla University Hospital. Department of Medicine and Psychiatry, University of Cantabria-Valdecilla Health Research Institute (IDIVAL). Santander (Spain)
2 Clinical Analysis Service. Marqués de Valdecilla University Hospital. Santander (Spain)
3 Rheumatology Service. Marqués de Valdecilla University Hospital. Department of Medicine and Psychiatry. University of Cantabria-IDIVAL. Santander (Spain)
4 Rheumatology Service. Sierrallana Hospital. Torrelavega (Spain)

Summary
Objective: To investigate the prevalence of hypercalcemia in patients with rheumatoid arthritis (RA) and analyze the clinical features and causes of hypercalcemia.

Material and methods: Retrospective case-based review study that included 500 patients with RA. Patients with increased calcium levels on at least two occasions were identified.

Results: Hypercalcemia was present in 24 of the 500 RA patients (4.8%). The age ranged between 50 and 80 years, with a mean of 68±10 years. The mean duration of the disease was 10±7 years. Of the patients with hypercalcemia, 22 were postmenopausal women (92%) and only two were men (8%). Hyperparathyroidism was found in 9 patients in the series; only one patient had malignant hypercalcemia due to multiple myeloma, and one case was a consequence of vitamin D intoxication. In one patient, hypercalcemia appeared to be related to calcium-alkali syndrome. In the remaining patients, hypercalcemia was idiopathic (8/24) or the study was incomplete (4/24). No obvious relationship was found between disease activity and the appearance of hypercalcemia.

Conclusion: As in the general population, primary hyperparathyroidism is the most common cause of hypercalcemia in patients with RA. In some patients, no other disorders causing hypercalcemia were identified, raising the possibility of a causal relationship between RA and hypercalcemia.

Key words: hypercalcemia, rheumatoid arthritis, hyperparathyroidism, vitamin D.

INTRODUCTION
Hypercalcemia is a relatively common clinical problem and a frequent laboratory finding, both in hospital and out-of-hospital practice. Calcium ions play a critical role in many cellular functions. Parathyroid hormone (PTH) and vitamin D are the most important hormones for regulating calcium. The main sources of serum calcium are intestinal absorption, stimulated by active vitamin D metabolites, and bone resorption, usually stimulated by PTH. Therefore, hypercalcemia can be classified as PTH-dependent (due to increased secretion of PTH by the parathyroid glands) and independent of PTH. The latter cases are attributable to increased bone resorption and/or increased intestinal absorption of calcium, induced by factors other than PTH. Among them, PTH-related protein (PTHrP) and locally produced cytokines are factors that often cause hypercalcemia in cancer patients. Unregulated extrarenal synthesis is of 1,25-dihydroxyvitamin D can also cause hypercalcemia, particularly in patients with chronic granulomatous disorders and in some patients with lymphoma.

Most reported cases of hypercalcemia are due to primary hyperparathyroidism or malignant neoplasms; together, these causes account for more than 90 percent of cases. Less common causes include granulomatous disorders, vitamin D poisoning, lithium or thiazide therapy, familial hypocalcidiuric hypercalcemia, etc. Among musculoskeletal diseases, sarcoidosis and metastatic bone tumors are well-known causes of hypercalcemia. However, the relationship between rheumatoid arthritis (RA) and hypercalcemia is unclear and conflicting results have been reported. Thus, while Ralston et al. found only 1 patient with hypercalcemia among 102 patients with RA, a much higher frequency, up to 30%, has been reported in some series. Therefore, our study aims to determine the frequency of hypercalcemia and its origin in unselected patients with RA.
PATIENTS AND METHODS
We investigated 500 unselected patients with a diagnosis of RA, seen in the Rheumatology consultation of the Marqués de Valdecilla Hospital. This tertiary hospital serves a population of about 350,000 people.

A computerized search of the blood tests carried out on these patients over a 15-year period (2002-2016) allowed us to identify total and ionized calcium values. Hypercalcemia was defined as a total serum calcium concentration greater than 10.4 mg/dl, and/or ionized calcium greater than 1.35 mmol/l (the limits of the normal range), in at least two determinations. The clinical records of patients with hypercalcemia were reviewed and clinical and biochemical data were extracted according to a standard protocol. The protocol was approved by the Cantabria Clinical Research Ethics Committee, which did not consider the written consent of the patients necessary due to the retrospective observational nature of the study.

A PubMed search was done on the terms "rheumatoid arthritis" and "hypercalcemia". Secondary references of relevant documents were also checked.

RESULTS
Patients’ characteristics
A total of 476 patients (95.2%) presented normal serum calcium levels, while 24 patients (4.8%) had hypercalcemia, according to the definition above.

The demographic, clinical and laboratory characteristics are listed in table 1. In summary, the RA sample with hypercalcemia (n=24) showed a preponderance of female gender (22 of 24, 92%) and had a mean age of 68±10 years (50-80). Most of the patients had long-standing RA (mean duration of the disease at the time of identification of hypercalcemia, 10±7 years; range 2-21), but in 5 cases the diagnosis of RA and hypercalcemia were simultaneous. Globally, 72% patients had positive rheumatoid factor and/or positive anti-citrullinated peptides. Not unexpectedly, the clinical spectrum was quite varied. Globally, 11 of the 24 patients with hypercalcemia (46%) had elevated inflammatory markers (CRP or ESR) at that time. Only 10 patients (42%) had evidence of arthritis at the time of hypercalcemia, and only 6 of them had arthritis and increased inflammatory markers. Four patients were taking vitamin D supplements and 9 were receiving calcium supplements. In all but one case, the doses were low and could not be considered as the cause of hypercalcemia.

Causes of hypercalcemia
After diagnostic studies, primary hyperparathyroidism was found in 9 patients (Figure 1). This represents 1.8% of the 500 RA patients, and 37% of the 24 hypercalcemia patients. Serum PTH levels ranged between 73 and 283 pg/ml (normal range <65 pg/ml). In 6 patients, a parathyroid adenoma was identified by scintigraphy or during surgical exploration. Three patients rejected the imaging studies. Two patients underwent surgery, 4 received antiresorptives and 3 did not receive any specific therapy.

Only one of the patients had a malignant hypercalcemia, due to multiple myeloma. In another patient, the hypercalcemia was due to vitamin D intoxication. In one patient, hypercalcemia could be due to the calcium-alkaline syndrome, a situation similar to the milk-alkaline syndrome. This diagnosis was based on the fact that hypercalcemia was associated with renal failure and the patient had been treated with calcium carbonate and thiazide supplements.

In the other patients in our series (8/24), the cause of hypercalcemia was unknown and, therefore, it can be considered idiopathic. Among this group, hypercalcemia was fluctuating (alternating normal and increased levels) in 5 patients, while in the other 3 it was transient. Hypercalcemia was always mild and asymptomatic. Although some patients showed elevated markers of inflammation, review of the cases did not reveal a relationship between hypercalcemia and clinical outbreaks of the disease. In 4 patients, follow-up studies and follow-up excluded disorders known to be associated with hypercalcemia (such as cancer, hyperparathyroidism, hyperthyroidism, adrenal insufficiency, etc.). However, in 4 other patients the study was limited, insufficient to establish with certainty the etiology of hypercalcemia.

None of the patients presented hypercalcemia secondary to granulomatous diseases (such as tuberculosis and sarcoidosis) or solid organ neoplasia. However, one patient had hypercalcemia mediated by increased 1,25-dihydroxyvitamin D levels, with suppressed PTH and increased angiotensin converting enzyme (ACE). Neither in the initial study (which included CT, PET and bone marrow biopsy), nor during follow-up were signs of neoplasia, adenopathy or granulomatous disease found. Corticosteroid treatment achieved full normalization of biochemical parameters, but the source of 1,25-dihydroxyvitamin D could not be identified.

DISCUSSION
RA is a chronic systemic inflammatory disorder. Although joint tissues are the main target of the inflammatory process, the disease also has consequences for bone tissue, both locally and systemically. In particular, RA causes increased bone resorption, which results locally in erosions and juxta-articular osteopenia, and systemically in reduced bone mass and increased risk of osteoporotic fractures. However, the association of RA with hypercalcemia is discussed (Table 2).

Table 1. Characteristics of patients with hypercalcemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at detection of hypercalcemia</td>
<td>68 ± 10 years</td>
</tr>
<tr>
<td>Duration of RA</td>
<td>10 ± 7 years</td>
</tr>
<tr>
<td>FR+</td>
<td>12/24 (50%)</td>
</tr>
<tr>
<td>ACP+</td>
<td>12/24 (50%)</td>
</tr>
<tr>
<td>Total serum calcium</td>
<td>10.6 ± 0.5 mg/dL</td>
</tr>
<tr>
<td>Serum ionic calcium</td>
<td>1.41 ± 0.1 mmol/l</td>
</tr>
<tr>
<td>PCR</td>
<td>2.3 ± 4.8 mg/dL</td>
</tr>
<tr>
<td>VSG</td>
<td>31 ± 33 mm/h</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.2 ± 0.7 mg/dL</td>
</tr>
<tr>
<td>PTH</td>
<td>87 ± 80 pg/ml</td>
</tr>
<tr>
<td>25-OH-vitamin D</td>
<td>46 ± 66 ng/ml</td>
</tr>
</tbody>
</table>

Variables expressed as mean ± standard deviation (SD) or number and percentage. RA: rheumatoid arthritis; RF: rheumatoid factor; ACP: anti-citrullinated cyclic peptide antibodies.
Almost 4 decades ago, Kennedy et al. noted the presence of hypercalcemia in 23 of 50 patients with RA (46%). In 7 cases (14%) the hypercalcemia was permanent. The cause was unclear. Many patients had active disease and some biochemical characteristics that suggested hyperparathyroidism, but serum PTH levels were within the normal range. However, Scott et al. reported a very low frequency of hypercalcemia among RA patients, 0.5% among outpatients and 0-2% among hospitalized patients. These findings are similar to those of Ralston et al., who found only one case of hypercalcemia in a group of 102 RA patients studied over a 3-month period. On the other hand, in a more recent study by Oelzner et al. which included 146 German RA patients, the frequency of hypercalcemia was 30%. Since high calcium levels were correlated with higher ESR and CRP values, as well as lower levels of PTH and 1,25-dihydroxyvitamin D, they suggested that hypercalcemia was probably due to increased bone resorption related to disease activity.

In our study, the frequency of hypercalcemia among RA patients was 4.8%, which is intermediate between those reported in previous studies. It is interesting to note that, unlike previous studies, we did not observe a clear association between RA activity and hypercalcemia. However, differences in patient characteristics, and specifically the availability of more potent disease-modifying drugs in recent years, make it difficult to compare the older series with the more recent ones.

Regarding the etiology of hypercalcemia, primary hyperparathyroidism seems to be the most common cause in patients with RA, similar to what happens in the general population. The prevalence of hyperparathyroidism in the Caucasian population is approximately 0.2-0.9%. Therefore, the 1.8% frequency that we found in RA may be somewhat higher than expected. However, the limited sample size does not allow us to firmly establish that the frequency of primary hyperparathyroidism is higher in RA than in the general population. However, a higher prevalence of hyperparathyroidism has recently been published in other RA cohorts, with a mean frequency of around 2.8%. On the other hand, it is worth mentioning that patients with hyperparathyroidism can have a variety of musculoskeletal manifestations, including pain and chondrocalcinosis, which must be properly interpreted and not be confused with the consequences of RA or other rheumatic disorders.

In the general population, cancer is the second most common cause of hypercalcemia. In our series, only one patient had hypercalcemia related to a malignancy, which is reassuring in the context of the increased cancer risk reported in RA.

The calcium-alkaline syndrome, an update of the picture previously known as milk-alkaline syndrome, characterized by the triad of hypercalcemia, metabolic alkalosis and kidney failure, secondary to the ingestion of variable amounts of calcium together with an absorbable alkaline, represents, according to data recent, the third most common cause of hypercalcemia. One patient in our cohort presented a picture consistent with this syndrome.

In a significant proportion of patients, the cause of hypercalcemia remained unclear. Patients with lymphoma and granulomatous disorders (such as tuberculosis or sarcoidosis) may have hypercalcemia due to unregulated extrarenal synthesis of 1,25-dihydroxyvitamin D. In the current series, one patient had recurrent hypercalcemia associated with high levels of 1,25-dihydroxyvitamin D. Consistent with an extrarenal source, 1,25-dihydroxyvitamin D and calcium levels normalized with glucocorticoid therapy. However, after a large study, which included repeated PET scans, CT scans, and bone marrow biopsies, no evidence of granulomatous disorder or cancer could be found. On the other hand, the patient’s age and the time course of serum calcium and 1,25-dihydroxyvitamin D levels do not fit within the spectrum of genetic deficiency of CYP24A1, an enzyme that metabolizes 25 and 1,25-dihydroxyvitamin D. Therefore, RA, although inactive, was the most likely explanation for the abnormal synthesis of 1,25-dihydroxyvitamin D. It should be noted.
that Gates published the case of a patient similar to this24. The mechanisms that relate RA to 1,25‐dihydroxyvitamin D synthesis are unclear, but could depend on cytokine‐mediated macrophage activation. Whatever the mechanisms involved, these appear to be very rare cases. In fact, RA was not among the underlying disorders in a series of 101 patients with 1,25‐dihydroxyvitamin D25 mediated hypercalcemia.

Future epidemiological studies, with larger cohorts of RA patients, would help to clarify whether the frequency of hyperparathyroidism increases in RA. Furthermore, careful clinical studies of patients in whom diagnostic analysis does not reveal causes of hypercalcemia other than RA can help to better understand the pathophysiology of these rare cases.

Treatment of hypercalcemia in RA must take into account the cause and the mechanisms responsible for the increase in serum calcium. General measures should include the withdrawal of calcium supplements and other drugs that induce hypercalcemia (such as lithium or thiazides) and maintaining adequate hydration. In acute severe cases, intravenous fluids, bisphosphonates such as zoledronic acid, and sometimes calcitonin are indicated26. For patients with a parathyroid adenoma, surgical removal is the therapy of choice, but non‐invasive procedures can be useful in patients with very high surgical risk27,28. In these patients, drug treatment with cinacalcet or antiresorptive agents can help control hypercalcemia9. In 1,25‐dihydroxyvitamin D‐mediated hypercalcemia, corticosteroids are usually very effective, but ketoconazole or antimalarials can also help control extrarenal vitamin D hydroxylation and, consequently, normalize levels2,29.

CONCLUSION

In this study of a cohort of 500 RA patients, hypercalcemia was present in 4.8%. As in the general population, primary hyperparathyroidism was the most common cause. In some patients, no other disorders causing hypercalcemia were identified, raising the possibility of a causal relationship between RA and hypercalcemia. However, in these cases we did not find a clear link between disease activity and calcium levels.

Although limited by its retrospective nature, our study thus adds useful information on the epidemiology of hypercalcemia and RA. These results suggest that hypercalcemia has a similar frequency in RA and in the general population and that the causes are similar. Although the study was incomplete in some cases, our data support that most patients have another underlying diagnosis as the cause of hypercalcemia. Therefore, if hypercalcemia is discovered in a patient with RA, a search should be made for underlying causes, particularly hyperparathyroidism and cancer.

### Conflict of interests

The authors declare no conflict of interest.
Bibliography


Association of biochemical parameters of bone metabolism with progression and/or development of new aortic calcifications

Summary
Objective: Biochemical parameters continue to be the most widely used option for the follow-up of patients with bone metabolic disorders. The objective of our study was to assess the association of some biochemical markers of bone metabolism with the appearance and progression of aortic calcifications.

Material and methods: In this study, 624 men and women older than 50 years were selected at random. The participants completed a questionnaire and underwent two lateral dorsal-lumbar x-rays and bone densitometry. Four years later, the same studies were repeated in 402 subjects along with a biochemical study.

Results: Age and the proportion of men were higher in those who had “global progression” of aortic calcification (progression of the existing ones plus new ones). The serum levels of calcium and calcitriol were significantly higher and those of osteocalcin significantly lower in which “global progression” of aortic calcification was observed. Multivariate analysis showed that only osteocalcin was independently associated with “global progression” of aortic calcification, with an 18% decrease for each 1 ng/mL increase in osteocalcin levels (odds ratio (OR)=0.82; 95% confidence interval (95% CI): 0.71-0.92).

The categorization of osteocalcin into tertiles showed that the subjects of the first tertile (<4.84 ng/mL) were associated with a higher proportion of new aortic calcifications: (OR=2.45; 95% CI: 1.03-3.56) with respect to the third tertile (>6.40 ng/mL).

Conclusion: Serum levels of osteocalcin could be a biochemical marker to evaluate the appearance and/or evolution of aortic calcification. However, it is necessary to determine with greater precision how it could exert this protective effect in the process of vascular calcification.

Key words: osteocalcin, vascular calcification, biochemical markers, bone mineral density.

INTRODUCTION
Atherosclerosis, arteriosclerosis, vascular calcification and osteoporosis are common age-related disorders associated with high morbidity and mortality. Due to the increased life expectancy in the Spanish population, these disorders are expected to become more and more frequent in the coming decades. Although recent work has been carried out on the development of non-invasive techniques for the early detection of vascular calcifications, such as pulse wave velocity and non-contrast carotid ultrasound, serum biochemical parameters continue to be the most widely used option for monitoring patients with bone metabolic disorders.

Having easily accessible non-invasive tools such as biochemical markers allow for the adoption of therapeutic measures in order to mitigate the deleterious effect of bone loss. Taking into account that osteoporosis and vascular calcification share etiopathogenic mechanisms, some biochemical parameters used to study bone metabolism could serve as possible markers of vascular calcification.
cification. Therefore, this study aims to assess the association of some biochemical markers of bone metabolism with the appearance and progression of aortic calcifications.

**Material and Methods**

This study used data from a European project designed to determine the prevalence of vertebral fracture (European Vertebral Osteoporosis Study - EVOS), in which the Bone and Mineral Metabolism Service of the Central University Hospital of Asturias took part.

For this work, 308 men and 316 women over 50 years of age were selected at random from the municipal registry of Oviedo. Our protocol involved patients' filling out a questionnaire on risk factors related to osteoporosis, two lateral dorsal-lumbar x-rays and a bone densitometry (DXA) (the radiographic study was not completed in only 2 cases), and collecting anthropometric measurements such as height and weight to determine body mass index (BMI). All subjects had sufficient stamina to go up two floors without an elevator and 99% lived in their own home.

After four years, they were invited to repeat the radiological study, bone densitometry, anthropometric measurements and respond to a questionnaire on risk factors for osteoporosis and a biochemical study. In the second control, 402 subjects participated (213 women and 189 men), of whom 335 agreed to carry out the biochemical study. A total of 67 subjects (16.7%) were excluded. The coefficients of variation (CV) were 0.78 and 0.73, data that indicate good reproducibility.

Evaluation of the progression of vascular calcification

Abdominal aortic calcification was evaluated by two independent investigators, and was defined and classified as grade 0 (absent), grade 1 (mild-moderate), and grade 2 (severe). Isolated punctate calcifications, a visible linear calcification of some biochemical markers of bone metabolism with the appearance and progression of aortic calcifications.

**Material and Methods**

This study used data from a European project designed to determine the prevalence of vertebral fracture (European Vertebral Osteoporosis Study - EVOS), in which the Bone and Mineral Metabolism Service of the Central University Hospital of Asturias took part.

For this work, 308 men and 316 women over 50 years of age were selected at random from the municipal registry of Oviedo. Our protocol involved patients’ filling out a questionnaire on risk factors related to osteoporosis, two lateral dorsal-lumbar x-rays and a bone densitometry (DXA) (the radiographic study was not completed in only 2 cases), and collecting anthropometric measurements such as height and weight to determine body mass index (BMI). All subjects had sufficient stamina to go up two floors without an elevator and 99% lived in their own home.

After four years, they were invited to repeat the radiological study, bone densitometry, anthropometric measurements and respond to a questionnaire on risk factors for osteoporosis and a biochemical study. In the second control, 402 subjects participated (213 women and 189 men), of whom 335 agreed to carry out the biochemical study. A total of 67 subjects (16.7%) were excluded. The coefficients of variation (CV) were 0.78 and 0.73, data that indicate good reproducibility.

**Evaluation of the progression of vascular calcification**

Abdominal aortic calcification was evaluated by two independent investigators, and was defined and classified as grade 0 (absent), grade 1 (mild-moderate), and grade 2 (severe). Isolated punctate calcifications, a visible linear calcification in less than 2 vertebral bodies, or a dense calcified plaque were defined as mild-moderate calcification. Therefore, this study aims to assess the association of some biochemical markers of bone metabolism with the appearance and progression of aortic calcifications.

**Material and Methods**

This study used data from a European project designed to determine the prevalence of vertebral fracture (European Vertebral Osteoporosis Study - EVOS), in which the Bone and Mineral Metabolism Service of the Central University Hospital of Asturias took part.

For this work, 308 men and 316 women over 50 years of age were selected at random from the municipal registry of Oviedo. Our protocol involved patients’ filling out a questionnaire on risk factors related to osteoporosis, two lateral dorsal-lumbar x-rays and a bone densitometry (DXA) (the radiographic study was not completed in only 2 cases), and collecting anthropometric measurements such as height and weight to determine body mass index (BMI). All subjects had sufficient stamina to go up two floors without an elevator and 99% lived in their own home.

After four years, they were invited to repeat the radiological study, bone densitometry, anthropometric measurements and respond to a questionnaire on risk factors for osteoporosis and a biochemical study. In the second control, 402 subjects participated (213 women and 189 men), of whom 335 agreed to carry out the biochemical study. A total of 67 subjects (16.7%) were excluded. The coefficients of variation (CV) were 0.78 and 0.73, data that indicate good reproducibility.

**Evaluation of the progression of vascular calcification**

Abdominal aortic calcification was evaluated by two independent investigators, and was defined and classified as grade 0 (absent), grade 1 (mild-moderate), and grade 2 (severe). Isolated punctate calcifications, a visible linear calcification in less than 2 vertebral bodies, or a dense calcified plaque were defined as mild-moderate calcification. The presence of a visible linear calcification along at least two vertebral bodies and/or the presence of two or more calcified dense plaques was defined as severe calcification. The degree of intra- and inter-observer concordance in the analysis of the x-rays was 92% and 90%, respectively, with a Kappa coefficient of 0.78 and 0.73, data that indicate good reproducibility.

The progression of aortic calcification was determined by comparing the x-rays taken at baseline with those at 4 years. It was defined as “global progression” of aortic calcification when an increase in the magnitude of baseline aortic calcification coexisted with the appearance of new calcifications, comparing the x-rays at the outset with those done 4 years later.

**Densitometric evaluation**

Bone mineral density (BMD) was measured with a Hologic® QDR-1000 DXA densitometer (Hologic Inc., Waltham, Massachusetts, USA). In all cases, the anteroposterior lumbar spine (L2-L4) and the proximal extremity of the right femur were analyzed. For the evaluation of lumbar BMD, 4 subjects with marked degenerative osteoarthritis were excluded. The coefficients of variation (CV) were 1.2% and 1.9%, respectively. The precision and quality control was performed daily with a lumbar spine phantom, with which a CV of 0.0±0.1% was obtained. In the fourth year, BMD was determined in the same areas used in the first study, and the percentage of change between both measurements was used to evaluate changes in BMD.

**Biochemical analysis**

In the baseline study, no biochemical study was carried out. At 4 years, a fasting blood and urine sample was taken from each subject participating in the study. Once the serum was separated, the latter and the urine were kept frozen at -80°C until quantification. Serum calcium, creatinine, phosphorus, total alkaline phosphatase, and acid resistant tartrate phosphatase were measured using an autoanalyzer (Hitachi Mod. 717, Rattgen, Germany). The serum levels of calcidiol (25OHD) were determined by prior extraction with acetonitrile (IDS, Ltd., Bolton, United Kingdom), whose intra- and inter-assay coefficients of variation (CV) were 5.2% and 8.2%, respectively.

Levels of 1,25-dihydroxyvitamin D were measured by radioimmunoassay (IDS, Ltd.); intra- and interassay CVs were 6.5% and 9%, respectively. The intact PTH and total osteocalcin levels were measured by radioimmunoassay (Nichols Institute, San Juan Capistrano, California, USA). Intra- and inter-assay CV values were 2.6% and 5.8% for PTH and 4.5% and 5.1% for osteocalcin, respectively.

All the tests carried out followed the principles set forth in the Declaration of Helsinki and were formally approved by the Committee for Clinical Trials of the Principality of Asturias.

**Statistic analysis**

Data analysis was carried out using SPSS version 17.0 for Windows. The quantitative variables were analyzed by Student’s-test and the qualitative variables by chi-square.

Multivariate analysis was performed using logistic regression adjusting for age, sex and BMI, in those serum or urinary markers in which the univariate analysis was significantly associated with progression and/or appearance of new abdominal aortic calcification.

Pearson correlations were performed between those biochemical parameters that, at the multivariate level, showed a significant association with the percentage of change in BMD between both cross-sectional studies.

**Results**

The mean age of those who had “global progression” of aortic calcification (progression of existing vascular calcifications plus new vascular calcifications) was higher than the age of those in whom this situation was not observed (Tables 1 and 2). However, there were no age differences in those who only presented a new aortic calcification as a change in the control at 4 years (Table 3). The BMI was similar in those with aortic calcification, both in those in which the calcification progressed, as in those with new calcifications, or considering both variations together (Tables 1-3).

Male sex was significantly more frequent in those in whom progression of existing aortic calcifications and/or new aortic calcifications was observed. In contrast, there were no differences in the smoking habit (Tables 1-3).
Table 1. Clinical and anthropometric variables and biochemical markers of bone and mineral metabolism in the presence or absence of “global progression” of vascular calcification (CV)

<table>
<thead>
<tr>
<th>Progression more new CV</th>
<th>Aortic calcification (n=118)</th>
<th>No aortic calcification (n=144)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>Male gender</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>77 (72.3%)</td>
<td>61 (42.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>Smoker</td>
<td></td>
<td>0.121</td>
</tr>
<tr>
<td>23 (19.5%)</td>
<td>18 (12.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Age (years)</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>69.6 ± 7.7</td>
<td>66.4 ± 8.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>BMI (kg/cm²)</td>
<td></td>
<td>0.367</td>
</tr>
<tr>
<td>28.0 ± 3.8</td>
<td>28.4 ± 4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>PTH (pg/mL)</td>
<td></td>
<td>0.460</td>
</tr>
<tr>
<td>54.0 ± 27.1</td>
<td>51.8 ± 20.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>Alkaline phosphatase (IU/L)</td>
<td></td>
<td>0.817</td>
</tr>
<tr>
<td>177 ± 89</td>
<td>175 ± 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>Calcium (mg/dL)</td>
<td></td>
<td>0.011</td>
</tr>
<tr>
<td>9.46 ± 0.30</td>
<td>9.35 ± 0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>Phosphorus (mg/dL)</td>
<td></td>
<td>0.964</td>
</tr>
<tr>
<td>3.45 ± 0.44</td>
<td>3.44 ± 0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcidiol (ng/mL)</td>
<td>Calcidiol (ng/mL)</td>
<td></td>
<td>0.092</td>
</tr>
<tr>
<td>15.5 ± 7.5</td>
<td>17.4 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcitriol (pg/mL)</td>
<td>Calcitriol (pg/mL)</td>
<td></td>
<td>0.025</td>
</tr>
<tr>
<td>43.9 ± 17.3</td>
<td>39.4 ± 14.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>Osteocalcin (ng/mL)</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>5.42 ± 1.76</td>
<td>6.22 ± 2.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FATR (U/L)</td>
<td>FATR (U/L)</td>
<td></td>
<td>0.212</td>
</tr>
<tr>
<td>2.02 ± 0.65</td>
<td>2.13 ± 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca/creatinine urine</td>
<td>Ca/creatinine urine</td>
<td></td>
<td>0.675</td>
</tr>
<tr>
<td>0.18 ± 0.11</td>
<td>0.17 ± 0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The variables are expressed in number (percentage) and in mean ± standard deviation; BMI: body mass index; PTH: parathormone; FATR: tartrate-resistant acid phosphatase.

Table 2. Clinical and anthropometric variables and biochemical markers of bone and mineral metabolism in the presence or absence of progression of vascular calcifications (VC)

<table>
<thead>
<tr>
<th>CV progression</th>
<th>Aortic calcification (n=62)</th>
<th>No aortic calcification (n=144)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>Male gender</td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>40 (64.5%)</td>
<td>61 (42.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>Smoker</td>
<td></td>
<td>0.119</td>
</tr>
<tr>
<td>13 (21.0%)</td>
<td>18 (12.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Age (years)</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>70.6 ± 8.2</td>
<td>66.4 ± 8.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>BMI (kg/cm²)</td>
<td></td>
<td>0.319</td>
</tr>
<tr>
<td>27.8 ± 4.0</td>
<td>28.4 ± 4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>PTH (pg/mL)</td>
<td></td>
<td>0.428</td>
</tr>
<tr>
<td>54.7 ± 29.2</td>
<td>51.8 ± 20.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>Alkaline phosphatase (IU/L)</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>175 ± 51</td>
<td>175 ± 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>Calcium (mg/dL)</td>
<td></td>
<td>0.009</td>
</tr>
<tr>
<td>9.48 ± 0.26</td>
<td>9.35 ± 0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>Phosphorus (mg/dL)</td>
<td></td>
<td>0.774</td>
</tr>
<tr>
<td>3.42 ± 0.45</td>
<td>3.44 ± 0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcidiol (ng/mL)</td>
<td>Calcidiol (ng/mL)</td>
<td></td>
<td>0.082</td>
</tr>
<tr>
<td>14.8 ± 7.6</td>
<td>17.4 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcitriol (pg/mL)</td>
<td>Calcitriol (pg/mL)</td>
<td></td>
<td>0.154</td>
</tr>
<tr>
<td>42.8 ± 17.2</td>
<td>39.4 ± 14.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>Osteocalcin (ng/mL)</td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>5.46 ± 1.87</td>
<td>6.22 ± 2.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FATR (U/L)</td>
<td>FATR (U/L)</td>
<td></td>
<td>0.692</td>
</tr>
<tr>
<td>2.09 ± 0.58</td>
<td>2.13 ± 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca/creatinine urine</td>
<td>Ca/creatinine urine</td>
<td></td>
<td>0.891</td>
</tr>
<tr>
<td>0.17 ± 0.10</td>
<td>0.17 ± 0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The variables are expressed in number (percentage) and in mean ± standard deviation; BMI: body mass index; PTH: parathormone; FATR: tartrate-resistant acid phosphatase.

Regarding the biochemical markers of bone metabolism, serum levels of calcium and calcitriol were significantly higher and those of osteocalcin significantly lower in those subjects in whom “global progression” of aortic calcification was observed (new calcifications plus progression of vascular calcification) (Table 1).

The logistic regression analysis adjusted for age, sex and BMI showed that the only biochemical marker that was independently associated with “global progression” of aortic calcification was osteocalcin, showing that increases of 1 ng/mL were associated with a decrease in 18% in the progression of aortic calcification (odds ratio (OR)=0.82; 95% confidence interval (95% CI): 0.71-0.95) (Table 4). Age and male sex were also significantly associated with progression of vascular calcification (OR=1.05; 95% CI: 1.01-1.08 and OR=2.06; 95% CI: 1.20-3.54, respectively) (Table 4).

In the univariate analysis, serum osteocalcin levels were significantly lower and calcium levels significantly higher in those subjects in whom aortic calcification had progressed (Table 2). The logistic regression analysis adjusted for age, sex and BMI confirmed that osteocalcin was the only parameter that showed a significant association:
Table 3. Clinical and anthropometric variables and biochemical markers of bone and mineral metabolism in the presence or absence of new vascular calcifications (VC)

<table>
<thead>
<tr>
<th>New CV</th>
<th>Aortic calcification (n=56)</th>
<th>No aortic calcification (n=144)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>37 (66.1%)</td>
<td>61 (42.4%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Smoker</td>
<td>10 (17.9%)</td>
<td>18 (15.0%)</td>
<td>0.327</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.5 ± 7.1</td>
<td>66.4 ± 8.9</td>
<td>0.082</td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>28.2 ± 3.6</td>
<td>28.4 ± 4.2</td>
<td>0.691</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>53.3 ± 24.8</td>
<td>51.8 ± 20.5</td>
<td>0.676</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>180 ± 119</td>
<td>175 ± 55</td>
<td>0.717</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.43 ± 0.34</td>
<td>9.35 ± 0.34</td>
<td>0.166</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.47 ± 0.44</td>
<td>3.44 ± 0.48</td>
<td>0.694</td>
</tr>
<tr>
<td>Calcidiol (ng/mL)</td>
<td>1.63 ± 7.3</td>
<td>17.4 ± 9.8</td>
<td>0.463</td>
</tr>
<tr>
<td>Calcitriol (pg/mL)</td>
<td>45.2 ± 17.4</td>
<td>39.4 ± 14.4</td>
<td>0.022</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>5.36 ± 1.65</td>
<td>6.22 ± 2.15</td>
<td>0.009</td>
</tr>
<tr>
<td>FATR (U/L)</td>
<td>1.95 ± 0.73</td>
<td>2.13 ± 0.64</td>
<td>0.103</td>
</tr>
<tr>
<td>Ca/creatinine urine</td>
<td>0.18 ± 0.11</td>
<td>0.17 ± 0.10</td>
<td>0.579</td>
</tr>
</tbody>
</table>

The variables are expressed in number (percentage) and in mean ± standard deviation; BMI: body mass index; PTH: parathormone; FATR: tartrate-resistant acid phosphatase.

In increases of 1 ng/mL were associated with a 16% increase in the progression of aortic calcifications (OR=0.84; 95% CI: 0.70-0.99) (Table 4). Sex (OR=1.95; 95% CI: 1.01-3.76) and also age (OR=1.06; 95% CI: 1.02-1.10) were associated in this multivariate model (Table 4).

When only those subjects who presented a new aortic calcification were analyzed, serum levels of osteocalcin were found to be significantly lower and those of calcitriol, significantly higher (Table 3). The logistic regression analysis adjusted for age, sex and BMI confirmed that only osteocalcin showed a significant association: increases of 1 ng/mL were associated with a 20% appearance of new aortic calcifications (OR=0.80; 95% CI: 0.67-0.97) (Table 4). Male sex (OR=2.30; 95% CI: 1.15-4.59), but not age, was associated in this multivariate model (Table 4).

The categorization of serum osteocalcin levels into tertiles showed that the lowest tertile (osteocalcin <4.84 ng/mL) was the one that showed the highest proportion of new aortic calcifications (22; 42.3%). The second tertile (osteocalcin between 4.84 and 6.40 ng/mL) showed the same trend, but in a lower proportion (18; 34.6%), while the third tertile (osteocalcin >6.4 ng/mL) showed the lowest proportion (12; 23.1%). A logistic regression analysis adjusted for age, sex and BMI showed that the subjects of the first tertile were associated with a higher proportion (2.45 times) of new aortic calcifications: (OR=2.45; 95% CI: 1.03-3.56). There were no differences with those of the second tertile (OR=1.48; 95% CI: 0.611-3.56).

The bi-variates correlations between the percentage of change in BMD at the lumbar and femoral neck level and the serum levels of osteocalcin showed a negative and significant correlation. Higher values of osteocalcin were associated with a lower loss of BMD, while lower values of osteocalcin were associated with greater losses of bone mass (Figure 1A and 1B).

**Discussion**

Our results confirm that, of the biochemical markers analyzed, osteocalcin was the only marker associated with the appearance and progression of aortic calcifications independently of age, sex and BMI. A 1 ng/mL increase in osteocalcin decreased the "global progression" of aortic calcification by 18%, a protection equivalent to being 3-4 years younger.

Osteocalcin, a vitamin K-dependent protein, is the most abundant non-collagen component in the mineralized matrix of bone. It is not only produced by bone, but also by vascular smooth muscle cells that show a phenotype similar to osteoblasts. It inhibits the precipitation of calcium phosphate and shows a strong affinity for hydroxyapatite. Initially, it was thought that osteocalcin inhibited the growth of hydroxyapatite crystals and limited bone formation.

Experimental studies have shown that decarboxylated osteocalcin can up-regulate nitric oxide synthesis in human endothelial cells with a protective effect against endothelial dysfunction. These findings support the opinion that decarboxylated osteocalcin is the biologically active form of the protein, with a protective function on the vasculature independent of its metabolic role, although more studies are required to confirm this fact.

Osteocalcin has been detected to a greater degree in calcified plaques and aortic valves than in healthy non-calcified vessels. The level of osteocalcin mRNA reportedly increases between 8 and 14 times in calcified aortic plaques compared to healthy aortas. The increase in total osteocalcin may occur as a result from the development of an osteogenic phenotype in atherosclerotic plaques. However, this requires further validation. Recently, osteocalcin has been found to play a crucial role in arterial calcification mediated by Wnt/β-catenin signaling through increased oxidative phosphorylation, and this finding may have clinical implications.
Table 4. Multivariate analysis of the independent variables significantly associated in the univariate analysis with the progression and/or presence of new aortic calcifications. The odd ratio (OR) and the 95% confidence interval (95% CI) are represented. Significant values are shown in bold.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>OR</th>
<th>IC 95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global progression of calcification (progression and new)</td>
<td>Age (every year)</td>
<td>1.05</td>
<td>1.01 - 1.05</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Gender (male)</td>
<td>2.06</td>
<td>1.20 - 3.54</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Calcium (each mg/dL)</td>
<td>1.87</td>
<td>0.79 - 4.42</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>Calcitriol (each pg/mL)</td>
<td>1.02</td>
<td>0.99 - 1.03</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>Osteocalcin (each ng/mL)</td>
<td>0.82</td>
<td>0.71 - 0.95</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Age (every year)</td>
<td>1.06</td>
<td>1.02 - 1.10</td>
<td>0.005</td>
</tr>
<tr>
<td>Progression of aortic calcification</td>
<td>Gender (male)</td>
<td>1.95</td>
<td>1.01 - 3.76</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Calcium (each mg/dL)</td>
<td>2.61</td>
<td>0.90 - 7.55</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>Osteocalcin (each ng/mL)</td>
<td>0.84</td>
<td>0.70 - 0.99</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>Age (every year)</td>
<td>1.02</td>
<td>0.98 - 1.07</td>
<td>0.250</td>
</tr>
<tr>
<td>New aortic calcifications</td>
<td>Gender (male)</td>
<td>2.30</td>
<td>1.15 - 4.59</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Calcium (each mg/dL)</td>
<td>1.51</td>
<td>0.56 - 4.03</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td>Calcitriol (each pg/mL)</td>
<td>1.02</td>
<td>0.99 - 1.04</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>Osteocalcin (each ng/mL)</td>
<td>0.80</td>
<td>0.67 - 0.97</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Significant values are shown in bold.

However, studies in patients are inconclusive. A relatively recent meta-analysis included 46 studies that examined the association between osteocalcin and atherosclerosis20. Of the studies that analyzed the association between osteocalcin and carotid intima-media thickness (CIMT), four reported that higher levels of osteocalcin were associated with greater CIMT, four reported that higher levels of osteocalcin were associated with a lower CIMT, and three did not find any correlation. However, studies that examined mononuclear cells positive for osteocalcin or histological staining for osteocalcin showed that higher levels of osteocalcin were associated with an increase in markers of atherosclerosis and calcification20. Thus, it is suggested that osteocalcin could be a marker of the calcification process.

Our results show that, in the 4-year period between both cross-sections, both the presence of new aortic calcifications and their progression were associated with lower levels of osteocalcin, regardless of age, sex and BMI. It is noteworthy that the lowest tertile of osteocalcin (<4.84 ng/mL) was associated with a significant increase in new aortic calcifications: 2.45 (1.03-3.56) compared to subjects with serum osteocalcin levels higher than 6.4 ng/mL.

Kim et al. found similar results in Asian women to those in our study, with an inverse correlation between osteocalcin and vascular calcification measured by the Agatston score, even after adjusting for age21. Similar results have also been shown in other cross-sectional22,23 and longitudinal studies, such as ours, in which raised levels of osteocalcin are found to be associated with less progression of abdominal aortic calcification24. These authors suggest that osteocalcin could be involved in the aortic calcification process indirectly by its action on insulin and insulin resistance. Fusaro et al. have recently observed in a population on dialysis that those diabetic patients with a higher prevalence of vascular calcification had lower serum levels of total and decarboxylated osteocalcin25. In fact, in a secondary analysis of our study, analyzing osteocalcin levels in those subjects diagnosed with diabetes, it was observed that the presence of diabetes (n=23) was associated with significantly lower levels of osteocalcin than those without diabetes (n=241) (4.89±1.80 ng/mL compared to 5.96±2.14 ng/mL; p=0.020).

It could also be conjectured that low levels of osteocalcin are associated with vascular calcification (VC) due to less bone remodeling, which could be a VC risk factor26,27. However, this possibility would not be supported by the results of this study, since the subjects with lower levels of osteocalcin and higher VC were those with lower BMD, which would be more indicative of high remodeling than low remodeling28.

On the other hand, the usefulness of osteocalcin as a serum marker remains controversial. There is still a long way to go to define whether osteocalcin can be used as a diagnostic or detection tool in the appearance of VC. It is noteworthy that no study has differentiated between forms of osteocalcin when it comes to VC. Consequently, it is necessary to study the effect that carboxylated and decarboxylated osteocalcin could have in this environment, as well as to consider the mechanisms associated with the increase of osteocalcin in calcified tissue29.
This study presents several limitations. First, osteocalcin determination was only carried out in the second cross section, which limits the associations found. Second, intact or total osteocalcin was determined without differentiating between carboxylated or decarboxylated. On the other hand, the evaluation of vascular calcification was carried out by simple X-ray imaging and not by more sensitive techniques. It is also possible that some of the people who attended the second check-up after 4 years would have done so because they were in a worse physical condition compared to those who did not attend it, although no clear selection biases were found29.

Despite these limitations, the study also has important strengths, such as the adequate response of the subjects who participated in the study, both at baseline (50%)30 and at 4 years of the follow-up period (70%). The degree of reliability among observers for the assessment of vascular calcification supports its use as a diagnostic criterion. Finally, unlike other studies, this study was prospective, and not cross-sectional like most of those cited. This reinforces the validity of the results found and their greater degree of association.

Thus, although new studies are needed to confirm these results, this study seems to indicate that serum levels of osteocalcin could be a promising biochemical marker associated with the appearance and/or development of aortic calcification.

Acknowledgments: This research study has been partially funded by the European Study on Vertebral Osteoporosis (EVOS), European Union (1991–1993); European Prospective Osteoporosis Study (EPOS), European Union (BIOMED 93–95), BMHI-CT 092-0182 (1993–1997); Health Research Fund (FIS 94/1901-E); Retic REDinREN of ISCIII (RD06/0016/1013, RD12/0021/0023 and RD16/0009/0017); National R + D + I Plan 2008-2011, State R + D + I Plan 2013-2016, European Regional Development Fund (ERDF), Science, Technology and Innovation Plan 2013-2017 and 2018-2022 of the Principality of Asturias (GRU-PIN14-028, IDI-2018-000152), Renal Foundation Íñigo Alvarez de Toledo (FRIAT). Augusto Antonio Díaz Sottolano has been funded by the Principality of Asturias Health Research Institute (ISPA). Sara Fernández Villabrille has been financed by IDI-2018-000152 and project FIS 17/00715 and Javier Rodríguez Carrio by a contract along with Juan de la Cierva and Sara Borrell.

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


Search for variants of the LRP4 gene in women with high bone mass and in patients with Chiari type I malformation

DOI: http://dx.doi.org/10.4321/S1889-836X2021000100004

Martínez-Gil N, Grinberg D, Balcells S
Genetics, Microbiology and Statistics Department. Biology Faculty. University of Barcelona. Center for Biomedical Research of the Rare Diseases Network (CIBERER). Institute of Biomedicine of the University of Barcelona (IBUB). San Joan de Déu Research Institute (IRSJD). Barcelona (Spain)

Summary
Objective: LRP4 is an essential facilitator in sclerostin-specific inhibition of the canonical Wnt pathway. Mutations in LRP4 have been associated with various conditions, including bone growth disease, sclerosteosis, and Chiari type I malformation (CMI).

Material and methods: The LRP4 has been re-sequenced in two patient cohorts with high bone mass phenotype (HBM) and with CMI aimed at finding causal variants.

Results: Among the mutations found, we would highlight: 1) a missense mutation in a patient with CMI, which does not co-segregate with the phenotype in the family; 2) a previously undescribed intronic mutation (c.3364+16A>C) in a woman with HBM; and 3) an intronic mutation in a woman with HBM with a very low frequency in the European control population.

Conclusions: Although we have not found variants in LRP4 to explain the HBM or CMI phenotype in the patients studied, we encourage other researchers to analyze the LRP4 gene in their patients as it is a good functional candidate for both phenotypes.

Key words: LRP4, HBM, Chiari malformation type I, bone mineral density, sclerostin.

INTRODUCTION

The Wnt signalling pathway is involved in a wide range of processes, including bone development and homeostasis. In accordance with this, mutations have been identified in various components of the Wnt pathway that cause different musculoskeletal diseases. The canonical Wnt pathway begins with the formation of a heterotrimeric complex between a co-receptor, LRP5/6, a ligand, WNT, and a receptor, FZD, which produces an accumulation of β-catenin in that, once in the nucleus, will activate the transcription of numerous important target genes for bone. This activation is finely regulated by a series of extracellular inhibitors such as DKK1 and sclerostin that bind to LRP5/6, preventing the formation of the heterotrimeric complex. For DKK1 and sclerostin to exert their inhibitory activity, they must form another heterotrimeric complex with LRP5 and KREMEN1/2 or LRP4, respectively. Although in the case of DKK1 the presence of KREMEN does not seem to be necessary to carry out a correct inhibition, the presence of LRP4 is essential for the inhibitory function of sclerostin.

LRP4 mutations have been described in humans which cause different diseases that affect not only bone mass, but also the regulation of the extremities and kidneys among other, depending on the position of where the mutation occurs. Specifically, mutations in the central cavity of the third β-propeller cause sclerosteosis, characterized by variable syndactyly and progressive...

Date of receipt: 22/12/2020 - Date of acceptance: 01/03/2021

This study was awarded a scholarship to attend the 2019 ASBMR meeting (Orlando, USA)
bone overgrowth, particularly severe in the facial skeleton and skull. These patients present an increase in the Wnt pathway in osteoblasts, generating an increase in bone formation. In addition to these mutations, in 2017, Merello et al. described a mutation in the second β-propeller domain (p.Thr851Arg) that cosegregated with the phenotype in a family with type 1 Chiari malformation (CMI). This is a malformation of the central nervous system characterized by a caudal displacement of the cerebellar tonsils, which generates varied symptoms both in onset and in severity (Orphanet, https://www.orphanet/consor/cgi-bin/index.php). Although some patients with CMI may be asymptomatic, others can present with a suboccipital headache and neck pain, among others. It is interesting to note that this malformation was described in a patient who presented a phenotype of high bone mass (HBM) due to gain-of-function mutations in LRP5, thus suggesting a possible relationship between CMI, the HBM phenotype and Wnt pathway. Taking into account the role of LRP4 on the Wnt pathway and the important role that this pathway has in determining bone mineral density (BMD) and in the development of the skull, we hypothesized that mutations in LRP4 could be the cause of the HBM in women with this phenotype or of causing disease in patients with CMI. In this study, we have carried out a re-sequencing of the LRP4 gene in a cohort of 10 women with the HBM phenotype and in a cohort of 12 patients with CMI.

**MATERIAL AND METHODS**

**Cohorts studied**

Sarrion et al. describe the HBM cohort, in a study of 10 women with HBM phenotype, in which this phenotype is defined as the sum of the Z-score values of the lumbar spine and femoral neck equal or greater than 4. The CMI cohort studied here consists of 12 patients with unrelated CMI, diagnosed and treated at the Hospital del Mar in Barcelona. The diagnosis of CMI is based on the position of the cerebellar tonsils by brain magnetic resonance imaging (Achieva 3.0 T, Philips, Amsterdam, The Netherlands) with a herniation equal to or greater than 5 mm on a mid-sagittal T1-weighted image in the presence of signs or symptoms indicating neural compression at the cranio-vertebral junction, syringohydromyelia, cerebellar dysfunction or intracranial hypertension. In addition, information is available on 8 relatives of 4 of the patients with CMI.

**LRP4 re-sequencing**

Genomic DNA from CMI cases and their relatives and from HBM women was isolated. women was isolated from peripheral blood leukocytes using the Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA), according to with the manufacturer’s instructions. Regarding the re-sequencing, specifically, we have amplified the exons that code for the mutations that cause Cenani-Lenz syndrome (p.D137N, p.C160Y, p.D49N, p.T461P, p.L473F, p.D529N, p.L953P, p.C1017R, p.R1277H, p.E1233K) and the third β-propeller domain where the mutations causing myasthenic syndrome and sclerosteosis are located. Amplification of each of these fragments was done by polymerase chain reaction (PCR) using GoTaq Flexi DNA polymerase (Promega). The PCR fragments were analyzed by agarose gel electrophoresis method and their purification was done on Multiscreen™ Vacuum Manifold 96-well plates (Merck Millipore). The purified PCR products were sequenced using the Sanger method in the genomics service of the CCITUB (Genómica, Parc Científic, Barcelona, Spain). The labeling kit used was BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher), detection and electrophoresis were carried out on the 3730 Genetic Analyzer and 3730xl Genetic Analyzer (ThermoFisher) automatic capillary sequencers models. The design of the primers was based on the consensus sequence ENSG00000134569 (LRP4; GRCh37.p13). Their sequence is presented in table 1.

**Bioinformatic analysis and in silico predictions of the effect of the variants**

To identify and characterize all the variants, we have used information culled from the Ensembl database GRCh37.p13 and ENCODE. The minor allele frequency (MAF) of each of the variants is extracted from the non-Finnish European population of gnomAD v2.1.1. We have used SIFT, (http://sift.bioc enforcing SG/edu.sg/) and PolyPhen (http://genetics.bwh.harvard.edu/pph2/) to test the effect of the change variants of amino acid (missense). The GTEx database (www.gtexportal.org/home/) has been used to identify variants that act as eQTLs.

**RESULTS**

Re-sequencing of LRP4 in women with HBM phenotype and in patients with CMI

In the LRP4 re-sequencing have identified 12 variants, of which one was not previously described (c.3364+16A>C; table 2). This variant has been found in heterozygosity in the HBM woman who presents a Z-score sum (lumbar spine and femoral neck) of 4.6. Six of the 12 variants identified are found both in the cohort of women with the HBM phenotype and in the cohort of patients with CMI, with a similar frequency in both cohorts to that of the European population. Furthermore, we have found 4 variants only present in the HBM cohort and 2 variants only present in the CMI cohort. All the variants described, except rs558515201 and the variant c.3364+16A>C, appear as eQTL of different genes and tissues in the GTEx database. Specifically, the rs17790156, rs61898529, rs2306028, rs964551 and rs2306032 variants are LRP4 eQTL.

**DISCUSSION**

Different studies have highlighted the role of the LRP4 gene in determining BMD, as mutations in it generate a phenotype of bone overgrowth. Furthermore, it has also been associated with CMI in a report on whole exome sequencing. In the study presented here, we have re-sequenced the regions of the gene that contain mutations associated with different conditions. In it, we have only found a missense mutation in LRP4 in a patient with CMI that does not co-aggregate with the phenotype in the family, thus ruling out its ability to cause the disease (Figure 1). It is interesting to note that we have found a variant not previously described (c.3364+16A>G) and the variant rs558515201, which has a very low frequency in the European population (MAF=0.00006480), in heterozygosity in two women with HBM. Furthermore, the rs3751097, rs340384558 variants, present in both HBM and CMI patients, are found in a cis regulatory element categorized as a distal enhancer-like signature by ENCODE. On the other hand, contrary to expectations, we found in both cohorts a slightly lesser frequency for the minor allele of the rs2306032 variant.
Table 1. Primers used in *LRP4* re-sequencing

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP4_Frag1</td>
<td>GGGCTTTAAGTCAGGCTTCC</td>
<td>CAACCCAACAGCCTGAGGT</td>
</tr>
<tr>
<td>LRP4_Frag2</td>
<td>GAGTGGGGAGGACAGACAGAG</td>
<td>TGGCAACACACTGGGCTTATT</td>
</tr>
<tr>
<td>LRP4_Frag3</td>
<td>ATAGTGCTGGGCCAAAAAA</td>
<td>GGGCACGCTACACACTTT</td>
</tr>
<tr>
<td>LRP4_Frag4</td>
<td>TGGCCTAAAATTATGGTGGC</td>
<td>ACCACTGGTATTGGGCTCC</td>
</tr>
<tr>
<td>LRP4_Frag5</td>
<td>AGTGGAGAGCTGCTTTTCTG</td>
<td>CCTCTGGAAGGAAGGAGA</td>
</tr>
<tr>
<td>LRP4_Frag6</td>
<td>TGGTTTTCTTTGATTTTCTT</td>
<td>GATGGAAGCTCTCTCACC</td>
</tr>
<tr>
<td>LRP4_Frag7</td>
<td>AAGCTGAGATAATGCACAGAA</td>
<td>ACAGTCACGGCTTTTTCAG</td>
</tr>
</tbody>
</table>

Table 2. Variants found in *LRP4* re-sequencing in women with HBM phenotype and in patients with CMI. The genotypes in the first column are indicated by the nucleotides of the coding strand of the *LRP4* gene, which is the reverse of that which is used in a standard way to indicate genomic SNVs. For this reason, for example, the A allele of c.431-12G>A is equivalent to the T allele of rs139371503, and the same for the other variants

<table>
<thead>
<tr>
<th>LRP4</th>
<th>Number rs</th>
<th>Type</th>
<th>MAF</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EUR</td>
<td>HBM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(T)</td>
<td>(T)</td>
</tr>
<tr>
<td>c.431-12G&gt;A</td>
<td>rs139371503</td>
<td>I</td>
<td>0.011</td>
<td>0.05</td>
</tr>
<tr>
<td>c.1309+24G&gt;A</td>
<td>rs3751097</td>
<td>I</td>
<td>0.101</td>
<td>0.2</td>
</tr>
<tr>
<td>c.1309+87_1309+91dup</td>
<td>rs540384558</td>
<td>I</td>
<td>0.038</td>
<td>0.05 (dup)</td>
</tr>
<tr>
<td>p.Asn501His</td>
<td>rs72897663</td>
<td>M</td>
<td>0.044</td>
<td>(G)</td>
</tr>
<tr>
<td>p.Lys546=</td>
<td>rs10838631</td>
<td>S</td>
<td>0.011</td>
<td>0.05 (T)</td>
</tr>
<tr>
<td>c.2507-73C&gt;T</td>
<td>rs558515201</td>
<td>I</td>
<td>&lt;0.01</td>
<td>(A)</td>
</tr>
<tr>
<td>c.2507-204C&gt;T</td>
<td>rs61898529</td>
<td>I</td>
<td>0.093</td>
<td>(A)</td>
</tr>
<tr>
<td>c.2612+104T&gt;A</td>
<td>rs17790156</td>
<td>I</td>
<td>0.09436</td>
<td>(T)</td>
</tr>
<tr>
<td>c.3004+18C&gt;A</td>
<td>rs2306028</td>
<td>I</td>
<td>0.127</td>
<td>(T)</td>
</tr>
<tr>
<td>c.3364+16A&gt;C</td>
<td>-</td>
<td>I</td>
<td>-</td>
<td>0.05 (C)</td>
</tr>
<tr>
<td>c.3536+22C&gt;A</td>
<td>rs964551</td>
<td>I</td>
<td>0.227</td>
<td>(G)</td>
</tr>
<tr>
<td>c.3700-21C&gt;G</td>
<td>rs2306032</td>
<td>I</td>
<td>0.329</td>
<td>(G)</td>
</tr>
</tbody>
</table>

MAF: minority allele frequency; EUR: non-Finish European population of gnomAD v2.1.1.; HBM: high bone mass phenotype; CMI: Chiari type I malformation; dup: duplication; M: missense variant; I: intronic variant; S: synonymous variant; eQTL: described as eQTL in GTex; T: tolerated by SIFT; B: benign by Polyphen-2.
which has been defined as a protector in whole genome association study with total BMD.

The low number of patients must be taken into account as a limitation of our study. This could explain both the absence of causal variants in the LRP4 gene in these two cohorts and the results contrary to those expected in allele frequency in the SNP rs2306032. Thus, increasing the size of the cohorts would provide us with a deeper understanding of the role of LRP4 on these phenotypes.

In conclusion, although LRP4 is undoubtedly an important gene for bone biology, we have not found any variant that could explain the HBM or CMI phenotype. Despite these negative results, it is interesting to consider LRP4 in the selection of candidate genes that may explain HBM or CMI-causing phenotypes.

**Figure 1. Pedigree of the family of the CMI CH10 patient.** NA: no data on the parent. Genotypes are indicated with the nucleotides of the coding strand of the LRP4 gene, which is the reverse of that used in a standard way to indicate genomic SNVs. Therefore, allele C is equivalent to allele G of rs72897663.

**Conflict of interests:** The authors declare no conflict of interest.
The cut-out phenomenon in intertrochanteric femur fracture: analysis using a finite element model

DOI: http://dx.doi.org/10.4321/S1889-836X2021000100005

Arias-Blanco A1, Marco M1, Giner E2, Miguélez MH1, Caeiro-Rey JR3, Larraínzar-Garijo R4
1 Department of Mechanical Engineering. University Carlos III of Madrid. Madrid (Spain)
2 Research Center in Mechanical Engineering (CIIM) - Department of Mechanical and Materials Engineering. Polytechnic university of Valencia. Valencia (Spain)
3 Department of Orthopedic Surgery and Traumatology. University Hospital Complex of Santiago de Compostela. A Coruña (Spain)
4 Traumatology Service. Infanta Leonor University Hospital. Complutense University of Madrid (Spain)

Summary

Objective: This work aimed to analyze the cut‐out phenomenon, which involves oblique displacements and/or rotations of the femoral head around the cephalic component of the intramedullary nail. The analysis was carried out using finite element numerical models. This technique seeks to understand the failure of this type of fixation and establish what positioning of the system favors or prevents failure due to cut-out.

Material and methods: The study was carried out on a numerical model of the proximal limb of an artificial femur and an intramedullary nail type PFNA (proximal femoral nail anti-rotation). In the numerical model, the position of the intramedullary nail was varied in the anterior/posterior and superior/inferior directions to analyze the influence of the position on the cut-out phenomenon. Stresses in critical areas and torque on the nail under normal position loading were analyzed.

Results: The most critical position was the one in which the intramedullary nail is placed in the superior position, due to the high compressions that appear in the trabecular bone of the femoral head. The centered position of the nail decreased the risk of bone damage and the torque that the intramedullary nail has to support.

Conclusions: This type of model allows us to simulate the influence of the nail position and obtain variables that are otherwise difficult to analyze. Although it is a simple model with static load, it confirms that a centered position of the intramedullary nail reduces the risk of cut-out.

Key words: femur, hip fracture, extracapsular fracture, intramedullary nail, cut-out, finite element model.

INTRODUCTION

Proximal extremity fractures of the femur are a very common problem in today's society and of great importance as there has been an increased incidence in the population. This increase is explained by the longer life expectancy in recent years, thus increasing the elderly population and, therefore, related diseases. This is particularly relevant in Spain which has, of late, seen a severe aging of the population.1

Several epidemiological studies describe the incidence of hip fracture in Spain. In most cases these are local studies and carried out over short periods of time. National studies have been carried out, although to a lesser extent.2 According to the Ministry of Health and Social Policy’s 2010 report “Hip fracture care in the hospitals of the National Health System”,3 a total of 487,973 cases of fracture were recorded between 1997 and 2008. In these figures and in those carried out in various local studies,4 a predominance of cases in the female sex and an increase in the incidence in age over the years has been found.

Date of receipt: 01/12/2020 - Date of acceptance: 15/03/2021

This study was funded by a 2018 FEIOMM Basic Research Grant
In addition to the large number of cases, it is worth noting the high in-hospital mortality rate (4.71-5.85%) and one year after the intervention (25-33%)\(^4\), and the fact that one in five patients will need permanent social and health care\(^2\). That is why proximal femur fractures pose a challenge that must be studied in depth.

Once the fracture has occurred, treatment, in most cases of extracapsular fractures, consists of internal fixation of the fragments using different osteosynthesis devices, including intramedullary nails.

The use of these mechanical fixations implies a series of complications that can appear after the intervention. There are two main phenomena that can lead to the failure of the fixation devices\(^5\): the mechanical failure of the fixation device itself and the so-called cut-out. The latter is defined as the collapse of the femoral neck, giving rise to an oblique displacement and/or rotation of the femoral head, thus causing damage to the trabecular bone, and facilitating the displacement of the cephalic screw\(^6\). An example of a cut-out failure is shown in figure 1.

The observed incidence is much higher in the case of cut-out. Caruso et al. report a 5.6% incidence of cut-out\(^8\). Wadhwani et al. also conclude that this phenomenon is the most common among the major complications in these interventions\(^9\).

Numerous studies report on the cut-out phenomenon. The most relevant for this work are those that analyze its incidence\(^9\) or the importance of nail position, either clinically\(^10\) or by means of numerical finite element models\(^11\). Other authors, such as Lenich et al., have developed mechanical systems to evaluate the mechanical behavior of the femur-nail structure and thus analyze the failure mechanisms that occur in it under fatigue tests\(^12\).

In this work, the study of the cut-out was approached using the finite element method. With this method, it is possible to analyze the efforts and displacements suffered by a femur due to an external load and under realistic conditions\(^13\), whether in artificial\(^14\) or human femurs\(^15\). In this case, an artificial femur was simulated, in which a 31A1 intertrochanteric fracture was generated numerically according to the AO/OTA\(^16\) classification, which would be treated with an intramedullary nail. The study on an artificial femur was chosen because it has already been characterized by several authors, who found that its behavior was very similar to the real human femur\(^17\)-\(^19\). In addition, the numerical model is easier to analyze, since it only consists of two clearly differentiated materials, thus avoiding geometric effects regarding real human femurs.

From these tests, in which different positions of the intramedullary nail were simulated, the variation of a series of parameters, such as global stiffness of the femur, tensions and torque, was studied and they were related to the risk of failure due to cut-out.

**Figure 1.** Intramedullary nail implanted as treatment for a 31A3 fracture according to the AO/OTA classification. Healthy specimen (left), fixation device failure due to cut-out (right)
MATERIAL AND METHOD

For the numerical modeling, an artificial femur (Model No. 3406, Sawbones, Pacific Research Laboratories Inc., Vashon, USA) was used, which is made up of two clearly differentiated materials that simulate trabecular bone and cortical bone (Figure 2a). Regarding the intramedullary nail, a PFNA model (Synthes GmbH, Oberdorf, Switzerland) has been used (Figure 2b).

Obtaining the geometry of the artificial femur from a scanner

To obtain the geometry of this femur with sufficient precision, a computerized axial tomography (CT) was chosen to generate it. The scanner was performed on a Somatom model, SIEMENS, with a resolution of 0.44 mm in the transverse plane and a thickness of 1 mm in the sections. Using this scanner, it is possible to generate the geometry of the femur and differentiate its two materials (cortical bone and trabecular bone) due to their difference in densities. Subsequently, by means of image segmentation and taking into account the different gray scales (Figure 3a), the geometry shown in figure 3b is obtained, consisting of the cortical and trabecular bone.

Generation of the CAD model of the intramedullary nail

In this case, the model was generated using Solid Edge 2019 software. For this, on a real PFNA-type intramedullary nail, the appropriate measurements were taken to obtain maximum precision in the geometric model. Figure 3c shows the geometric pattern of the nail.

Behavior of the materials to be used

The model consists of an artificial femur (trabecular bone and cortical bone) and a titanium alloy corresponding to the intramedullary nail.

a) Artificial femur

The artificial femur, as previously explained, is made up of two well differentiated regions, one corresponding to the trabecular bone and the other to the cortical bone. In the case of trabecular tissue, it is a rigid foam with properties similar to those of trabecular bone, in this case it is an isotropic material.

For cortical bone, a more complex material is used, a mixture of short glass fiber and epoxy resin, in this case being a composite material with different properties in different directions, that is, an orthotropic material. Since a main direction of the fibers is not shown and taking into account previous works\(^{14}\), the material is treated as isotropic, since it is more similar to its reality.

Regarding the material model adopted in the artificial femur, a linear elastic behavior was assumed. It is true that, in reality, human bone has an elastic regime and a plastic regime, and some authors have taken this into account when making numerical models\(^{20,21}\). However, in many other cases the femur has been analyzed as a linear elastic material until failure\(^{20,22,24}\). In this case, since it would work with relatively low loads that would not subject the bone to a critical state, a linear elastic model was considered valid. The properties used in this work for the artificial femur were those obtained experimentally by Marco et al.\(^{14}\) shown in table 1.

b) Intramedullary nail

All the components of the intramedullary nail are made of the Ti6Al7Nb\(^{25}\) alloy, which was modeled as an isotropic material and with a linear elastic behavior up to the elastic limit. The properties of this alloy are shown in table 2.

Meshing

The mesh used in the finite element models is formed by quadratic tetrahedral elements (code C3D10 in Abaqus) with a side of approximately 2 mm. The size of the element in the intramedullary nail is about 1.5 mm per side. These element sizes have been established through a mesh sensitivity analysis, reaching minimal variations between consecutive element sizes. Figure 4 shows the femur with the intramedullary nail and the intertrochanteric fracture modeled on the finite element mesh, to reproduce the real behavior of the specimen. The fracture was artificially generated, although there are also different numerical methods to simulate the initial fracture and its propagation\(^ {15,27}\).

Loading conditions

In this case, the scenario considered was that of an individual in an orthostatic position (standing and erect position), thus considering only the action of the individual's own weight on the femur. In the femur there are also the loads exerted by the muscles that are acting on it, such as the gluteus or the psoas. However, for the case of the study in which the magnitudes of interest are stresses and strains, as demonstrated by Cristofolini et al.\(^ {22}\), it was not strictly necessary to include the action of the muscles. In this model, a static load was analyzed to simplify the analysis, in which there is no movement of the patient, although this could induce critical loads in the femur that would be of interest.
The numerical value of the load was 75% of the weight of an average person, which is equivalent to 551 N for a 75 kg individual. The application of the same was carried out with an inclination of 8° with respect to the vertical and on a surface that simulates the region of contact of the femoral head with the acetabulum of the pelvis (Figure 5a).

Regarding the contour conditions, the lower region of the proximal femur was fixed so that it did not suffer displacement, as shown in figure 5b. These conditions are similar to the usual ones used in experimental tests in the proximal femur, in which the lower area is embedded in surgical cement14.

Placement of the intramedullary nail in the artificial femur: Study positions
In this work, the influence of the intramedullary nail position was analyzed. For this, a central-central position of the intramedullary nail was used as a reference, and its position was varied ±5 mm in the coronal and sagittal directions (Figure 6):
- Reference position: location of the intramedullary nail taken as reference (Ref.).
- 5 mm displacement in a posterior direction in the sagittal plane (SagPos5).
- 5 mm displacement in the anterior direction in the sagittal plane (SagAnt5).

Table 1. Mechanical properties of the synthetic femur14

<table>
<thead>
<tr>
<th></th>
<th>Trabecular bone</th>
<th>Cortical bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density, ( \rho ) (g/cm³)</td>
<td>0.27</td>
<td>1.64</td>
</tr>
<tr>
<td>Young’s modulus, ( E ) (MPa)</td>
<td>155</td>
<td>10,400</td>
</tr>
<tr>
<td>Poisson’s coefficient, ( \nu )</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Maximum compressive stress, ( \sigma_{\text{ult}} ) (MPa)</td>
<td>157</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. Properties of the Ti6Al7Nb alloy26

<table>
<thead>
<tr>
<th></th>
<th>Ti6Al7Nb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density, ( \rho ) (g/cm³)</td>
<td>4.52</td>
</tr>
<tr>
<td>Young’s modulus, ( E ) (MPa)</td>
<td>105,000</td>
</tr>
<tr>
<td>Poisson’s coefficient, ( \nu )</td>
<td>0.36</td>
</tr>
<tr>
<td>Yield strength, ( \sigma_y ) (MPa)</td>
<td>900</td>
</tr>
</tbody>
</table>
The cut-out phenomenon in intertrochanteric femur fracture: analysis using a finite element model


- 5 mm displacement in a superior direction in the coronal plane (CorSup5).
- 5 mm displacement in the inferior direction in the coronal plane (CorInf5).

In order to evaluate the possibility of failure due to cut-out that each of the configurations would present, a radiographic parameter that measured this risk was evaluated. These types of parameters are usually based on geometric relationships relative to the position of the intramedullary nail relative to the femur. In this case, the Parker parameter was chosen, defined as:

\[ PR = \frac{ab}{ac} \]

where \( ab \) and \( ac \) are the dimensions shown in figure 7, both in the anteroposterior radiograph (Figure 7a) and in the lateral (Figure 7b).

Table 3 shows the Parker parameter values for the different positions studied. As can be seen, the reference position showed a parameter close to 50%, while in the rest of the positions the Parker parameters deviated from this value.

Parameters to be analyzed in the model

Thanks to the finite element model, it is possible to analyze a large number of variables that can help us understand the cut-out phenomenon and what factors contribute to it. The results under study were:
- Maximum von Mises stress in the fixation device (\( \sigma_{\text{Mises}} \)). This parameter establishes how critical the conditions are for fixation, indicating the possibility that the nail may break.
- Minimal principal stress on the trabecular bone of the femoral head (\( \sigma_{\text{min.ppa}} \)). This variable determines the compression suffered by the trabecular bone due to the pressure exerted by the intramedullary nail. The higher this value, the greater the potential for small trabecular breaks, leading to nail-to-bone clearance and reducing fixation.
- Global stiffness of the femur. The overall stiffness of the femur may be affected by the inclusion of the intramedullary nail and the position of the nail. This parameter indicates the displacement suffered by a structure due to a given load, the higher the stiffness, the lower the displacement.
- Torsional moment experienced by the cephalic screw (\( \tau \)). It indicates the rotational force that the lag screw is undergoing due to the union that exists with the trabecular bone. The greater this torque, the more likely that the fixation will not be able to hold the femoral head in position and will cause the head to rotate over the nail.

Numerical model of damaged femur

Finally, a numerical model was developed that simulated the mechanical behavior of a human femur with areas already damaged due to the initial stages of the cut-out. In this model, the trabecular bone in the area superior to the lag screw would be microstructurally damaged, in such a way that it could not properly support the loads to which it was subjected. This damage was simulated by reducing the stiffness of the material in that area to 1% of the initial value. This would correspond to the early stages of cut-out failure, in which the trabecular bone is slightly damaged, such that the lag screw of the intramedullary nail is not able to properly fixate to the femoral head.

Results

Table 4 shows the parameters discussed in the previous section for each of the finite element models developed in this work.

Figure 5. Boundary forces and conditions in the femur. a) Region of application of the load simulating the contact area of the femoral head with the acetabulum of the pelvis. b) Contour conditions, angle of inclination of the load and fixation of the lower region of the proximal femur
Von Mises maximum stress
Considering the maximum von Mises tension in the fixation device, it was obtained that said tension was always located in the region where the intramedullary nail and the lag screw are connected (Figure 8). In this coincidence zone, a stress concentration was obtained and, in addition, the bending generated by the load in this region was also maximum. When the position of the intramedullary nail was lowered 5 mm in the coronal plane (CorInf5) a significant increase in tension was obtained with respect to the reference position. In all models the tension remained well below the elastic limit of the intramedullary nail material throughout ($\sigma_y = 900$ Mpa). From the latter, it would seem the intramedullary nail does not undergo critical stresses under normal load conditions. Furthermore, variations in position do not lead to its failure.

Minimal principal stress in trabecular bone
Continuing with the minimum principal stress in the femoral head, $\sigma_{\text{min.pupal}}$ figure 9 shows these stresses in the different positions studied. In this case, only the area of the trabecular bone above the lag screw is of interest. In all cases, the greatest compression (negative values imply greater compression) was found to take place at the end of the lag screw, since due to the applied load there was a compression between the load zone and the end of the screw. This compression was especially pronounced in the CorSup5 position. So, by positioning the intramedullary nail 5 mm in a superior direction in the coronal plane, the mass of trabecular bone tissue between the cortical bone and the cephalic screw is reduced, thus increasing its compression of this trabecular bone tissue area, which cannot adequately distribute the received load. This was also corroborated by other finite element models, such as the one carried out by Goffin et al.11, in which a positioning in the superior direction reportedly increased the compression in the trabecular bone and, consequently, the related damage.

Global stiffness of the femur
Regarding the global stiffness of the femur, it was not found to be significantly affected by varying the intramedullary nail position. This implied that, in the femur fixed by the intramedullary nail, analyzed as a structure in a global way, the variation in the position of the screw did not significantly affect the overall rigidity of the screw.

Torque
Finally, considering the torque experienced by the lag screw, a special increase was observed in the SagPos5 and CorInf5 positions. In the case of the SagPos5 position, based on figure 6, a reported increase in the eccentricity of the cephalic screw with respect to the center of the femoral head contributed to the increase in torsional moment. In the case of the CorInf5 position, the descent of the screw caused an instability in the fixation, which implied an increase in torque and, therefore, an increase in the possibility of rotation of the femoral head on the screw. In the case of the SagAnt5 position, the torque was drastically reduced, and this was due to the asymmetric geometry of the femur. Thanks to this asymmetry, this variation in screw position favored screw stability, although in this case a static load centered in the sagittal plane was being analyzed.

Figure 6. Study positions of the intramedullary nail. Trabecular bone tissue is shown in pink, cortical bone gray and intramedullary nail in gold. For each of the positions, the cross-sectional view is shown on the left and the coronal view is shown on the right.
Relationship with the RP parameter
The values of 46.47%±9.48 for the case of the antero‐posterior RP and of 53.38%±10.00 for the lateral RP obtained from Andruszkow et al.29 were taken as reference, and it was considered that the risk of cut‐out is increased both by increasing and decreasing these values.

The PR parameters studied and their relationship with the cut‐out were compared with the values presented in tables 3 and 4.

Comparing the results shown in table 3 with those mentioned in the previous paragraph, it was noticed that the position CorInf5 is the one with values closest to these. Therefore, this was later taken as the new reference position. The more the values of the rest of the positions differed with respect to this, the greater risk of cut‐out it was considered that they would entail.

Starting with the anteroposterior RP, it was noted that as it descended there was an increase in the von Mises tension experienced by the intramedullary nail and, on the contrary, when it increased there was an increase in the compression experienced by the trabecular bone tissue. No clear trends were observed between the anteroposterior RP and the torque experienced by the lag screw.

Considering the lateral RP only, it was found that its increase led to an increase in the torque experienced by the lag screw.

On the other hand, a relationship between global stiffness and Parker parameters was not observed.

Numerical model of damaged femur
Finally, the model that simulated the mechanical behavior of the femur in the early stages of cut‐out damage is presented. Figure 10 shows the results relative to the minimum principal deformation (equivalent to the highest compression values in terms of deformation) and the field of displacements in a global way. Figure 10a shows the area of trabecular bone that has been numerically damaged, in the upper part of the lag screw. This area could not support the load correctly. Therefore, it suffered large compression deformations, which differed from those obtained around it. Figure 10b shows the field of displacements in the trabecular bone. In this case, the damaged model suffered a maximum displacement in the area of the femoral head 0.02 mm greater than the healthy femur. This difference is small, although it can be critical and lead to greater damage in nearby areas. In this case, a small trabecular bone damaged area was simulated, hence the variation in displacements was not greater.

Table 3. Parker parameters of the studied positions

<table>
<thead>
<tr>
<th>Position</th>
<th>Anteroposterior PR [%]</th>
<th>Lateral PR [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref.</td>
<td>56.8</td>
<td>53.8</td>
</tr>
<tr>
<td>SagPosS</td>
<td>55.9</td>
<td>65.4</td>
</tr>
<tr>
<td>SagAntS</td>
<td>56.5</td>
<td>45.5</td>
</tr>
<tr>
<td>CorSupS</td>
<td>63.8</td>
<td>57.0</td>
</tr>
<tr>
<td>CorInfS</td>
<td>48.0</td>
<td>55.7</td>
</tr>
</tbody>
</table>
Based on the conclusions drawn from this work and corroborating them with the work of Lenich et al., it is quite evident that the best position that favors the biomechanics of the femur with intramedullary nail fixation is the one in which the screw is in the position center with respect to the two planes of the femoral neck. Lenich et al. suggested that this positioning minimizes the effect of possible rotations that may appear between the femoral head and the screw.

Considering the ranges of the anteroposterior and lateral RP values for which there is a risk of failure due to cut-out, Parker establishes that the anteroposterior RP value for the cases without incidents was 45%, while for the failure cases per cut-out was 58%. In the cases of lateral RP, they were 45% and 36%, respectively.

Andruszkow et al. distinguishes between fractures treated with sliding screws and with intramedullary nails, in addition to differentiating the type of fracture according to the AO/OTA classification. In this study, a mean of 46.47%±9.48 was obtained in the case of anteroposterior RP and 53.38%±10.00 for lateral RP in AO/OTA 31A1 fractures treated with an intramedullary nail. There has been no failure due to cut-out, and no case of failure due to cut-out has been reported in this type of fractures treated with these fixation devices.

There is a discrepancy on whether the risk of cut-out increases or decreases by increasing or decreasing this parameter. For example, Parker established that the risk of cut-out increased when the fixation device tended to be positioned more towards the posterior direction, which is equivalent to increasing the lateral PR value. However, Baumgaertner et al. stated that the risk of cut-out increased when the fixation device was positioned more in the anterior direction.

**Figure 8. Zone of maximum von Mises tension in the intramedullary nail. Position: Ref. Values in MPa**

![Image of Zone of maximum von Mises tension in the intramedullary nail.](image)

**Figure 9. Minimal principal stress on the femoral head. Values in MPa**

![Image of Minimal principal stress on the femoral head.](image)
Other studies based on finite element models, such as that by Goffin et al., opt for a screw position in the lower zone, to minimize damage to the trabecular bone. This conclusion may be valid in simple models in which the load is fixed, but in the real biomechanics of the hip, a position different from the central-central position can lead to high torsional torques at certain moments of gait or other positions, resulting in rotations of the femoral head that can damage the area around the screw. The numerical model of Goffin et al. did not consider the torque depending on the position, a parameter that in this work we consider relevant in the analysis of this phenomenon.

Although an artificial femur was analyzed in this work, these have been widely studied in the literature, and their mechanical behavior is similar to that of human femurs, as Cristofolini et al. of static numerical models, in which variations in the load position and dynamic effects are not taken into account. In the near future, it is expected that the numerical model will be improved and will be able to include these aspects.

**CONCLUSIONS**

The main conclusions obtained in this work are the following:

- The low von Mises stresses experienced in the fixation device in relation to its elastic limit explain the low incidence of failures in these compared to failures by cut-out, therefore, the position of the screw does not affect at any time the integrity of the nail.

- Considering the sagittal plane, cut-out failure is more likely when the nail is displaced in both the anterior and posterior directions, with movements in the posterior direction being accompanied by an increase in the torsional

---

**Table 4. Parameters obtained in the different models using finite element models**

<table>
<thead>
<tr>
<th>Position</th>
<th>$\sigma_{\text{Mises}}$ [MPa]</th>
<th>$\sigma_{\text{min.ppal}}$ [MPa]</th>
<th>Global stiffness [N/mm]</th>
<th>$T_t$ [N-mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref.</td>
<td>240</td>
<td>-2.6</td>
<td>936</td>
<td>670</td>
</tr>
<tr>
<td>SagPos5</td>
<td>187</td>
<td>-1.5</td>
<td>914</td>
<td>1,147</td>
</tr>
<tr>
<td>SagAnt5</td>
<td>219</td>
<td>-2.3</td>
<td>928</td>
<td>74</td>
</tr>
<tr>
<td>CorSup5</td>
<td>183</td>
<td>-4.1</td>
<td>988</td>
<td>267</td>
</tr>
<tr>
<td>CorInf5</td>
<td>326</td>
<td>-2.3</td>
<td>911</td>
<td>962</td>
</tr>
</tbody>
</table>
moment experienced by the lag screw. This implies that the nail cannot fix the femoral head, leading to its rotation.

Considering the coronal plane, cut-out failure will tend to occur when the intramedullary nail is displaced in the superior direction, a failure related to compression of the trabecular bone tissue. Offsets in the lower direction would help avoid this failure, but would lead to a higher load on the fixture and increased torque under realistic biomechanical conditions.

Taking into account the actual biomechanics of the hip and its rotations in activities of daily living, the safest position of the cephalic component in rotationally unstable fractures is center-center.

Using numerical models, it is possible to simulate the first stages of cut-out, in which a damaged area can give rise to small displacements, which may later increase the damage, finally triggering the aforementioned phenomenon.

Acknowledgments: The authors are grateful for the funding received through the 2018 Spanish Society for Bone Research and Mineral Metabolism research grant. They also appreciate the Ministry of Science and Innovation funding and the ERDF Program through the projects DPI2017-89197-C2-1-R and DPI2017-89197-C2-2-R.

Conflict of interests: The authors declare that they have no conflict of interest in relation to this work.
1. Instituto Nacional de Estadística. Índice de natalidad por ciudad. 2010.


Pathophysiology of osteoporosis in chronic inflammatory joint diseases

DOI: http://dx.doi.org/10.4321/S1889-836X2021000100006

Castañeda S1,2, García-Puentes MV3, Bernad Pineda M4
1 Rheumatology Department. La Princesa University Hospital. Princesa Health Research Institute (IIS-Princesa). Madrid (Spain)
2 UAM-Roche, EPID*-Future (*Diffuse Interstitial Lung Diseases). Autonomous University of Madrid (UAM). Madrid (Spain)
3 Comprehensive Services for Health Management (SIGEMED). Madrid (Spain)
4 Rheumatology Department. La Paz University Hospital. La Paz University Hospital’s Research Institute (IdiPAZ). Madrid (Spain)

Summary
The immune system and the bone often share the same anatomical niches and spaces, as there is a close functional relationship between both of them. As a consequence, there is a constant interaction between them and a bidirectional flow of information between the immune cells and those of the bone tissue (osteoclasts, osteoblasts and osteocytes) often unknown, in which multiple inflammatory mediators and various growth and cell differentiation factors are involved. This leads to a very close interaction between inflammation and bone loss. In fact, osteoporosis (OP) is one of the most frequent systemic complications in chronic inflammatory diseases (CIDs). The prevalence of OP in CIDs depends on each pathological scenario. Rheumatoid arthritis (RA) is a paradigmatic disease which causes chronic inflammation, where the presence of OP is frequent and shows even prior to the appearance of the first symptoms of the RA. The pathogenesis of RA-associated OP is complex and includes the cooperation of multiple pro-inflammatory cytokines that promote osteoclastogenesis and inhibit bone formation. Tumor necrosis factor alpha (TNF-α) and different interleukins (IL), such as IL-1, IL-6 and IL-17, stand out among all, IL-6 having a relevant hierarchical role. In this study, we review the role of pro-inflammatory cytokines in bone and joint destruction in different CIDs, giving special emphasis to RA, as we set out the bases of possible pathways that open new therapeutic horizons in their framework.

Key words: osteoporosis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, pathophysiology, interleukins, IL-6, treatment.

CHRONIC INFLAMMATORY DISEASES
Chronic inflammation is a nonspecific response against aggressor agents mediated by the body's immune system. In such a scenario, an infiltrate of predominantly mononuclear cells, such as lymphocytes, macrophages and plasma cells, is produced. Under certain conditions or when the aggressor agent persists, a sustainable accumulation and activation of immune cells occurs. Then, the secretion of cytokines, agents that prolong the life of lymphocytes and macrophages, is increased, what leads to chronic inflammation.

Inflammation is the main mechanism involved in bone destruction in chronic inflammatory diseases (CIDs)1, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA), systemic lupus erythematosus (SLE), multiple sclerosis and/or inflammatory bowel disease (IBD). These diseases show a chronic systemic inflammation that can affect different organs, caused by an alteration of the immune system2.

One of the characteristics of CIDs is the common symptoms that patients present: malaise, fatigue, daytime sleepiness, weakness, nonspecific arthromyalgia, hyporexia, anxiety and low mood2.

Inflammatory joint diseases encompass more than 100 different and heterogeneous disorders that affect the joints and cause disability. However, RA and spondyloarthritis (SpA: AS, reactive arthritis, PsA and SpA associated with IBD) are the most frequent1.

RA is an autoimmune disease considered the prototype of destructive inflammatory arthritis and characterized by chronic inflammation of the synovium in multiple joints and tendon sheaths. The synovial membrane is the target organ where the immune system interferes with bone homeostasis, producing severe structural damage and bone destruction there where joint and peri-articular inflammation exist4,5. In fact, in patients with inflammatory rheumatic diseases, bone destruction occurs together with erosions, periarticular osteopenia and/or generalized osteoporosis (OP)1,4.
The cause of RA-associated OP has its origin in an alteration of bone remodeling, which is the common pathophysiological mechanism of both diseases. The loss of bone mass in RA can be periarticular or generalized. Periarticular loss, commonly called juxta-articular OP, affects the trabecular and cortical bone and is one of the first radiological manifestations. It can precede both the appearance of erosions and damage to the joint space, and is easily detected on X-rays of the hands. Accelerated bone loss in the hands has been associated with the development of RA in patients suffering undifferentiated arthritis and presenting progressive joint disease in the hands and feet at the onset of RA.

Another form of RA bone loss involves erosion of the marginal bone as a consequence of inflammation of the synovial membrane. The erosion, generally irreversible, may begin before arthritis symptoms appear and is related to the severity of the disease and functional impairment. Finally, and due to autoimmune mechanisms, RA usually produces generalized bone loss (systemic OP), inclusively in those regions of the skeleton located far from the inflamed joints even in the initial stages of the disease.

The bone system and the immune system

The musculoskeletal system and the immune system closely interact in the homeostasis of hemopoietic and lymphopoietic cells, acting in the pathogenesis of CIDs-associated OP as well as in postmenopausal OP, but it remains to be explained how adaptive immune responses affect the bone tissue. However, recent evidence has revealed that the reverse is also true: bone cells regulate immune cells, a concept consistent with the established role of bone marrow in the development and homeostasis of the immune system.

Due to its anatomical characteristics, both the in and out of the bone tissue are closely related to the immune system. In the inside, in the bone marrow, hematopoiesis occurs, so bone and immune cells locally work together in an indisputable way. At the outside, the skeleton is in direct contact with the periosteum, enthesis, and juxta-articular bone, where it connects with determining structures that have a role in the joint destruction process that characterizes chronic inflammatory joint diseases (CIDs).

Likewise, the immune system and bone tissue are connected to the general circulation by nutritional and periosteal vessels that cross the cortical bone, and, within the bone compartment, this connection is produced through fibrous enthesis junctions and calcified components of cartilage and fibrocartilage.

This permanent interaction between bone and immune system is of great importance in maintaining bone homeostasis and is also key in bone pathology. Throughout adult life, bone remodeling occurs in basic multicellular units (BMUs) or bone remodeling units, where osteoclasts reabsorb a certain amount of bone, and osteoblasts form the osteoid matrix and mineralize it to fill the previously created cavity. There are osteoclasts, macrophages, preosteoblasts and osteoblasts inside BMUs that are governed by a series of factors, both general and local, and allow the normal functioning of bone tissue and the maintenance of bone mass.

Bone cells interact with the immune system cells in the bone marrow development during growth and the healing of fractures. Apart from that, osteoblasts play an important role in controlling the renewal and differentiation of hemopoietic stem cells and B cells in places close to the endosteum.

A series of substances synthesized by bone cells, immune system cells or bone marrow have an effect on bone growth and remodeling. The most important local factors are growth factors, cytokines, and bone matrix proteins (Table 1).

The musculoskeletal and the immune systems interact with each other, sharing molecules and generating a collaborative regulatory system called "osteimmune system". The most representative and well-known molecule of this system is the receptor activator of nuclear factor kappa B ligand (RANKL), which fulfills multiple functions, both under physiological conditions and in conditions as different as RA and bone metastases. Based on current evidence showing great mutual dependence, it is accepted that the relationship between bone and the immune system does not develop by accident, but as a necessary consequence of evolution.

RANKL expression in osteoblasts is stimulated by various factors or molecular mediators, such as the interleukin (IL) 1, IL-6, IL-11, IL-15, IL-17, TNF-α, prostaglandin E2, parathormone (PTH), calcitriol, interferon and glucocorticoids (GCs), and is suppressed by the transforming growth factor-β (TGF-β) (Table 1). On another note, the
osteoprotegerin (OPG) expression is stimulated by the TGF-β, bone morphogene-
tic proteins (BMPs), interferon (IFN), IL-6, IL-11 and IL-13 and is inhibited by the PTH, IL-17, calcitriol and GCs. Estrogens inhibit RANKL production and increase OPG and TGF-β secretion (Table 1).5,14,15,16. The immune and bone systems share, indeed, a wide range of regulatory mechan-
isms, and today we can assert this influence to be bidirectional, not only of
the immune system on the bone, but also in the opposite direction.6,17. In fact, in the
bone marrow microenvironment, bone cells and immune system cells are so clos-
ely located that their interaction is logical.12,

The RANK/RANKL/OPG system is the promoter of most of the factors that regu-
late bone resorption. It belongs to the group of proteins related to tumor necro-
sis factorα (TNF-α) and actively participates in the control of bone resorption and activation of osteoclasts.15. Although the RANK/RANKL/OPG pathway remains the basis for understanding the coupling of the immune system cells with those of the bone system, some research suggests that there may be additional stimuli and unique pathways that act independently or in concert with RANK.13,

Systemic inflammation in RA increases the production of inflammatory cytokines, such as TNF-α, IL-1, IL-6 and IL-17, which act on the RANK/RANKL/OPG system, activating osteoclastogenesis and increasing bone resorption, due to RANKL’s wide expression in synovial fibro-
blasts and in the T cells of inflamed joints of RA patients.17. This abnormal activation of osteoclasts in the absence of equivalent levels of osteoblastic activity results in a generalised decrease in bone mass and a higher incidence of vertebral and non-
vertebral fractures.18,19.

The immune regulation of osteoclasts is closely related to the RA pathogenesis. There is evidence that RA bone destruction is primarily caused by the increased in osteoclast activity as a result of the activation of a unique subset of T helper cells, the T helper 17 cells (Th-17). These cells have a low production of IFN gamma (IFN-γ) and are capable of causing local inflammation through the production of pro-inflammatory cytokines.20,

Mature Th-17 cells produce IL-17, IL-21, and IL-22, all of them cytokines with high pro-inflammatory activity. In RA, IL-17 produced by Th-17 cells exerts its os-

teoestrogenic effect by stimulating RANKL expression in synovial fibroblasts.21. In immune precursor cells such as macrophages, IL-17 also stimulates the production of inflammatory cytokines, including TNF-α, IL-1β and IL-6, and get in contact with the RANK of osteoclast precursors, all this leading to the differentiation of osteoclasts, provoking them to migrate towards the marginal zone where erosions begin.22,23. Furthermore, synovial cells sti-
mulated by inflammatory cytokines also produce matrix-degrading enzymes that play an important role in articular cartilage destruction.24,

### Table 1. Main mediators involved in bone remodeling

<table>
<thead>
<tr>
<th>Bone resorption stimulation factors</th>
<th>Main effects on bone tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>Osteoclast activation, Osteoblast inhibition</td>
</tr>
<tr>
<td>IL-6</td>
<td>Osteoclast activation, Osteoblast inhibition</td>
</tr>
<tr>
<td>IL-1</td>
<td>Stimulates osteoclastogenesis</td>
</tr>
<tr>
<td>IL-8</td>
<td>Stimulates osteoclastogenesis</td>
</tr>
<tr>
<td>IL-11</td>
<td>Stimulates osteoclastogenesis</td>
</tr>
<tr>
<td>RANKL</td>
<td>Osteoclast activation</td>
</tr>
<tr>
<td>IL-17</td>
<td>Osteoclast activation</td>
</tr>
<tr>
<td>IL-23</td>
<td>Osteoclast activation</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>Osteoclast activation</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Stimulates osteoclastogenesis</td>
</tr>
</tbody>
</table>

### Bone resorption inhibition factor

| IFN-gamma                          | Inhibition of osteoclasts |
| IL-2                               | Inhibition of osteoclasts |
| IL-4                               | Inhibition of osteoclasts |
| OPG                                | Inhibition of osteoclasts |

### Bone formation inhibition factor

| DKK-1                              | Inhibition of osteoblasts |
| Sclerostin                          | Inhibition of osteoblasts |
| TNF-α                              | Inhibition of osteoblasts (dual effect: they also activate osteoclasts) |
| IL-6                               | Inhibition of osteoblasts (dual effect: they also activate osteoclasts) |

DKK-1: Dickkopf-1; IFN: interferon; IL: interleukin; M-CSF: macrophage colony stimulating factor; OPG: osteoprotegerin; RANKL: receptor activator of nuclear factor kappa B ligand (NF-kB); TNF: tumor necrosis factor. Modified from ref. 5 (Lorenzo I et al. Front Med. 2020).

### ROLE OF INTERLEUKIN-6 (IL-6) IN RA AND OSTEOPOrosis

IL-6 plays an essential role in the pathophysiology of RA and associated bone destruction. Through cell signalling, which can be initiated in the cell membrane or by soluble forms of its receptor, IL-6 acts both locally, pro-
moting joint inflammation and destruction, and systemically, causing some of the extra-articular and systemic manifestations of the disease, including pain, fatigue, morning stiffness, anemia, depression, low mood and weight loss.15,21,

IL-6 actions are mediated through the interaction between its non-signalling receptor-α, the IL-6 receptor (IL-6Rα), with which it interacts first, and subsequently forms a bond with the transduction receptor of signals, the glycoprotein (gp) 130.22. IL-6Rα is expressed in hepatocytes, monocytes/macrophages, neutrophils, and some types of T cells.23

Through intracellular signalling by binding to its mem-
brane receptor or by the classical pathway, IL-6 regulates normal processes related to the immune and neuroendo-
ctrine systems, hematopoieses, bone metabolism, lipid and glucose metabolism and acute phase responses. When IL-6 binds to its soluble IL-6R receptor, it predominantly regu-
lates systemic pro-inflammatory effects, including mono-
ocyte recruitment, macrophage differentiation, and T cell recruitment and differentiation. Its bond with cell
membrane’s gp130 prolongs its life average, which is why elevated IL-6 values are observed in the serum and synovial fluid of rheumatoid patients 19.

Apart from this, IL-6 is an effective stimulator of osteoclast-induced bone resorption and is essential for the pathogenesis of bone loss in the context of chronic inflammation, as occurs in other pathologies such as IBD 22. Elevated IL-6 values in patients with RA induce an increase in osteoclastogenesis and an imbalance of bone remodeling in favor of resorption, which leads to a generalized bone mass loss and, secondarily, osteoporosis 23.

In the preclinical stage of RA, IL-6 binds to various cell lines and causes neutral protein migration to the joints, contributing to the development of chronic inflammation, impaired B and T cell differentiation, and angiogenesis. Subsequently, preosteoclasts are stimulated to produce acute phase reactants such as C-reactive protein, fibrinogen, and serum amyloid A19,20.

In summary, IL-6 is an essential mediator in the pathogenesis of RA, acting indirectly on the bone and controlling the inducing effects of TNFα and IL-1 bone resorption. IL-6 increases RANKL production, induces the RANKL mRNA expression and increases bone resorption through RANK/RANKL/OPG interaction. The resulting bone erosion and cartilage destruction, together with inflammation and thickening of the synovial membranes, cause the development of inflammatory pannus that causes irreversible damage to the joint19,21. Therefore, IL-6 inhibition is an important aspect of the treatment of RA that minimizes joint and bone damage. IL-6 inhibitors target both IL-6 ligand and IL-6R 19,23,24.

SECONDARY OSTEOPOROSIS CAUSED BY THE TREATMENT OF CIDs

The iatrogenesis produced by GCs also plays a relevant role in OP associated with CIDs 25,26. In fact, the GC treatment used in RA, IBD and in 50% of premenopausal women with SLE is the most frequent cause of secondary OP and the first cause of OP in the population under 50 years of age 27, mainly caused by the inhibition of bone formation, provoked by a decrease in the number and activity of osteoblasts and because GCs favor osteocytic apoptosis, and primarily due to abnormal osteoclastogenesis. GCs block the action of vitamin D in the absorption of calcium 22. Patients with RA have a risk of vertebral and hip fracture 2 to 3 times higher than the general population of the same age and sex 27. Furthermore, the dose and time of exposure to GC are keys to the risk of fracture 25.

In RA, the coexistence of comorbidities is frequent and is related to the disease itself, inflammatory activity or treatment, resulting in an increase in physical disability. The decrease in physical activity that sometimes leads to prolonged immobilization periods also induces bone and muscle mass losses (“typical sarcopenia in RA”).

OP TREATMENT IN PATIENTS WITH RHEUMATOID ARTHRITIS

In RA patients it is advisable to periodically evaluate the risk of fracture using fracture risk scales such as FRAX® (Fracture Risk Assessment Tool; https://www.sheffield.ac.uk/FRAX/tool.aspx?lang=sp) and/or periodic determination of bone mineral density (BMD) by dual X-ray densitometry (DXA). This recommendation is even more important in patients older than 50 years of age, suffering severe RA and/or who have received prolonged treatment with GCs 26.

The main objective of the treatment of primary OP or as comorbidity of RA is fracture prevention 28,29. The action of the treatment can be antiresorptive or bone forming. The most widely used antiresorptive drugs are oral bisphosphonates and denosumab. Teriparatide is the treatment of choice when bone-forming treatment is to be started 30.

To our knowledge, no randomized controlled trials with bisphosphonates have been published regarding patients with RA-associated OP with fracture as primary endpoint, but only regarding patients with GC-induced RA and OP 32. In these patients, bisphosphonates prevent bone loss in the lumbar spine and femoral neck and reduce the risk of vertebral fracture after 24 months of treatment, but have no effect on the prevention of non-vertebral fractures 33.

Although bisphosphonates are the first-line treatment for OP, denosumab has demonstrated its antiresorptive efficacy in patients with primary and secondary OP 34. Denosumab is a human monoclonal antibody (IgG2) that targets and binds with high affinity and specificity to RANKL, preventing the activation of its receptor, RANK, on the surface of osteoclasts and their precursors. By preventing the RANKL/RANK interaction, osteoclast formation, function, and survival are inhibited, which in turn causes decreased bone resorption in trabecular and cortical bone 34. In a randomized and controlled trial, treatment with denosumab and calcium and vitamin D supplements significantly increased lumbar spine and total hip BMD after 6 and 12 months, reducing the risk of fracture and also reducing the radiological progression of arthritis in RA patients treated with methotrexate 33.

Teriparatide acts as an anabolic drug by increasing bone formation, stimulating osteoblastogenesis and decreasing the osteoblasts and osteocytic apoptosis 32. In clinical practice, it has been observed that teriparatide treatment reflects a significant increase in BMD and a decrease in vertebral fractures in RA patients under GCs treatment 35. These results were endorsed by the same authors in an integrated analysis consisting of four observational studies under clinical practice conditions, in which a reduction in non-vertebral fracture incidence was also observed. However, these results should be viewed with caution, since they are uncontrolled studies 36.

EFFECT OF IL-6 INHIBITORY THERAPIES ON BONE LOSS DURING RA

As mentioned previously, one of the deleterious effects induced by RA chronic inflammation is the bone mass loss produced by the imbalance in bone remodeling in favor of resorption 3. Likewise, the use of GCs in RA patients for more than three months increases the bone mass loss, especially trabecular mass loss, raising vertebral and hip fracture risk 37. A more recent study concluded that the incidence of fractures in patients receiving GC treatment is even higher than is known, especially at the beginning of the treatment. Thus, an annual incidence of vertebral fracture of 5.2% was detected in patients in an early stage of the treatment, this incidence decreased to 3.2% in those under prolonged treatment 38.

Treatment of RA with IL-6 antagonists is effective in controlling inflammatory activity, since this cytokine not only causes local inflammation, but also damage to bone structures due to its ability to stimulate the expression of the RANKL and osteoclastogenesis 3. Sarilumab and
tocilizumab are two biological drugs approved in Spain for the treatment of RA. Their mechanism of action is based on blocking the IL-6 receptor.

The effect of tocilizumab and sarilumab on the biochemical markers of bone remodeling, both formation and resorption, has been analyzed in some clinical trials. In the MONARCH study, monotherapy with sarilumab compared with that with adalimumab, achieved a significantly greater reduction in the RANKL biomarker for bone resorption, and a greater increase in the markers of procollagen type 1 N-terminal propeptide (P1NP) and osteocalcin\(^39\). In this and other trials, it has been shown that the decrease in RANKL levels and in RANKL/OPG ratio begins in the early stages of the treatment (week 2 after the start of the treatment with sarilumab), and that the decrease is maintained and even progresses during the 24 weeks of the study\(^39,41\).

Tocilizumab treatment over one year has not shown some significant changes in BMD in patients with normal baseline values, but in those with osteopenia\(^42\). After two years of treatment, tocilizumab has shown a significantly increased BMD in the femoral neck in patients with positive cyclic anti-citrullinated peptide antibodies (ACPA)\(^43\). Regarding bone remodeling biomarkers, tocilizumab significantly increases bone formation, achieving a 25% reduction in the carboxy-terminal telopeptide of type I collagen (CTX-I)/osteocalcin ratio after 16 weeks of treatment\(^44\), a small decrease (<15%) in the CTX-I and cross-linked carboxy-terminal telopeptide of type I collagen resorption biomarkers generated by matrix metalloproteinases (ICTP)\(^45\) at 24 weeks, and a significant increase in osteocalcin levels in 100% of patients at the end of the 52 weeks of treatment\(^46\).

All these data together suggest that the specific blocking of IL-6 could produce a direct anti-osteoporotic effect which is added to its indirect beneficial effects, such as the clinical control of the disease activity or the reduction, and even withdrawal of the systemic inflammatory. Anti-TNF agents have also shown some efficacy in reducing systemic bone loss in RA\(^47\,48\).

SECONDARY OSTEOPOROSIS CAUSED BY ANKYLOSING SPONDYLITIS AND OTHER CIJDs

25% of patients with AS present OP and an increased risk of vertebral and non-vertebral fractures. Although bone loss depends on multiple factors, the effect of pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) on osteoclast activation appears to be one of the main ones. In advanced stages of the disease, the decrease of BMD and the occurrence of fractures are also influenced by mechanical factors due to immobility, and spine stiffness.

The assessment of BMD in AS in the spine using traditional DXA is more difficult to carry out because of the appearance of ossification/syndesmophytes, especially in late stages of the disease, which can overestimate the assessment of the subject’s calcium mineral content, although bone loss has been detected in other anatomical regions such as the hip even in the initial stages of the disease\(^47\). A decrease in bone mass has been also detected in the spine using other techniques, complementary to DXA, such as DXA assessment using lateral projection, less sensitive to artifacts\(^49\), or by applying the trabecular bone score (TBS)\(^50\), which allows visualization of the bone micro-texture of the vertebral body avoiding the addition of calcium that supposes the presence of syndesmophytes or other juxta-vertebral ossifications, and which is a good predictor of clinical vertebral fracture and major osteoporotic fracture in patients with AS, regardless of the FRAX\(^51\).

The treatment with TNF-α inhibitors, the most widely used biological treatment, in these patients, not only reduces the inflammatory activity of the disease, but also improves the quantification of remodeling biomarkers and increases BMD\(^52\,53\), although it is not yet clear that they reduce the incidence of new fractures\(^51\).

The prevalence of OP and fracture risk in patients with psoriasis and PsA is a widely debated issue, still unclear at the present time. Traditionally, there is a higher prevalence of OP in patients with psoriasis and PsA, when compared with the control population\(^52,53\). Regarding fractures, in a population-based study carried out by Ogdie et al. patients with psoriasis and PsA were reported to have a higher risk of fractures, with an adjusted hazard ratio (HR) of 1.26 (1.06-1.27) in patients with PsA, while patients with severe or severe psoriasis would have increased risk of any type of OP fracture as well as vertebral fractures: adjusted HR of 1.26 (1.15-1.39) and 2.23 (1.54-3.22), respectively\(^54\).

However, other authors do not seem to find in patients with PsA a prevalence of OP higher than in the general population, except for those presenting more severe polyarticular involvement and poorer functional grade\(^55,56\).

Finally, SLE patients, a prototype of chronic systemic autoimmune disease, also have a higher incidence of OP and fractures than the general population, due to the confluence of several factors such as: prolonged treatment with GCs, use of anticoagulants and immunosuppressants, periods of transient amenorrhea suffered by many patients with SLE in flare-ups, vitamin D deficiency\(^57\) and low physical activity, in addition to the inflammatory activity of the disease caused by various cytokines and pro-inflammatory mediators\(^58\).

CONCLUSIONS

CIJDs frequently present associated OP, although with different prevalence and severity depending on the type of underlying disease. CIJDs include RA, AS and PsA. RA is the prototypical disease that appears together with chronic inflammation and OP and, therefore, the only one included in different fracture risk assessment scales such as FRAX\(^5\). In fact, RA is a disabling disease, frequently associated with localized and generalized OP, in approximately one third of patients. The incidence of OP in RA patients depends on multiple factors, such as disease severity, age, prolonged use of corticosteroids, sarcopenia, and periods of prolonged immobilization. IL-6 is a crucial pro-inflammatory cytokine that plays a relevant role in the pathogenesis of joint inflammation and RA-associated OP. Treatment with IL-6 neutralizing agents improves both the joint and systemic symptoms, as well as the associated OP.

AS and PsA are also chronic inflammatory diseases that are associated with OP to a lesser extent, at least in its early stages, and which involved molecular mechanisms are less understood. The use of anti-TNF drugs in these patients have increased BMD and improved bone remodeling biomarkers, although their effect on fractures is more doubtful, so longitudinal clinical studies are needed to corroborate these incipient findings.
In all patients with a diagnosis of CIDs, especially RA, BMD and the risk of fractures should be early assessed, in order to start preventive treatment, at least consisting of calcium and vitamin D supplements and/or to administer a basic anti-osteoporotic treatment to try to prevent this dreaded complication, especially in cases with a higher risk of fracture.

Acknowledgments: To patients with chronic diseases, for everything they convey and teach us every day.

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

Bibliography


20. Fuentes JC, Santos MJ, Cahué H, Choy E. Interleukin-6 as a key player in systemic...


29. Leppäenaho E, Charpentier R. High fracture risk patients with glucocorticoid-induced osteoporosis should get an anabolic treatment first. Osteoporos Int. 2020;31(1):1829-34.


Unusual case of bone proliferation: Nora's lesion

We present the case of a 43-year-old man who presents pain and functional impotence in the left wrist of one year of evolution. Upon examination, an indurated tumor adhered to deep planes is found in this location. Following findings on computerized axial tomography (CT) of images consistent with osteochondroma versus peripheral chondrosarcoma (Figure 1), a bone scan was requested. This bone scintigraphic study in three phases of the upper limbs and a subsequent full-body image (Figure 2), showed the early arrival of the tracer with an increase in the vascular pool of slight-moderate intensity in the distal portion of the left radius (arrow), which persisted with greater intensity in late images. No other diseased findings were observed in the rest of the skeleton. These findings revealed increased vascularity and osteoblastic activity at the distal end of the left radius.

A biopsy was carried out, with a pathological result of osteochondromatous proliferation compatible with Nora's lesion, confirming this diagnosis after surgical resection. Nora's lesion occurs predominantly in the second or third decade of life, without gender differences, mainly affecting the extremities. Of uncertain etiology, it consists of an excretory and exophytic lesion that originates in the bone cortex, formed by bone, cartilaginous and fibrous tissue, with nuclear atypia. Bone scan allows us to know the metabolic characteristics of this lesion. Given its aggressive nature, a differential diagnosis should be made with malignant lesions such as osteosarcoma.

Correspondence: Ana Moreno-Ballesteros (anamoreno_ballesteros@hotmail.com)
Conflict of interests: The authors declare no conflict of interest.

Bibliography

Care profile of susceptible patients with osteoporosis by telemedicine visits in the post-COVID-19 era

Summary

Objective: Define the profile of the candidate and non-candidate osteoporosis patient for assistance in osteoporosis teleconsultation, in the post-COVID-19 era. Proposal of a management protocol for outpatient follow-up.

Methods: We have carried out a bibliographic review through a systematic search in the Pubmed.gov databases of the available evidence of articles in English and Spanish with an inclusion date until October 2020, following the recommendations of the GRADE system (Grading of Recommendations, Assessment, Development and Evaluation). Database is aimed at locating and accessing relevant information for this review in an updated way.

Results: The profile of the patient candidate for teleconsultation would be of those who present a history of osteoporosis, previously diagnosed, with treatment and requiring follow-up. COVID-19 has occurred in a context in which the main causes of mortality are chronic diseases and the need to protect against transmission.

Conclusions: We propose a consensus for managing this patient, with differentiated sections for the different stages of the telematic care process. This will help in clinical decision-making and also in the process of follow-up and therapeutic adherence and, therefore, in optimal use of healthcare resources.

Key words: osteoporosis, telemedicine, COVID-19, outpatient care.

INTRODUCTION

Osteoporotic fractures represent a growing public health problem worldwide. At present, we lack adequate procedures for prevention, diagnosis, assessment, intervention and patient follow-up.

New technologies have provided new communication tools and have changed our mentality, with the possibility of carrying out, virtually, actions or procedures in our daily lives that until now required our physical presence, either for technical, cultural or social reasons.

The COVID-19 pandemic, caused by the SARS-COV-2 virus, has triggered a global public health emergency with rapid evolution and tragic consequences. The fight against this disease is forcing to modify the forms of care, which includes transforming some face-to-face consultations into remote ones.

Prior to the COVID-19 pandemic, telemedicine, in its different forms, was used in exceptional situations. One of the first uses was the tele-nursing practice that emerged in the UK and Canada at a primary care level.

During the alarm or lock-down period and the ensuing care crisis caused by the pandemic, which began in March 2020, we have been forced to carry out inquiries electronically. Osteoporosis, as a chronic disease, is a health care challenge in this new situation in our consultations, which we should take advantage of to assess its benefits and be able to overcome its barriers. For this reason, the definition of a clinical profile of the patient and the creation of a care circuit are absolutely necessary to ensure the correct care of these patients. However, today, the information available on the possible benefits or safety of this type of telematic care is quite limited.
The objective of our proposal is to develop a protocol for the management of the patient in telematic follow-up, where it is essential to promote coordinated action between the different levels of care, Primary Care, Social Healthcare and Hospital, with the participation of doctors from the various specialties involved, as well as Nursing and Community Pharmacy, which facilitate detection, assessment and treatment, ensuring good adherence.

**PROFILE OF THE PATIENT WITH OSTEOPOROSIS WHO ATTENDS OUTPATIENT CONSULTATIONS FOR VARIOUS MEDICAL SPECIALTIES**

The profile of the patient with osteoporosis is diverse and various specialties converge in their care, developing their activity at all levels of care (rheumatologists, internists, family doctors, gynecologists, trauma specialists, geriatricians, physical therapists), different specialties for the same patient with osteoporosis. What varies is the reason for consultation, the vital moment and the location of the patient. Medical societies have collected the characteristics of patients with osteoporosis, making it possible to establish the characteristic profile in each specialty. The RETOSS (Rheumatology and Osteoporosis) study, promoted by the Spanish Society of Rheumatology (SER), analyzed the profile of patients with postmenopausal osteoporosis in the rheumatology consultation. These were patients with low calcium intake, a family history of fracture, a previous history of fractures, insufficient calciudial levels, and aged over 70 years, in addition to a high incidence of back pain.

Most were referred from Primary Care (63%), Gynecology (13.8%) or Trauma (10%).

The OSTEOMED (Osteoporosis in Internal Medicine) registry, promoted by the Osteoporosis Working Group (GTO) and under the aegis of the Spanish Society of Internal Medicine (SEMI), showed that the main reason for consultation in Internal Medicine services were confirmed vertebral fractures (17.2%), non-vertebral fractures (9.1%), back pain or lyphosis (9.8%), musculoskeletal pain (11.4%), and suspected secondary osteoporosis (7.2%). Overall, it was a sedentary patient profile (36%), with low sun exposure (23%). An active search for patients receiving corticosteroids was recommended. In this the Internal Medicine and Primary Care consultations play an important role. The patients were referred from Primary Care, other hospital services and other consultations of the Internal Medicine service itself.

From the geriatrics perspective, the National Registry of Hip Fractures (NRHP) collects hip fracture patients older than 75 years admitted in 2017 in 54 hospitals in Spain. It is the Spanish prospective study of a patient with postmenopausal osteoporosis (PMO) according to the patient’s profile. Adaptado de Blanch et al.

**A. Osteoporosis patient without fracture**

The consensus agrees that in patients without risk of fracture, follow-up should be carried out by Primary Care or Rheumatology. In case of high risk of fracture, the follow-up by Rheumatology would be prioritized. Patients with early surgical or symptomatic menopause are subject to Gynecology care. In case of associated thyroid disease, she would be treated by Endocrinology.

Those patients with osteoporosis where a significant loss of bone mineral density is observed, despite correct pharmacological treatment, would require rheumatology care.

In all patients whose musculoskeletal condition also conditions pain or functional loss, an assessment and subsequent follow-up by Rehabilitation would be recommended.

As complementary examinations, bone densitometry (dual radiological absorptiometry –DXA–) and lumbar spine radiography are recommended every 2 years. The consultation should also serve to underline adherence to treatment.

**B. Patient with osteoporosis with vertebral fracture**

In acute vertebral fracture, especially involving the posterior wall, it is the Traumatology Service that must assess the surgical option, which must be accompanied by a medical evaluation by Rheumatology, Internal Medicine or Geriatrics according to age and comorbidities.

The importance of having an FLS (Fracture Liaison Service), or a Bone Metabolism Unit, as a device that should assume responsibility for such fractures is highlighted.

At the follow-up level after the acute moment, follow-up by rheumatology and rehabilitation or geriatrics is recommended in case of advanced age or functional or cognitive deficit. At the level of outpatient follow-up, primary care is recommended, provided there are specifically trained professionals.

**Figure 1. Medical care circuits for patients diagnosed with postmenopausal osteoporosis (PMO) according to the patient’s profile.**

**WITHOUT fracture**

- Risk free: - Primary care - Internal Medicine - Gynecology
- With risk: - Rheumatology

**WITH vertebral fracture**

- Acute: - Orthopedic surgery and traumatology
- Chronic: - Rheumatology - Rehabilitation

**WITH non vertebral fracture**

- Acute hip fx: - Orthopedic surgery and traumatology
- Non-acute hip fx: - Rheumatology - Internal Medicine - Geriatrics
Table 1. Advantages and disadvantages of telematic consultation\textsuperscript{15,16}

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results similar to those obtained in the face-to-face visit are achieved</td>
<td>Demands a series of technological skills</td>
</tr>
<tr>
<td>Provides a more efficient management for the professional</td>
<td>Not all households have the structure, technology and skills necessary to carry out these consultations</td>
</tr>
<tr>
<td>Saves patient time, as it avoids travel and waiting time</td>
<td>Older people with sensory limitations (visual, hearing), cognitive impairment</td>
</tr>
<tr>
<td>It can be useful to solve problems and to solve doubts about medication, administrative tasks, monitoring of chronic patients</td>
<td>Difficulty correct identification of the patient</td>
</tr>
<tr>
<td>It contributes to reducing face-to-face visits and facilitating more time per person for care</td>
<td>Loss of non-verbal communication</td>
</tr>
</tbody>
</table>

C. Osteoporosis patient with non-vertebral fracture

These fractures correspond mainly to hip and distal radius fractures. The acute phase care corresponds to Traumatology, but the care by interdisciplinary teams integrated in an FLS or orthogeriatric unit have shown improvements in both complications and mortality, as well as functional improvement at discharge. They also improve coordination with primary care and secondary prevention of new fractures\textsuperscript{10}.

To sum up, regardless of the referring specialty, the recommendation is:

- Attention to the fracture by multi-disciplinary units.
- To highlight the role of the FLS.
- Coordination between levels of care, especially highlighting the role of Primary Care in secondary prevention.
- Establish standardized evaluation and follow-up measures (risk of fracture, pain, functional capacity, quality of life).

IMPACT OF COVID-19 INFECTION FOR OSTEOPOROSIS OUTPATIENT CLINICS

During the period of the recent health crisis, telemedicine has been the main contact tool for patients of hospitals and outpatient clinics. Previously, its use had been rather sporadic\textsuperscript{11}.

One of the most affected hospital environments has been outpatient facilities, both due to the limitation of face-to-face visits by patients and the availability of doctors given the great overload in hospital wards.

Furthermore, patients have limited or reduced attendance at face-to-face consultations for fear of being infected, which may lead to follow-up problems, cancellation of diagnostic tests or delay in requesting initial specialist visits\textsuperscript{12,13}.

According to the Catalan Health Service data, in Catalan hospitals over the second quarter of 2020, initial visits of any specialty decreased by 50% compared to the same quarter of the previous year without recove-ring in the third quarter (70% of those made in 2019), the majority being by telemedicine. Before the health crisis, 13,500 face-to-face visits, 14,500 telephone visits and about 1,000 telematic consultations were attended daily in Primary Care centers; now face-to-face visits are around 18,000 a day, telephone visits around 86,000 and telematic consultations around 17,000\textsuperscript{14}.

TELEMEDICINE IN THE COVID-19 ERA

Digital tools offer important opportunities to reshape current healthcare systems, and are not incompatible with face-to-face visits throughout the doctor-patient relationship. There will be times when the face-to-face consultation makes more sense (first visits) and at other times it will be more convenient to use telemedicine (follow-up).

There are different formats of telematic consultation:

- By telephone. Through a phone call from the doctor to the patient.
- Video call. It consists of calling the patient through a device that allows the patient and the doctor to see each other, which facilitates both identification and non-verbal communication.
- Electronic consultation or e-consultation. Telematic contact between professionals and with the patient that would allow resolution of doubts.

Telemedical consultation has advantages and disadvantages (Table 1)\textsuperscript{15,16}. The Catalan Health Service has published a document affirming that telematic care may be more useful in the follow-up visits of stable chronic patients who are well aware of their condition and do not require a physical examination\textsuperscript{17}.

There is scientific evidence regarding telemedicine use in the field of osteoporosis\textsuperscript{18}. Some telematic experiences have been carried out in countries with great distances between populations such as Australia and Canada, where the geography and distribution of health workers require a remote approach to the ambulatory care of health problems in the population\textsuperscript{19}. A recent study from a Canadian osteoporosis telemedicine program found that participating patients perceived a number of benefits such as high-quality care, valued the experience and credibility of the treating physician, but also posed some drawbacks such as coordination of their care with the results of the complementary examinations carried out and, sometimes, the sub-optimal follow-up of other health professionals, such as physiotherapists\textsuperscript{20}.

In the United Kingdom, telephone consultations are promoted in the clinical guidelines for follow-up consultations in the Fracture Liaison Service (FLS) with the aim of promoting adherence to osteoporosis treatment\textsuperscript{21}.

The great challenge for the future is in the transition to the post-pandemic phase, since the key transformation of telemedicine is to change from a crisis mode (where
the use of provisional or unapproved technologies has been allowed) to a sustainable mode where it is necessary for the different health centers to invest in systems that guarantee the safe transfer of data, the encryption of emails, guaranteeing patient privacy along with long-term technical support.

Directive 2011/24/EU on the rights of patients in remote cross-border healthcare using telemedicine, describes them. Although future challenges must be posed in interoperability and compliance with the General Data Protection Regulation (GDPR).

PROFILE OF THE SUSCEPTIBLE PATIENT REGARDING TELEMEDICINE CARE

The profile of the patient with osteoporosis who comes to our consultations is getting older, with more associated chronic diseases and, probably, with a greater limitation for face-to-face access to follow-up medical consultations.

There are different formats of telematic consultation such as telephone consultation, video call, specific telematic platforms. The success of this communication lies in choosing, in a personalized way, the proposal that best suits each patient. Thus, prior to the consultation, the person’s functional and cognitive situation, their cultural level, socio-family environment and the presence of sensory or compression deficits that would favor the presence of a relative during the consultation should be assessed.

An important aspect is the role of nursing in telematic consultation. As in the FLS model, it would be to establish initial contact with the patient who meets the inclusion criteria (Table 2) into inform them that the planned medical consultation will be telematic (specifying date and time), and preparing the patient in relation to relevant information for this visit (Table 3).

Subsequently, the osteoporosis specialist will carry out the telematic consultation in the available format, preferably following a template that allows structuring the visit in anamnesis, assessment of complementary tests carried out, summary of the case and decision-making in relation to the diagnosis and/or therapeutic plan.

All this should be recorded in the clinical history, to facilitate continuity of care. Finally, the administrative staff will manage, within the healthcare circuit, the complementary tests that proceed, and will schedule a new follow-up visit, if necessary.

CONCLUSIONS

Osteoporosis is a very prevalent chronic disease with great social and health repercussions that requires prolonged treatment over time, an aspect that sometimes complicates adherence to it.

This situation requires us to actively redesign the current healthcare of our patients to ensure a correct identification, assessment and monitoring in a safe and effective way. Knowing the clinical profile of patients referred to an osteoporosis consultation will help to improve the management of this disease.

COVID-19 has occurred in a context in which the main causes of mortality are chronic diseases, and where there are social inequalities, and this also has to do with the expansion of the pandemic. For all these reasons, the long-term care approach that should be used should make it easier for people to protect themselves against...

---

**Table 2. Inclusion criteria**

- Confirmed diagnosis of osteoporosis (bone densitometry vs. previous fragility fracture)
- No hearing, cognitive or visual impairment problems
- Present minimal knowledge of health condition
- No comorbidities that prevent the development of telecare
- Acceptance of healthcare technology by patient
- Sufficient Internet access vs. telephone coverage
- No economic cost for the patient

**Table 3. Structural proposal to carry out telematic consultations. Adapted from Barrios V et al.**

<table>
<thead>
<tr>
<th>Steps</th>
<th>Who</th>
<th>Responsibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initial contact with the patient</td>
<td>Personal administrative/case management</td>
<td>Patient location (write down valid contact number)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preparation of the patient (annotated treatments, helping family member if necessary)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recent taking of constants, weight, analytical if applicable)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patient notes. Establishment of appointment (day, time)</td>
</tr>
<tr>
<td>2. Consultation medical telematics</td>
<td>OP specialist</td>
<td>Use of template (recommended)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case summary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Evolution: emergency services or hospitalizations, other consultations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Current treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anamnesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Analytical or other test results</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Timely therapeutic changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Therapeutic plan and circuit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Continuity of care: recommendations for primary care</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Document in medical history</td>
</tr>
<tr>
<td>3. Flow of the patient</td>
<td>Personal administrative/case management</td>
<td>Healthcare circuit: request for tests or new consultation if applicable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Current query record</td>
</tr>
</tbody>
</table>
transmission. Important changes have been caused in the form of attention in our consultations, some of them will surely be reversed with the passage of time; Others, such as the increase in non-contact visits at the expense of face-to-face visits, are presumably to last once the pandemic is over.

The need to limit access to hospitals and infections has imposed telemedicine, but it is here to stay. There are many advantages that it can offer to professionals and users, becoming a key assistance tool. Faced with this scenario, it is imperative to move towards a more secure and protocolized telematic attention. This care must be carried out in a structured way to be cost-effective. For this, we will previously review the clinical and functional situation in which the patient is located and thus be able to correctly plan said assistance, indicating its need and suitability.

A posteriori, its articulation will be based on a series of guidelines divided into three major steps: the initial contact with the patient, the telematic medical consultation as such and the care flow of the process. The privacy of clinical data is ensured.

It is essential that health authorities make use of scientific knowledge to act in the complex and difficult situation we currently face, to maintain outpatient care for our patients with osteoporosis, prioritizing the evaluation of their results on the health of patients, as well as their impact on health systems in the short and long term.

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