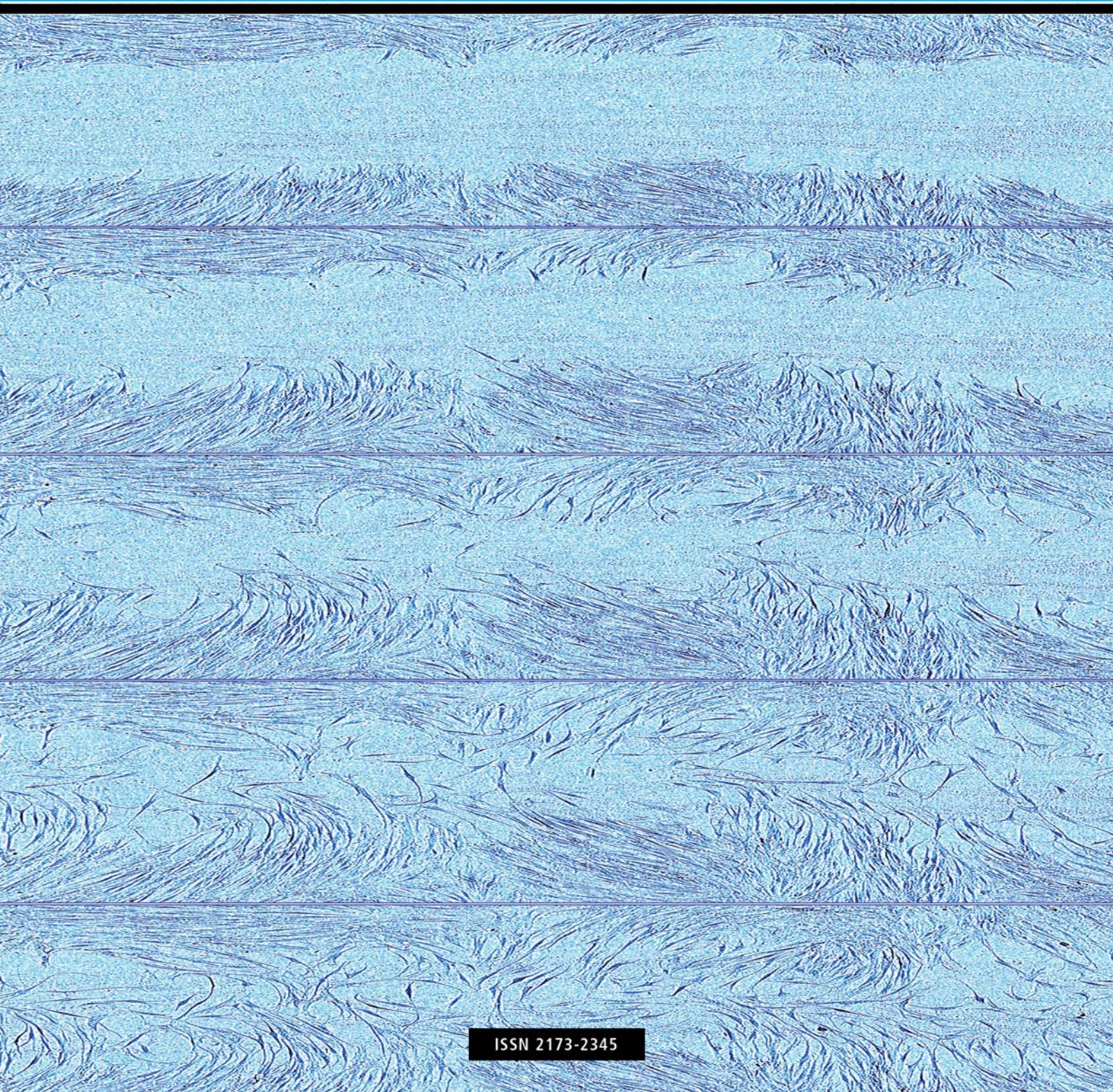
seiomm
Sociedad Española de Investigación Ósea y del Metabolismo Mineral

feiomm
Fundación Española de Investigación Ósea y del Metabolismo Mineral

Volume 9 · Number 2 · April-June 2017

Revista de Osteoporosis y Metabolismo Mineral

www.revistadeosteoporosisymetabolismomineral.com



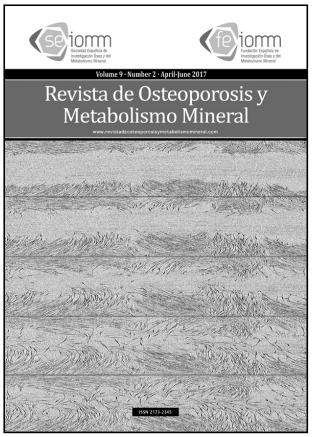
ISSN 2173-2345

XXII Congreso de la SEIOMM

Elche, 25-27 de octubre 2017

seiomm
Sociedad Española de Investigación Ósea y del Metabolismo Mineral

feiomm
Fundación Española de Investigación Ósea y del Metabolismo Mineral



Our cover
Images of a 24 hour migration experiment performed with a human mesenchymal stem cell culture derived from bone marrow. From top to bottom, the images show how cells invade the free space of artificially created cells in the culture

Autors:
Antonio Casado Díaz, Joel Cabezas, José Manuel Quesada Gómez. CIBER de Fragilidad y Envejecimiento Saludable. Servicio de Endocrinología y Nutrición. Hospital Universitario Reina Sofía. Instituto Maimónides de Investigación Biomédica de Córdoba (Spain)

Velázquez, 94 (1ª planta)
28006 Madrid (Spain)

Telf: +34-625 680 737
Fax: +34-917 817 020

e-mail: seiomm@seiomm.org

http://www.seiomm.org

Editing

ibáñez & Plaza Asociados, S. L.
EDITORIAL TÉCNICA Y COMUNICACIÓN

Avda. Reina Victoria, 47 (6º D)
28003 Madrid (Spain)
Telf. +34-915 538 297
e-mail: correo@ibanezyplaza.com
http://www.ibanezyplaza.com

Graphic design
Concha García García

English translation
David Shea

ISSN: **2173-2345**

Submit originals:
romm@ibanezyplaza.com

SUMMARY

Vol. 9 - Nº 2 - April-June 2017

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

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

Editorial Committee

Committee of experts

The Garvan calculator and fragility fracture risk

DOI: http://dx.doi.org/10.4321/S1889-836X2017000200001

Hernández Hernández JL, Olmos Martínez JM

Unidad de Metabolismo Óseo y Lipídico - Departamento de Medicina Interna - Hospital Marqués de Valdecilla-IDIVAL (Instituto de Investigación Marqués de Valdecilla) - Universidad de Cantabria - Santander (España)

e-mail: hernandezjluis@gmail.com

Loss of bone mass is only part of the syndrome which, in addition to densitometric osteoporosis, sarcopenia and other risk factors, eventually contributes to fragility fracture. The low sensitivity and specificity of bone mineral density (BMD) measurement in predicting fracture risk has led to the development of tools that include several known risk factors such as demographic variables, physical examination, personal and/or family history of fracture, presence of diseases or medications with influence on bone metabolism and risk factors for falls[1]. Some of these algorithms for predicting the risk of fracture have not been validated in external populations, others lack methodological deficits and only a few have been integrated into national clinical guidelines for osteoporosis.

Validation, both internal and external, is one of the keys to developing a risk calculator. In particular, external validation generalizes the scale to populations beyond those in which it was generated. The work of Reyes Domínguez et al.[2], published in this issue of the Journal of Osteoporosis and Mineral Metabolism, is the first in Spain to validate the Garvan calculator in a sample of 121 individuals without basal densitometric osteoporosis, monitored over 10 years and who had not received anti-osteoporotic treatment during that time.

Furthermore, the discriminative capacity of a predictive model or tool, that is, its ability to distinguish between subjects with or without the event (in this case, osteoporotic fracture), is usually assessed by the area under the ROC curve (AUC). Its value varies between 0 and 1, with a figure between 0.7 and 0.8 considered acceptable. Reyes Domínguez et al.[2], reported an AUC value of 0.72 for any fragility fracture, which gives the Garvan calculator an acceptable predictive capacity. These results are superimposable to those published by Langsetmo et al.[3] in a validation study of the Garvan calculator in Canada. These authors find an AUC for any brittle fracture of 0.69 in females and 0.70 in males. The AUC for hip fracture was higher (0.80 and 0.85, respectively). Only in the quintile at highest risk of fracture did the model overestimate the 10 year risk of any fragility fracture in males and hip fracture in females.

The GLOW study included 19,586 postmenopausal women 60 years of age or older without previous anti-osteoporotic treatment, recruited in 723 primary care centers in 10 countries and followed over a two-year period. Three predictive models that did not include the BMD value were evaluated; the FRAX®, the Garvan calculator, and a model that only considered the age and antecedent of a previous fracture. An AUC of 0.64 was found to predict major osteoporotic fracture and 0.76 for prediction of hip fracture. However, neither of the two models (FRAX® and Garvan) was better than the one that only included age and previous fracture, which fuels the debate about the utility of more complex risk scales[4]. Indeed, in a recent systematic review, tools that predict the risk of osteoporotic fracture and that include few risk factors, such as the Garvan calculator, often have equal or even greater discrimination capacity which include many risk factors (FRAX®, QFracture®)[5].

In general, the predicted risk with the Garvan calculator in the validated work is close to or slightly higher than the observed risk of osteoporotic fracture and better predicts the risk of hip fracture than that of any fragility fracture[1,3,4-8]. In the work of Reyes Domínguez et al.[2], the risk of hip fracture could not be analyzed because of the limited number of incident fractures in the analyzed population.

The significance of the absolute risk of fracture should be related to the threshold value of therapeutic intervention recommended in each country, to provide the patient with adequate information about their risk. In order to calculate the validity criteria of the Garvan calculator, Chen et al.[9] used the American FRAX® cut-off points (20% in the case of the major osteoporotic fracture), finding a sensitivity of 20%, a specificity of 96% and a negative predictive value of 89%. In the study of Reyes Domínguez et al.[2], the authors' optimal cut off point considers a high risk of osteoporotic fracture to be 18.5%, with a sensitivity and specificity of 67% and a negative predictive value of 86%, similar to that found by Chen et al.[9]

In summary, the work of Reyes et al.[2] has the importance of being the first to validate the Garvan calculator in Spain and, in addition, the interest of its possible use as a screening tool to identify subjects with low risk of fracture. Its greater discriminative capacity has been demonstrated with respect to the negative predictive value of any osteoporotic fracture. Its usefulness as a predictor of hip fracture has not been assessed in this study, as has already been noted.

Further validation studies of the simplest risk calculators, such as Garvan, are required, with prospective population cohorts including participants with different risk factors. Given that no predictive tool captures all the known risk factors for fragility fracture or temporal relationships, clinical judgment should remain a key factor in applying the results of these scales to an individual patient.

Bibliography

1. Dagan N, Cohen-Stavi C, Leventer-Roberts M, Balicer RD. External validation and comparison of three prediction tools for risk of osteoporotic fractures using data from population based electronic health records: retrospective cohort study. BMJ. 2017;356:i6755.
2. Reyes Dominguez AI, Sosa Cabrera N, Saavedra Santana P, Gómez de Tejada Romero MJ, Jódar Jimeno E, Sosa Henríquez M. Valoración de la capacidad predictiva de la calculadora Garvan del riesgo de fractura a 10 años en una población española. Rev Osteoporos Metab Miner. 2017;9(2):55-61.
3. Langsetmo L, Nguyen TV, Nguyen ND, Kovacs CS, Prior JC, Center JR, et al. Canadian Multicentre Osteoporosis Study Research Group. Independent external validation of nomograms for predicting risk of low-trauma fracture and hip fracture. CMAJ. 2011;183:E107-14.
4. Sambrook PN, Flahive J, Hooven FH, Boonen S, Chapurlat R, Lindsay R, et al. Predicting fractures in an international cohort using risk factor algorithms without BMD. J Bone Miner Res. 2011;26:2770-7.
5. Rubin KH, Friis-Holmberg T, Hermann AP, Abrahamsen B, Brixen K. Risk assessment tools to identify women with increased risk of osteoporotic fracture: complexity or simplicity? A systematic review. J Bone Miner Res. 2013;28:1701-17.
6. Ahmed LA, Nguyen ND, Bjørnerem Å, Joakimsen RM, Jørgensen L, Størmer J, et al. External validation of the Garvan nomograms for predicting absolute fracture risk: the Tromsø study. PLoS One. 2014;25;9:e107695.
7. Bolland MJ, Siu AT, Mason BH, Horne AM, Ames RW, Grey AB, et al. Evaluation of the FRAX and Garvan fracture risk calculators in older women. J Bone Miner Res. 2011;26:420-7.
8. van Geel TA, Eisman JA, Geusens PP, van den Bergh JP, Center JR, Dinant GJ. The utility of absolute risk prediction using FRAX® and Garvan Fracture Risk Calculator in daily practice. Maturitas. 2014;77(2):174-9.
9. Chen SJ, Chen YJ, Cheng CH, Hwang HF, Chen CY, Lin MR. Comparisons of different screening tools for identifying fracture/osteoporosis risk among community-dwelling older people. Medicine (Baltimore). 2016;95:e3415.

Reyes Domínguez AI[1], Sosa Cabrera N[1], Saavedra Santana P[1,2], Gómez de Tejada Romero MJ[1,3], Jódar Gimeno E[1,4], Sosa Henríquez M[1,5]

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

2 Departamento de Matemáticas - Universidad de Las Palmas de Gran Canaria - Las Palmas de Gran Canaria (España)

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

4 Servicio de Endocrinología - Hospital Quirón - Madrid (España)

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

Assessment of the predictive capacity of the Garvan calculator of 10 year risk of fracture in a Spanish population

DOI: http://dx.doi.org/10.4321/S1889-836X2017000200002

Correspondence: Manuel Sosa Henríquez - C/Espronceda, 2 - 35005 Las Palmas de Gran Canaria (Spain)
e-mail: manuel.sosa@ulpgc.es

Date of receipt: 13/01/2017
Date of acceptance: 06/03/2017

Summary

Introduction: Several calculation tools or scales have been developed in recent years to assess the risk of fracture due to long-term fragility. The Garvan calculator has not been validated in the Spanish population. This study aims to observe their predictive capacity in a population sample of the Canary Islands and, therefore, of the Spanish population.

Material and Methods: We included 121 patients who were followed up for 10 years in our consultations. All were assessed the risk of fracture using the Garvan calculator and based on the data obtained in the first visit.

Results: Of the 121 patients, 30 suffered at least one osteoporotic fracture over the 10-year follow-up period. The group of patients with fractures had on the Garvan scale an average risk value to suffer any fracturing fracture of 27%, compared to 13% of those who did not suffer fracture ($p<0.001$). The area under the corresponding ROC curve was 0.718 (CI-95% = 0.613 ; 0.824). Based on this, the estimated optimal cut-off point to consider a high risk fracture was 18.5%. This value corresponded to a sensitivity of 0.67 (CI-95% = 0.47 ; 0.83) and a specificity of 0.67 (CI-95% = 0.56 ; 0.77).

Conclusions: Our results show that the Garvan scale adequately predicts the risk of 10-year osteoporotic fracture in our population. A value lower than 18.5% would allow us to establish a low fracture risk and could be used as a screening tool.

Key words: *osteoporosis, risk, fracture, scale, Garvan calculator, Spanish population.*

Introduction

Osteoporosis is a very prevalent disease, which produces the so-called "fragility fractures" as the only clinical complication[1]. In recent years, several calculation tools or scales have been published which, based on clinical data and with or without the aid of bone densitometry, estimate the risk of a fracture in the long term, up to 10 years[2-6].

Although these scales share many clinical data such as age or history of previous fractures, they also differ in the methodology and population in which they have been developed, as well as whether or not they include bone densitometry or other risk factors. For example, the more widely used FRAX® scale, published in more studies and sponsored by the World Health Organization (WHO)[3], apparently underestimates the risk of fracture in both patients with certain diseases[7-12] as well as globally in some countries, such as Spain[13], Argentina[14] or Canada[15].

The Garvan fracture risk calculator or Garvan scale was devised by Australian researchers at the Garvan Institute of Medical Research. It has been less widely used than the FRAX®, showing often divergent results in some studies which compared both scales[16-18]. It has not been validated in Spain, which led us to carry out this study, with the aim of observing its validity in a Canary Island population of both sexes. We have considered extending it to the Spanish population.

Material and methods

Design: This prospective study initially included 400 people of both sexes whose densitometries at the time of the first visit showed no osteoporotic values. The subjects had attended at least a second follow-up visit. Subsequently, those patients who were monitored over 10 years and who had not undergone pharmacological treatment for osteoporosis in those years were selected. The 121 who met this criterion were included in the follow-up study.

Fractures in the first 10 years of follow-up: All 121 individuals included in the study presented fragility fractures that occurred during the 10-year follow-up period.

Application of the Garvan calculator: All the patients included in our study were assessed for fracture risk due to long-term fragility using the Garvan calculator based on the data obtained during the first consultation. The tool considers a total of 5 calculation variables: sex, age, presence of fragility fractures beyond 50 years of age and falls in the last 12 months. The determination of bone mineral density by densitometry may be added if we have it. Otherwise, the calculation is also carried out, but the program requires including weight. In our study, all patients underwent bone densitometry screening at the first visit. This scale is freely available, without registration, on-line at: https://www.garvan.org.au/promotions/bone-fracture-risk/calculator/

Once the data has been entered, the calculator shows the risk of frailty fracture for: a) any fragility fracture, and b) specifically hip fracture, and both at 5 and 10 years.

Statistical Study

Univariate analysis: Categorical variables were expressed as frequencies and percentages, and the continuous variables as means and standard deviations when the data followed a normal distribution, and as medians and interquartile ranges (percentiles 25-75) when the distribution followed was not normal. The percentages were compared using the chi-square test, the means with Student's t test, and the medians with the Wilcoxon test for independent data.

Survival analysis: To explore the predictive ability of the fracture risk of the Garvan calculator, patients were classified according to the tertiles corresponding to this predictor. In each of these groups the survival curves were estimated up to the appearance of the first fracture using the Kaplan-Meier method. The difference between them was contrasted using the log-rank test.

Receiver Operating Characteristics (ROC) Curves: In order to evaluate the discriminatory capacity of any frailty fracture risk, the 121 patients who were monitored over 10 years were classified according to whether or not they suffered at least one fracture during this time period. For this classification, a ROC analysis was carried out, estimating the area under the corresponding ROC curve with a 95% confidence interval. The Garvan scale's discriminatory optimal threshold was selected as the value associated with the point of the ROC curve that minimized the quantity:

$$(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$$

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were estimated for this threshold with 95% confidence intervals.

A hypothesis test was considered statistically significant when the corresponding p value was less than 5%. Data were analyzed using the R program (version 3.1.0.).

Results

Table 1 shows the baseline characteristics of the 400 patients initially recruited for this study. It is observed that there is a greater proportion of women than men and that the mean age was 63 years, without obtaining statistically significant differences between both sexes. As expected, males were larger in size and weight than females, but body mass index (BMI) was similar in both groups, with an overweight average. The overall median risk of fracture fractures at 10 years when Garvan was applied was 15%, significantly higher in females than in males ($p<0.001$).

Table 2 shows the characteristics of the studied population over 10 years from the time of the Garvan estimation. The total number of patients was 121, of which 30 had at least one fracture due to fragility in this time frame. None of the patients

received anti-osteoporotic treatment, although the patients with fractures were indicated after the fracture occurrence was reported. Of all the osteoporotic fractures (vertebral, hip, Colles, humerus, tibia, and ribs) only two were of the hip. At the outset of the study, the fractured patients had a mean risk of suffering any fragility fracture of 27%, compared to 13% of those who did not suffer a fracture (p <0.001). The same significant result was observed with the risk of hip fracture, since patients who suffered a new osteoporotic fracture (of any type) during follow-up showed an average value of 8% versus 3% of non-fractured ones.

Table 3 shows the statistical parameters used to assess the ability of the Garvan scale to predict any fracturing fracture within 10 years after its determination in the study population. The area under the corresponding ROC curve was 0.718 (CI-95% = 0.613 ; 0.824) (Figure 1). Considering this ROC curve, and looking for the value that offered the best statistical conditions to predict the risk of fracture, we set the optimum cutoff point at 18.5%. This value corresponds to a sensitivity of 0.67 (IC-95% = 0.47 ; 0.83), a specificity of 0.67 (CI -95% = 0.56, 0.77), a predictive value of 0.86 (CI-95% = 0.76 ; 0.93) and a positive predictive value of 0.40 (CI-95% = 0.26 ; 0.55).

Figure 2 shows the survival curves for the period between the estimation of the risk of frailty fracture and the first fragility fracture in each of the cohorts determined by the tertiles of the Garvan scale. According to these tertiles, the groups were divided according to whether the value obtained was less than 11% between 11 and 22%, and higher than 22%. The log-rank test showed statistically significant differences at 5 years (p<0.001).

The limited number of hip fractures (only 2) prevented an ROC analysis and one of survival for this type of fracture.

Discussion

In recent years, the diagnosis and treatment of patients with osteoporosis have changed, as a series of calculation tools or risk scales have been developed that allow us to estimate the probability of suffering a fracture due to fragility in the future, usually 10 years. This differs from the risk estimation offered by bone densitometry, which, in isolation, reports only a part of the fracture risk, which is clearly multifactorial[19,20]. Therefore, the combination of fracture risk factors and the results of densitometry have a greater specificity and sensitivity than each of them separately[21]. The FRAX® and Garvan scales, in contrast to QFracture®, include the value of bone mineral density per DXA in calculations for the likelihood of fracture risk.

The definitive role of these scales has not been established, although their presence is increasing in position papers and clinical guidelines.

Currently, FRAX® is the most accepted scale. It was the first to be published and is sponsored by the WHO[22]. It allows researchers to calculate fracture risk in a large number of countries. It is the tool with the greatest amount of literature published, with a treatment threshold of more than 20% for any fragility fracture and 3% for a hip fracture[23]. However, the FRAX® scale also has its limitations. On the one hand, it does not include falls, a very important risk factor in the production of most fragility fractures[24,25]. On the other hand, several authors have expressed their concern as it underestimates the risk of fracture in diabetic patients and in the Spanish population[12,13], because this scale has not yet been corrected for Spain. Finally, the formula with which the FRAX® calculator has been developed has not been published, a fact that has generated great controversy and suspicion in the scientific community.

Another fracture risk calculator is the QFracture®[5,26], developed by English authors, who added additional risk factors such as falls, diabetes mellitus and other diseases to variables already included in the FRAX® scale (http: //www.qfracture.org). In addition, the degree of alcohol and tobacco consumption was incorporated in more detail, and it has the novelty of making it possible to estimate fracture risk from 1 to 10 years, very useful for those individuals whose life expectancy is lower.

As for the limitations of QFracture® tool, it does not include calculations of bone densitometry and contains many variables[5,26], so the time required to complete the questionnaire is significantly longer. In addition, the QFracture® scale is not as widely used as FRAX®, which may be because it has not been validated outside the UK, and therefore there is less published material about this tool. On the other hand, the optimal cutoff points for the clinical management of patients with osteoporosis have not been established. Its website suggests a risk estimate for women of 11.1% in 10 years and for men, 2.6% over the same period of time.

Finally, there are few comparative studies between the QFracture® and FRAX® scales. We have been able to find only the work of Johansen et al. Who considered QFracture® better as a tool for estimating hip fracture risk, since it includes the history of falls[27]. On the other hand, Kanis et al published a review of the Scottish Intercollegiate Guidelines Network (SIGN), which concluded that the use of QFracture® should be used for estimating hip fracture risk and not for the risk of fragility fractures[19].

The Garvan fracture risk calculator was published by a group of Australian researchers from the Garvan Institute of Medical Research to predict in a given patient the absolute risk of having any osteoporotic fracture within 5 and 10 years. The study included a sample of more than 2,500 individuals, men and women, over 60 years of age from data collected by the Dubbo study[28]. They included the following four risk factors: age, number of previous fractures after 50 years of age, number of falls in the last year and the value of bone mineral density or weight (if bone densitometry is not available).

Table 1. General characteristics of the population recruited at the beginning of the study

	Total N = 400	Men N = 38	Women N = 362	Value p
Age, years (#)	63.3 ± 8.9	63.8 ± 9.1	63.3 ± 8.9	0.736
Weight, kg (#)	67.9 ± 13.2	78.7 ± 13.7	66.8 ± 12.6	<0.001
Size, cm (#)	157.1 ± 7.3	169.7 ± 6.1	155.7 ± 6.0	<0.001
BMI*, kg/m² (#)	27.5 ± 4.9	27.3 ± 4.2	27.6 ± 5.0	0.741
Garvan value for any 10 year frailty fracture, % (&)	15 (10 ; 29)	8 (4 ; 14.7)	15 (10 ; 29)	<0.001
Garvan value for 10 year hip fracture, % (&)	3 (1 ; 8.25)	0.95 (0.42 ; 3)	3 (1 ; 9)	<0.001

Data expressed as #: means ± standard deviations; &: medians (interquartile ranges).
*BMI: body mass index.

Table 2. Characteristics of the studied population for 10 years from the time of the estimation of the Garvan value

		Fractures*		
	Total N = 121	No N = 91	Yes N = 30	P
Age, years (#)	59.3 ± 6.8	58.2 ± 6.4	62.8 ± 6.7	0.001
Weight, kg (#)	66.8 ± 11.7	67.4 ± 12.5	64.9 ± 8.8	0.309
Size, cm (#)	156.4 ± 6.0	156.6 ± 5.9	155.7 ± 6.3	0.439
BMI, kg/m² (#)	27.3 ± 4.7	27.5 ± 5.0	26.8 ± 3.6	0.503
Garvan value for any 10 year frailty fracture, % (&)	15 (10 ; 28)	13 (9.5 ; 23)	27 (14.2 ; 43.2)	<0.001
Garvan value for 10 year hip fracture, % (&)	3 (1 ; 8)	2 (1 ; 6.5)	8 (3 ; 17)	<0.001

*Fractures occurring within 10 years of follow-up.
Data expressed as #: means ± standard deviations; &: medians (interquartile ranges).

Table 3. Capacity of the Garvan scale to predict an osteoporotic fracture within 10 years of being calculated

Parameter	Estimate (IC-95%)
Area under the ROC curve	0.718 (0.613 ; 0.824)
Cut off point	**18.5**
Sensitivity	0.67 (0.47 ; 0.83)
Specificity	0.67 (0.56 ; 0.77)
Positive predictive value	0.40 (0.26 ; 0.55)
Negative predictive value	0.86 (0.76 ; 0.93)
Positive likelihood ratio	2.02 (1.37 ; 2.98)
Reason for negative likelihood	0.50 (0.29 ; 0.84)

The Garvan scale, although apparently very practical and easy to use, is hampered by the limited relevant bibliography and that it has not been validated outside Australia.

In the main, existing publications compare the FRAX® scale with QFracture®, and FRAX® with the Garvan calculator[29]. Several studies have concluded that the FRAX® tool with bone mineral density (BMD) measurement underestimates the incidence of osteoporotic fractures, while both FRAX® without BMD and the Garvan scale overestimate the incidence of these fractures[6,30]. However, although the FRAX® and Garvan calculators include different risk factors, the therapeutic recommendation is the same[18].

As the Garvan scale has not yet been validated in Spain, the main contribution of our study is to give reliability to its predictive capacity in our population, which would allow its use in our patients, and with this the estimation of the risk of fracture due to fragility of A faster way than with the QFracture® scale, and a transparent methodology in its elaboration and with the inclusion of the falls, facts that the FRAX® does not offer.

With the FRAX® and QFracture® scales, an attempt has been made to identify a cutoff point from which we would consider the patient to be at high risk of fracture due to fragility and, therefore, it would be advisable to initiate some treatment. As we mentioned earlier, in the FRAX® scale, this value has been set at 20% for any fragility fracture and 3% for the hip, whereas in QFracture®, the authors recommend considering cut-off points for women and Men at 11.1% and 2.6%, respectively.

In the Garvan calculation tool this cut-off point has not yet been clearly established. According to our study results, an estimate of the risk of suffering any frailty fracture below 18.5% would be indicative of a very low risk, so starting treatment would not be necessary.

Figure 1. ROC curve for the risk of suffering any fracturing fracture calculated with the Garvan scale

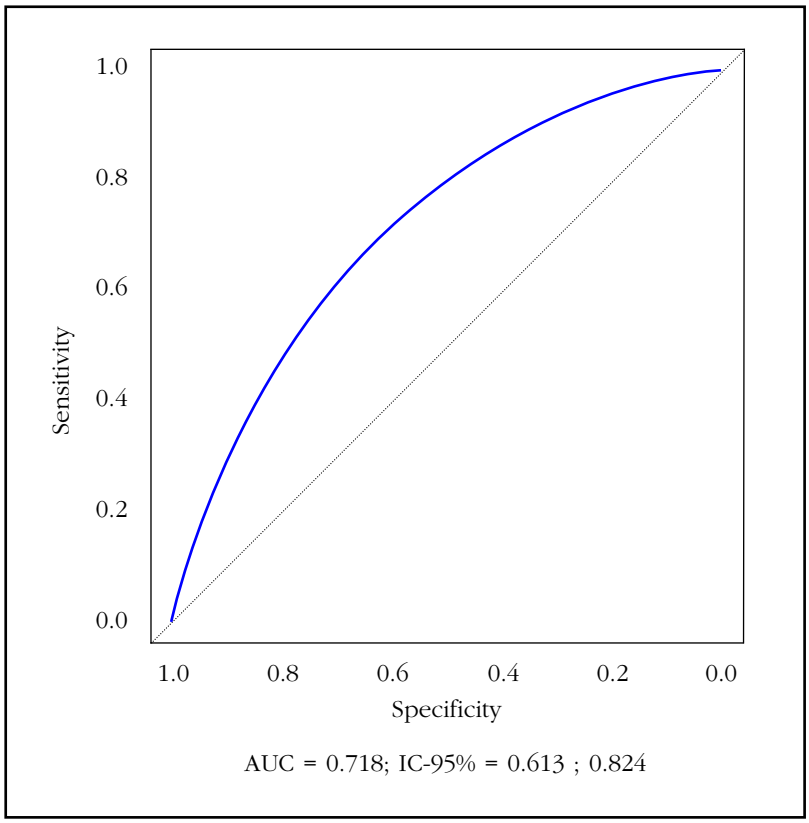
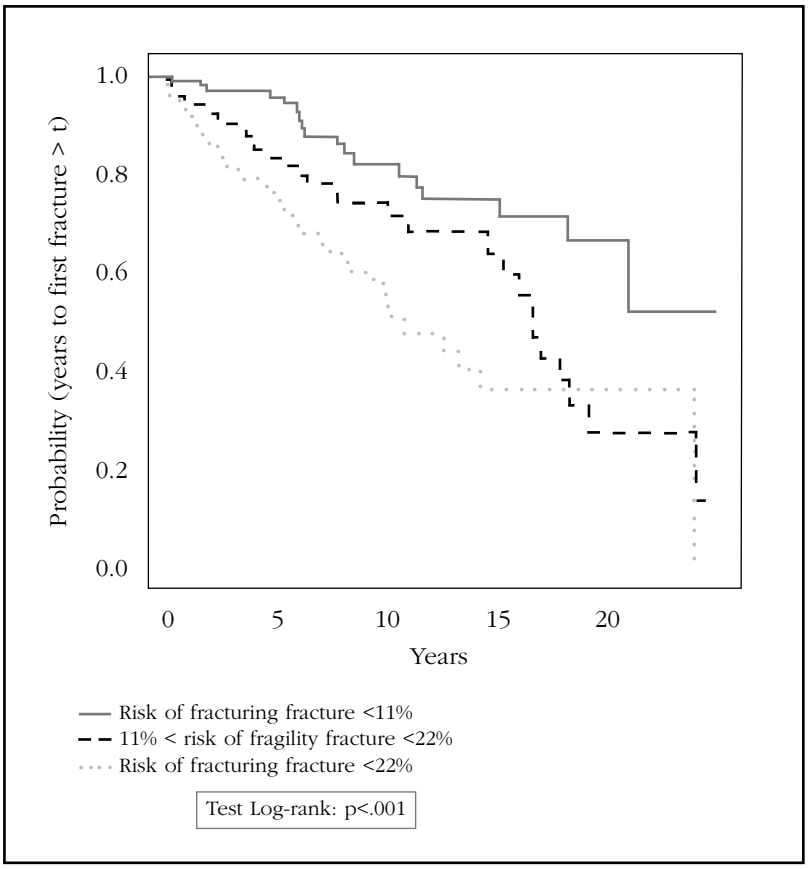


Figure 2. Survival curves up to the first fracture according to the groups defined by the tertiles of the Garvan value for risk of any fragility fracture



The main weakness of our study is the small sample size, due to the enormous difficulty found in our consultations of patients without densitometric osteoporosis and with a follow-up over so many years, besides not having received anti-osteoporotic treatment until the first fracture. The same reason has prevented us from performing the calculations for hip fracture risk, since the number of fractures incident at this location was insufficient to obtain a conclusive statistic. Despite this, the statistical study performed had enough robustness to be able to validate our findings.

In conclusion, according to the results of our study, the Garvan calculator can be used to a

ccess osteoporotic fracture risk in our population. Likewise, it could be used as a screening tool, since, according to the statistical calculations obtained, a value lower than 18.5% would allow us to establish in a given patient a very low risk of suffering any fragility fracture in the following 10 years.

Conflict of interest: The authors declare that there is no conflict of interest in this research study.

Funding: This study was funded in part by a research grant from the Canary Island Osteoporosis Society.

Bibliography

1. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 2009;9:1137-41.
2. Black DM, Steinbuch M, Palermo L, Dargent-Molina P, Lindsay R, Hoseyni MS, et al. An assessment tool for predicting fracture risk in postmenopausal women. Osteoporos Int. 2001;12:519-28.
3. Kanis JA, Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Synopsis of a WHO report. Osteoporos Int. 1994;4:368-81.
4. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. The Lancet. 2002;359:1929-36.
5. Hippisley-Cox J, Coupland C. Derivation and validation of updated QFracture algorithm to predict risk of osteoporotic fracture in primary care in the United Kingdom: prospective open cohort study. BMJ. 2012;344:e3427.
6. Bolland MJ, Siu AT, Mason BH, Horne AM, Ames RW, Grey AB, et al. Evaluation of the FRAX and Garvan fracture risk calculators in older women. J Bone Miner Res. 2011;26:420-7.
7. Stephens KI, Rubinsztain L, Payan J, Rentsch C, Rimland D, Tangpricha V. Dual-energy X-Ray absorptiometry and calculated FRAX risk scores may underestimate osteoporotic fracture risk in Vitamin D deficient veterans with HIV infection. Endocr Pract. 2016;22:440-6.
8. Caffarelli C, Alessi C, Nuti R, Gonnelli S. Divergent effects of obesity on fragility fractures. Clin Interv Aging. 2014;9:1629-36.
9. FRAX from WHO underestimates fracture risk in diabetes. Bonekey Rep. 2012;1:69.
10. Dede AD, Tournis S, Dontas I, Trovas G. Type 2 diabetes mellitus and fracture risk. Metabolism. 2014;63:1480-90.
11. González Reimers E, Negrín A, Santolaria Fernández F, Martín González MC, Hernández Betancor I, Fernández Rodríguez CM, et al. Utilidad del FRAX® en el estudio de las fracturas en el paciente alcohólico. Rev Osteoporos Metab Miner. 2011;3:149-56.
12. Giangregorio LM, Leslie WD, Lix LM, Johansson H, Oden A, McCloskey E, et al. FRAX underestimates fracture risk in patients with diabetes. J Bone Miner Res. 2012;27:301-8.
13. González-Macías J, Marin F, Vila J, Díez-Pérez A. Probability of fractures predicted by FRAX® and observed incidence in the Spanish ECOSAP Study cohort. Bone. 2012;50:373-7.
14. Camporro F, Redondo L, Bulacio E, Gutiérrez Magaldi I, Chamale E, Sáenz F. Comparison of FRAX Score without bone mineral density determination and the criteria proposed by the Argentine Osteoporosis Society for the use of antiresorptive therapy in postmenopausal women. Medicina (Mex). 2015;75:155-8.
15. Roux S, Cabana F, Carrier N, Beaulieu M, April P-M, Beaulieu M-C, et al. The World Health Organization Fracture Risk Assessment Tool (FRAX) underestimates incident and recurrent fractures in consecutive patients with fragility fractures. J Clin Endocrinol Metab. 2014;99:2400-8.
16. Marques A, Ferreira RJO, Santos E, Loza E, Carmona L, da Silva JAP. The accuracy of osteoporotic fracture risk prediction tools: a systematic review and meta-analysis. Ann Rheum Dis. 2015;74:1958-67.
17. Crandall CJ. Risk assessment tools for osteoporosis screening in postmenopausal women: a systematic review. Curr Osteoporos Rep. 2015;13:287-301.
18. Billington EO, Gamble GD, Reid IR. Reasons for discrepancies in hip fracture risk estimates using FRAX and Garvan calculators. Maturitas. 2016;85:11-8.
19. Kanis JA, Compston J, Cooper C, Harvey NC, Johansson H, Odén A, et al. SIGN Guidelines for Scotland: BMD versus FRAX versus QFracture. Calcif Tissue Int. 2016;98:417-25.
20. Cummings SR, Bates D, Black DM. Clinical use of bone densitometry: scientific review. JAMA. 2002;288:1889-97.
21. Kanis JA, Oden A, Johnell O, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. 2007;18:1033-46.
22. Hillier TA, Cauley JA, Rizzo JH, Pedula KL, Ensrud KE, Bauer DC, et al. WHO absolute fracture risk models (FRAX): Do clinical risk factors improve fracture prediction in older women without osteoporosis? J Bone Miner Res. 2011;26:1774-82.
23. The Advisory Board of the National Osteoporosis Guideline Group, Kanis JA, Harvey NC, Cooper C, Johansson H, Odén A, et al. A systematic review of intervention thresholds based on FRAX: A report prepared for the National Osteoporosis Guideline Group and the International Osteoporosis Foundation. Arch Osteoporos [2016 Dec. consultado en: http://link.springer.com/10.1007/s11657-016-0278-z
24. Masud T, Binkley N, Boonen S, Hannan MT. Official positions for FRAX® clinical regarding falls and frailty: can falls and frailty be used in FRAX®? J Clin Densitom. 2011;14:194-204.
25. Grisso JA, Kelsey JL, Strom BL, Ghiu GY, Maislin G, O'Brien LA, et al. Risk factors for falls as a cause of hip fracture in women. N Engl J Med. 1991;324:1326-31.
26. Hippisley-Cox J, Coupland C. Predicting risk of osteoporotic fracture in men and women in England and Wales: prospective derivation and validation of QFracture scores. BMJ. 2009;339:b4229.
27. Johansen A. QFracture is better than FRAX tool in assessing risk of hip fracture. BMJ. 2012;345:e4988.
28. Nguyen ND, Frost SA, Center JR, Eisman JA, Nguyen TV. Development of prognostic nomograms for individualizing 5-year and 10-year fracture risks. Osteoporos Int. 2008;19:1431-44.
29. Leslie WD, Lix LM. Comparison between various fracture risk assessment tools. Osteoporos Int. 2014;25:1-21.
30. van Geel TACM, Eisman JA, Geusens PP, van den Bergh JPW, Center JR, Dinant G-J. The utility of absolute risk prediction using FRAX® and Garvan Fracture Risk Calculator in daily practice. Maturitas. 2014;77:174-9.

Blanch J[1], Nogués X[2], Moro MJ[3], Valero MC[4], del Pino-Montes D[5], Canals L[6], Lizán L[7]

1 Servicio de Reumatología - Hospital Universitario del Mar - IMIM (Instituto Hospital del Mar de Investigaciones Médicas) - Barcelona (España)

2 Servicio de Medicina Interna - IMIM (Instituto Hospital del Mar de Investigaciones Médicas) - Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF) - Instituto de Salud Carlos III FEDER - Barcelona (España)

3 Servicio de Medicina Interna - Hospital Universitario Infanta Leonor - Madrid (España)

4 Servicio de Medicina Interna - Hospital Universitario Marqués de Valdecilla - Santander (España)

5 Servicio de Reumatología - Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF) - Instituto de Salud Carlos III FEDER - Salamanca (España)

6 Laboratorios Amgen S.A. - Barcelona (España)

7 Outcomes'10 - Universidad Jaume I - Castellón (España)

Medical care circuits for postmenopausal patients in Spain

DOI: http://dx.doi.org/10.4321/S1889-836X2017000200003

Correspondence: Josep Blanch - Hospital del Mar - Passeig Marítim, 25-29 - 08003 Barcelona (Spain)
e-mail: 20143@parcdesalutmar.cat

Date of receipt: 10/10/2016
Date of acceptance: 12/02/2017

Summary

Objectives: To reach a consensus on the medical care circuits of patients with postmenopausal osteoporosis (PMO), including derivation and management (assessment tools and medical tests), identifying profiles according to the opinion of bone metabolism experts, from Spain's Health Service.

Material and methods: The Delphi technique was used with two successive consultation rounds, with 38 experts in PMO management belonging to 14 scientific societies taking part in the study. Review of literature and the opinion of the scientific committee rounded out the questionnaire. The experts expressed their "desire" (1=total rejection, 9=stronger desire) and "forecast" (1=will absolutely not occur; 9=will occur with maximum probability) about the issues raised. A consensus was reached when 75% or more of the participants scored 1-3 (disagreement) or 7-9 (agreement). In addition, experts were divided up into 3 discussion groups to complement the information according to patient profiles found previously in the Delphi method.

Results: Consensus was reached on 75% of the questions. The experts established three profiles of PMO patients: no fracture, vertebral fracture and non-vertebral fracture, as well as the diagnostic and therapeutic resources recommended for these patients.

The patient without a fracture should be managed in Primary Care or Rheumatology and scales will be used to evaluate fracture risk in early stages of the disease. The patient with chronic vertebral fracture should refer to Rheumatology and Rehabilitation, and will be Rheumatology, whereas the patient with acute vertebral fracture should be treated in Orthopedic Surgery, and this is how it will possibly happen. Diagnosis of vertebral fracture patients will be based mainly on x-rays.

To assess progress, questionnaires on the functional capacity and pain scales are recommended. However, these will not be used due to the lack of time involved. The patient with non-vertebral fracture should be and will be referred to Orthopedic Surgery, with 3-4 radiographs recommended to ensure fracture consolidation.

Conclusions: Delphi method results indicate that referral of PMO patients are concentrated in Primary Rheumatology, when there is no fracture, and Orthopedic Surgery, in the case of fracture.

Key words: *postmenopausal osteoporosis, vertebral fracture, non-vertebral fracture, derivative circuits.*

Introduction

Osteoporosis is a global health problem with clinical, economic and social consequences that mainly affect postmenopausal women[1]. More than 200 million people have osteoporosis, and the aging of the population may increase in this prevalence[2].

The most significant clinical manifestations of osteoporosis are fragility fractures, especially those of the hip, spine, forearm and humerus. However, other fractures in patients older than 50 years are considered osteoporotic, including tibia, pelvis and femur[3].

In Europe, in 2000, an incidence of 3.1 million osteoporotic fractures was estimated in men and women over 50 years of age, with 620,000 hip fractures, 574,000 in the forearm, 250,000 in the proximal humerus and 490,000 vertebral fractures, among others, representing 34.8% of all osteoporosis fractures worldwide[3]. In 2010, the number of new fractures amounted to 3.5 million, and this number is expected to increase by 28%, with 4.5 million fractures in 2025[1].

In Spain, 35% of women over 50 years of age are affected by osteoporosis, a percentage that increases to 52% in those older than 70 years[4]. Additionally, almost 50% of women with postmenopausal osteoporosis (PMO) present one or more risk factors for osteoporotic fractures[5], which explains an estimated incidence of 250,000 osteoporotic fractures per year, representing direct and indirect costs of osteoporotic fractures. 126 and 420 million euros, respectively[6].

Spain is one of the countries with one of the most efficient National Health Systems, offering two well differentiated levels of care, Primary Care (PA) and Specialized Care. In general, Primary Care is the gateway to the system, except in the case of emergencies. However, given the decentralization of health services in each of the Autonomous Communities, the coordination between these two levels of care may not be as homogeneous as expected[7].

Rheumatology (RHEU), Obstetrics and Gynecology (GYN) and Orthopedic Surgery and Traumatology (OST) are some of the specialties involved in the management of PMO. However, there is little national or international information on the referral circuit for patients with osteoporotic fractures and the professionals involved[8,9]. The lack of consensus on referral protocols between specialized units in the management of different profiles of patients with PMO has revealed the importance of defining roles and establishing joint action protocols between specialties[10,11]. The absence of these protocols may make it difficult to establish adequate treatments and obtain clinical benefits for patients[8,12].

In qualitative research, there are different methodologies available to generate discussion among experts that results in the convergence of opinions and the deduction of consensus. The Delphi technique is an efficient technique for exploring policy issues, with the aim of organizing communication between groups to reach consensus on a particular topic[13-15]. On the other hand, the discussion group is a methodology that allows exhaustive approaches to a specific topic of study, where participants' perceptions facilitate in-depth understanding of the issues under study, based on the experiences and beliefs of the participants[16-18].

The aim of this study was to reach a consensus on the medical care circuits of the patient with PMO, including the circuits of derivation and management (evaluation tools and medical tests), identifying profiles according to the opinion of experts in bone metabolism who work in Spain's Health System.

Material and methods

The Delphi technique was used with two successive rounds of consultation. In addition, three discussion groups, according to the profile of the patient with PMO, were carried out to complement the conclusions reached by this method (Table 1). These societies were responsible for selecting the participants in the study, according to the following criteria: working in the Spanish National Health System, experience related to PMO and availability to participate in the study. Thirty-eight medical specialists, experts in the clinical and therapeutic management of patients with PMO, with extensive experience in PMO prevention, diagnosis, treatment and follow-up in Spain's public health system were invited to participate. These experts belonged to different medical specialties: PC (n=6), OST (n=6), Endocrinology and Nutrition (END) (n=3), Geriatrics and Gerontology Rehabilitation (REH) (n=3), Internal Medicine (MI) (n=5), GIN (n=6) and REU (n=6). None of the participants received remuneration for responding to the questionnaire.

The Delphi Survey Method

The Delphi technique is a consensus method whose goal is to achieve general agreement or convergence of opinion on a particular topic. It is based on a highly-structured group interaction to collect data through self-completed questionnaires by participants[19].

The questionnaires used during the two consultation rounds were elaborated and designed by the coordinating team of the study, under the supervision of the scientific committee or study group of the study, made up of 6 medical professionals with extensive experience, either in the management of the patient with PMO or in the study methodology. They were a series of questions that the interviewee had to rate according to a Likert scale. The content of the statements came from the systematic review of the literature[8] and contributions from the scientific committee (Figure 1). Likewise, time was allotted so participants could comment and make suggestions on the issues raised.

The questionnaire used during the first round consisted of 35 questions, each consisting of 1 to 10 questions. The issues were organized into 5

blocks: general aspects; PMO (primary prevention, diagnosis, treatment, follow-up and rehabilitation of the patient); Fractures in PMO patients (diagnosis and outpatient fracture management, fracture hospital admission and prevention of a second fracture); derivation circuits; and observations and comments. The questions explored different aspects associated with the prevention, diagnosis, treatment and follow-up of the patient with PMO with and without fracture, as well as the criteria that should be followed to derive patients among professionals.

According to the different profiles of patients with PMO (with and without fractures) and their clinical situation, the questionnaire presented various referral circuits in a way that defined the specialties that should be involved in its driving. In addition, the use of assessment tools and medical tests was also explored in these patient groups.

Participants rated the questions on a 9-point Likert scale, according to each of the questions presented, from two perspectives: "desire" (1=total rejection, 9=strongest desire) and "prognosis" (1=no will occur at all, 9=will occur with maximum probability). A consensus was reached when at least 75% of the participants scored the questions between 7-9 (agreement) or between 1-3 (disagreement) (Figure 2).

The questionnaire used during the second round was individually designed for each of the experts. It contained those issues for which no consensus was reached during the first round, as well as the suggestions made by participants. The questionnaire presented the participant's own individual scores and the position described by the majority of the group (rank in which was the highest percentage of answers), for each of the questions. After considering these qualifications, the respondents re-scored the questions, having the opportunity to either re-award the previous grade or modify their initial responses in accordance with the results shown, in order to reach a consensus at the maximum number of questions. Thirty-seven experts participated in the second round, since one of the subjects decided not to continue in the study.

The questionnaire used during the first round of the Delphi method was answered using a restricted access web platform (June 2011), while the second-round questionnaire was sent and received via email (September 2011).

Study Groups

Taking into account the conclusions obtained through the Delphi technique, and to define and complement them with aspects not explored in

Table 1. Scientific Societies collaborated in this study

Spanish Society of Bone and Mineral Metabolism Research (SEIOMM)
Spanish Society of Rheumatology (SER)
Spanish Association for Research into Menopause (AEEM)
Study Group on Osteoporosis of the Spanish Society of Orthopedic Surgery and Traumatology (GEIOS-SECOT)
Spanish Society into Osteoporotic Fractures (SEFRAOS)
Spanish Society of Endocrinology and Nutrition (SEEN)
Ibero-American Society of Osteology and Mineral Metabolism (SIBOMM)
Osteoporosis Study Group of the Spanish Society of Internal Medicine (GTO-SEMI)
Spanish Society of Family and Community Medicine (SEMFYC)
Spanish Society of Primary Care Physicians (SEMERGEN)
Spanish Society of General and Family Physicians (SEMG)
Spanish Society of Rehabilitation and Physical Medicine (SERMEF)
Spanish Society of Geriatrics and Gerontology (SEGG)
Hispanic Foundation of Osteoporosis and Metabolic Diseases (FHOEMO)

detail, three discussion groups were held with the participating experts. Three meetings were defined according to the three profiles of PMO patients that emerged from Delphi responses. Each discussion group consisted of 6 to 8 experts, according to the most representative specialties due to their involvement in each of the profiles (Table 1):

a) patient with PMO without fracture: AEEM (n=1), SEMERGEN (n=1), SEMFYC (n=1), SIBOMM (n=1), SEMG (n=1), y SEIOMM (n=1).
b) patient with PMO with vertebral fracture: SEIOMM (n=1), FHOEMO (n=1), SEEN (n=1), GTO-SEMI (n=2), y SER (n=1).
c) patient with PMO with nonvertebral fracture: SECOT-GEIOS (n=2), SEFRAOS (n=2), SEGG (n=2), y SERMEF (n=2).

Our aim was to explain and define the habitual referral circuits of the patient with PMO according to the specialties available in each center, and to specify the frequency of use of assessment tools and medical tests during the follow-up of the PMO according to the profile of the patient. Participants in the Delphi method were invited to take part in the discussion groups according to the specialties mainly involved in managing each patient profile: GIN n=2, IM n=1, PC n=3); Patient with vertebral fracture (IM n=3, REU n=2, END n=1); Patient with non-vertebral fracture (OST n=4, GER n=2, REH n=2).

Figure 1. Diagram of the study methodology

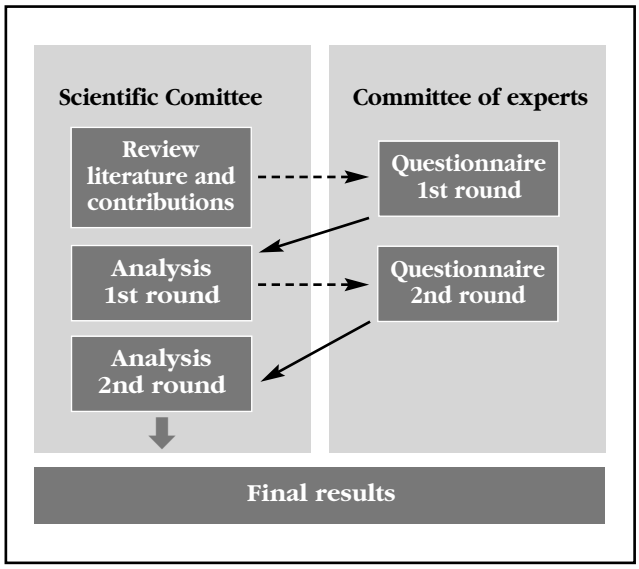
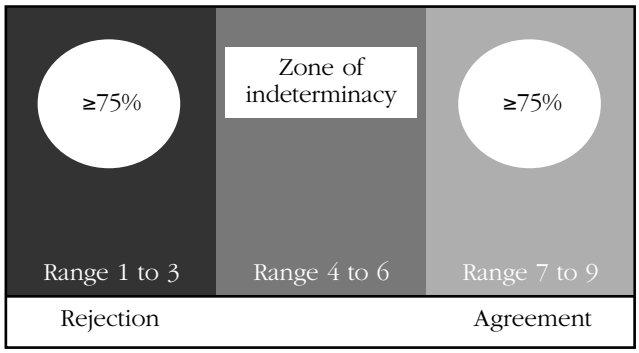


Figure 2. Definition of consensus



Results

In all, 100% (n=38) of the experts invited to participate in the study responded to the questionnaire in the first round, whereas 97.4% (n=37) did so during the second round. The experts participating in the study had an average of 24 years (SD=9) of experience in the clinical practice of their specialty, an average of 18 years (SD=8) involved in the management of patients with PMO and visited a median of 40 patients with PMO per month (Range: 10 - 200).

A consensus was reached in 75% of the questions posed by the Delphi technique, 73.6% from the "desire" perspective and 76.4% from the "prognosis" perspective.

As a result of the comments provided by the Delphi participants in the space provided in the questionnaire for this purpose, three distinct profiles of patients with PMO were identified: patients without fracture, those with vertebral fracture and those with non-vertebral fracture.

Patient with PMO without fracture

Derivation Circuits

In Delphi, experts reached consensus that AP (83.3%) and Rheumatology (77.8%) should be the specialties preferably involved in managing patients with PMO without a fracture, without achieving a consensus in the "prognosis" (Figure 3). Additionally, in the discussion group it was detailed that, in clinical practice, the high prevalence of this patient profile implies that the described specialists would not be able to treat the entire population, so these patients should be managed by PA when Possible without being referred to other specialties. Moreover, GER should be the specialty responsible for the management of elderly patients when possible, and in case of absence of Geriatrics Service in the health center, these patients should be followed by PA.

Regarding clinical situations such as early symptomatic or surgical menopause, Delphi demonstrated that these patients should be and will be referred to Gynecology (86.5% and 83.3%, respectively) (Figure 3). The discussion group added that in the event that this clinical situation is associated with thyroid disease, the patient should be referred to NDT.

In the Delphi consultation, the experts reached consensus in both "desire" (78.4%) and "prognosis" (75.78%) that REU should be and will be the reference specialty of patients with PMO and high risk of fracture. The discussion group argued that AP should be the reference specialty, but in case patients require specific treatment or monitoring that cannot be assumed by PA, the reference specialty would become Rheumatology or a specialized referral unit (when available).

When patients with PMO present a significant loss of bone mineral density despite receiving pharmacological treatment, REU should be (81.1%) and (88.9%) the reference specialty; While in patients with poor physical condition, muscle weakness, functional restriction, risk of falls, need for orthopedic evaluation, vertebral deviation or chronic refractory incapacitating pain, Delphi participants pointed out that REH should be the reference specialty (up to 80%), Without reaching consensus in the "prognosis" perspective (Figure 3).

Assessment Tools

The experts indicated that the osteoporosis evaluation scales should be used (89.2%), without reaching a consensus in the "prognosis". In addition, therapeutic adherence (89.2%), fracture risk (88.9%) and functional capacity (78.4%) should be evaluated during follow-up of patients with PMO, reaching consensus in the "prognosis "Only with respect to the use of fracture risk scales (75.7%). In addition, the discussion group recommended and specified the frequency with which these tools should be administered: the Morisky-Green questionnaire should be used to assess adherence one month after the start of treatment and during each follow-up visit; FRAX® or QFracture® would be used to assess the risk of fractures in the early stages of the disease (prior to initiating drug treatment); The functional capacity would be evaluated during the initial visit and annually, without specifying any specific tool (according to availability); Analogue visual scales for examining pain should be applied as often as possible. It was commented that no tool is usually used to evaluate satisfaction with treatment, indicating that it is usually not evaluated; No specific instrument is used to assess health-related quality of life (HRQL), indicating that it is only assessed during clinical research. In all cases, the results of these evaluations should be incorporated into the patients' medical records.

Medical Tests

Regarding medical tests, the results of the Delphi method showed that the bone densitometry, used for the evaluation of the evolution of the PMO, should be performed in periods less than two years. However, no consensus was reached on the definition of a specific period. For its part, the discussion group specified that it would be necessary to perform a bone densitometry and a dorso-lumbar x-ray every two years and to measure the size of the patient at each visit.

Patient with PMO and vertebral fracture

Derivation Circuits

In patients with acute vertebral fracture, the Delphi consultation indicated that OAT should be the reference specialty from both perspectives ("desire": 86.5% and "prognosis": 80.6%) (Figure 3). The discussion group established that in case of hospital admission, OST should be the specialty, but that the management of the patient with PMO should be under the responsibility of REU, IM or GER, or of a Bone Metabolism Unit or Fracture Liaison Service (FLS), where available.

In patients with chronic vertebral fractures, the experts pointed out in the Delphi that REU (83.8%) and REH (77.8%) should be the reference specialties. However, all patients will be referred to REU (75%) (Figure 3). The discussion group indicated that the patient diagnosed with PMO with chronic vertebral fracture should be managed by REU and REH.

In addition, in the discussion group, the experts mentioned that COT together with the specialists in bone metabolism should diagnose the vertebral fracture. Additionally, if the patient needed hospitalization, it would require multidisciplinary units (Fracture Unit or FLS). The experts detailed that these units should consist mainly of specialists in TOC and bone metabolism, as well as by REH, Pain Unit (if available) and GER or IM (for the management of clinical situations that are not exclusive to bone metabolism, such as co-morbidities) (Figure 4). As for outpatient management of vertebral fractures (including treatment), the Primary Care unit should be responsible, should there be experienced staff. Otherwise, the patient should be referred to an expert in bone metabolism. Finally, the specialist diagnosing vertebral fracture should be involved in the prevention of subsequent fractures.

The discussion group also pointed out that REH should be the reference specialty in the case of patients with vertebral fracture and functional restriction secondary to immobilization and pharmacological treatment, or if orthopedic measures are required. Those patients with vertebral fracture and chronic pain refractory to pharmacological treatment should be managed by two groups of specialists, Unit of Pain (or IM, depending on availability) in coordination with REH for pain management and by REU in coordination with IM or experts in bone metabolism (depending on hospital availability).

Assessment Tools

For the evaluation of the progression of the patient with fracture, questionnaires should be used on functional capacity (83.9%) and pain scales (80.7%); However, in Delphi, no consensus was reached on the "prognosis". The discussion group explained that functional capacity, pain and HRQoL are usually measured in the clinical research setting, but in standard practice this involves substantial time investment, although it is considered to be very useful.

Medical Tests

The diagnosis of fracture should be based on radiographs (97.3%), symptoms (89.2%), physical examination (86.5%) and medical history (83.7%). From the "prognosis" perspective, the experts mentioned that the diagnosis of fracture will be based on radiographs (91.9%), symptoms (83.8%), and physical examination (83.8%). The discussion group concluded that the most important medical tests to evaluate the patient with vertebral fracture should include radiographs and bone densitometry during the first year and size (measured by stadiometer) at each medical visit. Subsequently, a bone densitometry every two or three years.

Patient with PMO and without vertebral fracture

Derivation Circuits

In the Delphi consultation, OST was mentioned as the reference specialty for patients with non-vertebral fracture (hip or distal radius) and with acute femoral fracture (100% for both perspectives), and for patients with fractures in other locations ("desire": 91.7% and "prognosis": 94.4%) (Figure 3).

The discussion group established that during the acute phase OST should be the reference specialty. The diagnosis of non-vertebral fracture in patients with PMO should be performed by OST in such a way that the severity of the fracture can be assessed and appropriate treatment and rehabilitation recommended. Hospital admission requires multidisciplinary units (Fracture Unit or FLS) that include OST, REH, GER or IM, Social Services and Nursing (Figure 5). Primary care should be involved in the outpatient management of these patients once the acute process has been controlled. In addition, PC should be the specialty in charge of preventing successive non-vertebral fractures.

Assessment Tools

Experts agreed in the focus groups that functional capacity should be systematically evaluated until stability is achieved. Minimal revisions should be made at the beginning, during and at the end of the fracture process. The evaluation of HRQoL should be done systematically, although the experts recognize that it consumes a lot of consultation time.

Medical Tests

The experts recommended 3 to 4 radiographs per year (first, third, sixth and twelfth month), especially when the fractures are located in the hip or tibia, in such a way as to ensure consolidation of the fracture after discharge.

Discussion

This study provides new information on referral circuits and specialties that should be involved in the management of patients with PMO with and without fracture. To define reference criteria between specialties, it has been shown that a distinction should be made between the type and location of fractures, defining three patient profiles: patients without fracture, vertebral fracture, and non-vertebral fracture.

The most significant conclusion obtained from the consensus is the importance of defining bypass circuits that should be followed during each phase of the management of patients with PMO according to the profile of each patient and their clinical situation. However, discussion groups have pointed out that in clinical practice the selection of the referring physician and the patient care process depends on the local availability of the services.

The high prevalence of patients with PMO without fracture makes it difficult for specialties such as REU to assume full responsibility for their management; Therefore, much of the attention to this population is delegated to Primary Care. However, the extensive knowledge required for this task and the constant overload of work to which PC professionals are subjected means that monitoring of patients with PMO in clinical practice depends greatly on the situation of the health center, staff availability and professional experience[20].

Regarding patients with PMO and vertebral fracture, the experts point out that it is necessary to involve different specialties, highlighting the role of orthopedic surgeons and experts in bone metabolism in the diagnosis of fracture. The availability of experts in bone metabolism, defined as "the specialists with the most knowledge about osteoporosis in the health center", will depend on the local situation of each center. From the point of view of patient management, the acute or chronic nature of the fracture leads us to consider a distinction of roles between the different specialties. In the consensus of experts, the OST should be and will continue to be the reference specialty in the case of acute fracture. On the other hand, in the supplementary discussion groups, experts emphasize the importance of OST in those patients requiring hospital admission, whereas REU, IM or GER and Bone Metabolism Units would be the specialties of choice for fracture management. Finally, REU and REH would be the reference specialties for patients with chronic fractures. This may be explained by the differences of opinion in the experts regarding the management of the PMO itself, which requires specialists with high knowledge on osteoporosis, its treatment and associated comorbidities; as well as in terms of fracture management, which will require knowledge about fracture treatment and pain control, a distinction of knowledge clearly identified in the literature[21,22].

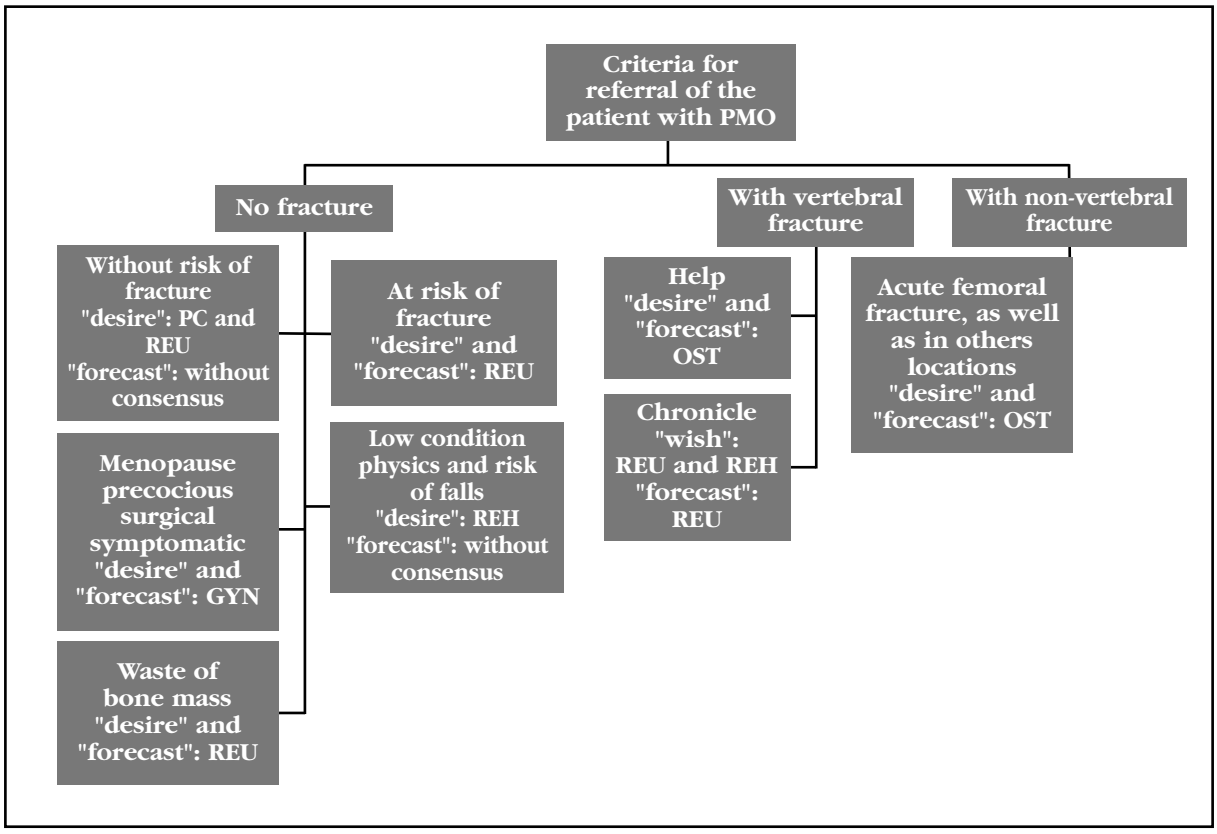
Furthermore, patients with non-vertebral fracture should be referred to OST for stabilization of the fracture, but Primary Care should be responsible for its management and follow-up once the acute process is completed. Another fundamental aspect identified by the experts is the need to create multidisciplinary units for the management of patients with PMO and fractures, particularly during hospital admission (Fracture Unit or FLS). The creation of these multidisciplinary teams could be useful in the design of new strategies to optimize the use of health resources and improve the clinical management of patients with PMO[23,24]. Fracture Units or FLS provide clinically and cost-effective care in patients with osteoporosis with fragility fractures[25]. In Glasgow, UK, the Fracture Unit has contributed to a 7.3% reduction in hip fractures over 10 years, compared with a 17% increase in England25. In Italy, the implementation of a Fracture Unit made up of multidisciplinary teams has been shown to reduce major complications from 21% to 45%, while readmissions to the hospital at 6 months decreased by 20% and the mortality rate by 3%[26]. Patients treated at the Fracture Unit in the Netherlands had a significantly lower mortality and a lower risk of non-vertebral fractures than those not treated in this service, with a reduction of 35% and 56%, respectively, for more than two years follow-up[27]. Therefore, the Fracture Unit or FLS seems to be a successful

method for reducing the number of subsequent fractures and premature mortality after fracture.

Coordination among specialists is paramount during the management of patients with PMO and fractures, since the specialist who must assume patient management once the fracture has consolidated is still not defined. This lack of standardization of roles of each specialist may be associated with a delay in the treatment of this patient profile[12]. Therefore, programs are needed for the detection and study of patients with fractures, who establish guidelines for care and follow-up[28].

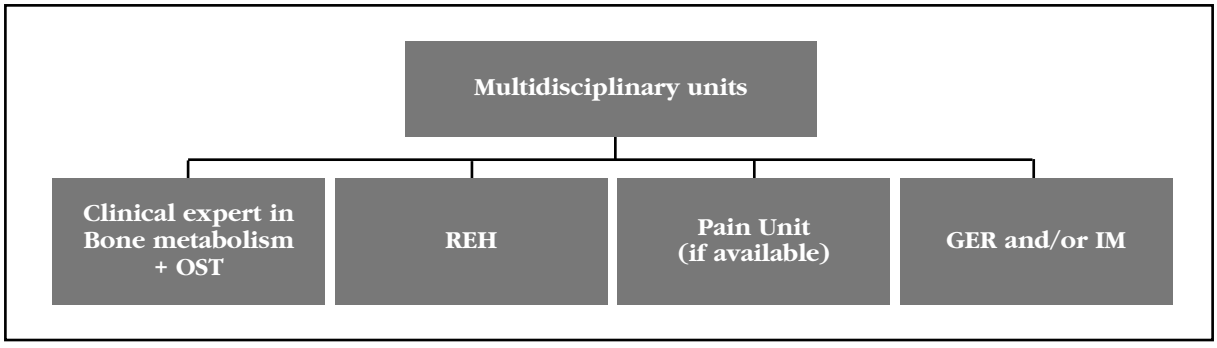
Finally, another aspect highlighted in this study is the need to use and standardize evaluation tools to explore the evolution of the patient with PMO, as well as the risk of fractures, functional capacity, pain, therapeutic adherence, treatment satisfaction or HRQL. Regarding this last aspect, the experts identified the work overload as the cause of the insufficient use of HRQL measurement instruments in the usual clinical practice. However, the recommendations arising from the discussion groups allow establishing minimum requirements for the future.

Figure 3. Consensus reached ("desire" and "prediction") in the Delphi Method concerning the derivation circuits of the patient with PMO



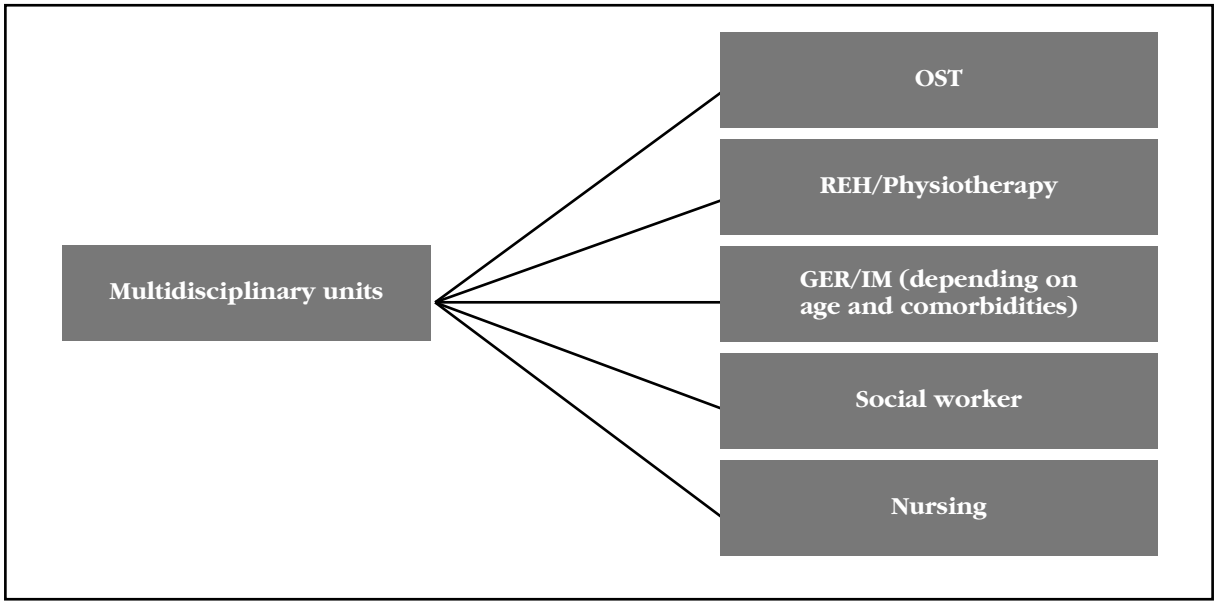
PMO: postmenopausal osteoporosis; PC: Primary Care; OST: Orthopedic Surgery and Traumatology; REU: Rheumatology; REH: Rehabilitation; GYN: Obstetrics and Gynecology.

Figure 4. Specialties proposed in the study group of experts for the formation of multidisciplinary units in managing the patient with vertebral fracture



OTS: Orthopedic and Traumatology Surgery; REH: Rehabilitation; GER: Geriatrics and Gerontology; IM: Internal Medicine.

Figure 5. Specialties proposed in the discussion group of experts for the formation of multidisciplinary units in the management of patients with non-vertebral fractures that require hospitalization



OST: Orthopedic Surgery and Traumatology; REH: Rehabilitation; GER: Geriatrics and Gerontology; IM: Internal Medicine.

The study is subject to the advantages and disadvantages of the consensus technique used[29,30]. The characteristics of the Delphi technique allow minimizing reciprocal influence among the participants and allow a good functioning with a heterogeneous group of participants, also preserving their anonymity[19]. The participation of physicians of different specialties involved in the management of patients with PMO reflects the usual practice and provides extensive information on the clinical and therapeutic management of osteoporosis. However, the panel of experts may not necessarily be representative of the usual clinical practice in Spain, given the differences between Autonomous Communities. Thus, the information presented must be analyzed in context, since the data included represent the Spanish population and may not be extrapolated to other populations. Another limitation of this study is that the list of items presented in the questionnaires reflects the scientific evidence and the opinion of the experts at the time of its elaboration and may require an update as soon as new scientific information on the management of patients with PMO. However, there are still gaps in the medical care of the patient with fracture and the referral of patients with difficulties to the corresponding specialists[31]. Therefore, the information provided by this study contributes to the literature on managing patients with PMO. It highlights the multiple opportunities for improvement in the field of follow-up of patients with PMO.

On the one hand, the lack of referral circuits per patient profile in each department or health area and, on the other hand, the need to specify the criteria for conducting clinical tests and evaluating patient-centered results for each profile. In conclusion, the information gathered in both Delphi and in the discussion groups provides a guide to optimize patient care with PMO in Spain's Health System.

Acknowledgments: The authors wish to express their gratitude for their participation in the study: Dr. Rafael Sánchez-Borrego, member of the Spanish Association for the Study of Menopause (AEEM); Dr. Manuel Díaz Curiel, member of the Hispanic Foundation of Osteoporosis and Metabolic Diseases (FHOEMO); Dr. Manuel Mesa, member of the Spanish Society of Orthopedic Surgery and Traumatology (SECOT); Dr. Esteban Jodar, member of the Spanish Society of Endocrinology and Nutrition (SEEN); Dr. Pedro Carpintero, member of the Spanish Society of Osteoporotic Fractures (SEFRAOS); Dr. Carmen Navarro, member of the Spanish Society of Geriatrics and Gerontology (SEGG); Dr. Carmen Valdés, member of the Spanish Society of Primary Care Physicians (SEMERGEN); Dr. Vicente Giner, member of the Spanish Society of Family and Community Medicine (SEMFYC); Dr. José Carlos Bastida, member of the Spanish Society of General Practitioners and Family (SEMG); Dr. José Luis Pérez, member of the Spanish Society of Internal Medicine (SEMI); Dr. Alberto Garcia, member of the Spanish Society of Rheumatology (SER); Dr. Elena Martínez, member of the Spanish Society of Rehabilitation and Physical Medicine (SERMEF); And Dr. Santiago Palacios, member of the Ibero-American Society of Osteology and Mineral Metabolism (SIBOMM).

Funding: This study was funded by Amgen S.A. laboratories.

Conflict of Interest: Authors Javier del Pino Montes, Josep Blanch, Xavier Nogués, María Jesús Moro and María del Carmen Valero declare that they have no conflict of interest in this study. Laura Canals works in Amgen S.A. Luis Lizán works for an independent research institution and was paid compensation for his contribution in developing and coordinating the original Research Project, as well as for the drafting of this manuscript.

Bibliography

1. Hernlund E, Svedbom A, Ivergård M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. Arch Osteoporos. 2013;8:136.
2. Reginster JY, Burlet N. Osteoporosis: a still increasing prevalence. Bone. 2006;38:4-9.
3. Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzoli R, Reginster JY, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int. 2013;24;23-57.
4. Naves M, Díaz-López JB, Gómez C, Rodriguez-Rebollar A, Cannata-Andia JB. Determinants of incidence of osteoporotic fractures in the female Spanish population older than 50. Osteoporos Int. 2005;16:2013-17.
5. Palacios S, Neyro JL, Puertas JC, Fernandez de Cabo. Clinical profile of Spanish postmenopausal women with a diagnosis of osteoporosis and risk factors for endometrial pathology, breast cancer and cardiovascular disease. Menopause. 2013;20:852-59.
6. Del Pino-Montes J. Epidemiología de las fracturas osteoporóticas: las fracturas vertebrales y no vertebrales. Rev Osteoporos Metab Miner. 2010;2:8-12.
7. Ojeda Feo JJ, Freire Campo JM, Gervás Camacho J. La coordinación entre Atencion Primaria y Especializada: ¿reforma del sistema sanitario o reforma del ejercicio profesional? Rev Adm Sanita. 2006;4(2):357-82.
8. Del Pino-Montes J, Blanch J, Lizán L, Marín N. Paciente con fractura por osteoporosis posmenopáusica en España: circuito de atención médica. Rev Osteoporos Metab Miner. 2012;41:27-35.
9. Quintana O, Torres M. Osteoporosis: una mirada hacia el futuro desde atención primaria. Rev Osteoporos Metab Miner. 2014;6(4):79-82.
10. Sheehan J, Mohamed F, Reilly M, Perry IJ. Secondary prevention following fractured neck of femur: a survey of orthopaedic surgeons practice. Ir Med J. 2000;93:105-7.
11. Feldstein AC, Schneider J, Smith DH, Vollmer WM, Rix M, Glauber H. Harnessing stakeholder to improve the care of osteoporosis after a fracture. Osteoporos Int. 2008;19:1527-40.
12. Skedros JG. The orthopaedic surgeon role in diagnosing and treating patients with osteoporotic fractures: standing discharge orders may be the solution for timely medical care. Osteoporos Int. 2004;15:405-10.
13. De Loe RC. Exploring complex policy questions using the policy Delphi. A multi-round, interactive survey method. Applied Geography. 1995;15:53-68.
14. Powell C. The Delphi technique: myths and realities. J Adv Nurs. 2003;41:376-82.
15. Hsu CC, Sandford BA. The Delphi Technique: Making Sense of Consensus. Practical Assessment Research & Evaluation. 2007;12:1-8. Available online: http://pareonline.net/getvn.asp?v=12&n=10.
16. Morgan DL, Krueger RA editors. The Focus Group Kit. Thousand Oaks (CA): Sage Publications; 1998.
17. Pope C, Mays N, editors. Qualitative Research in Health Care. London: BMJ books; 2000.
18. Green J, Thorogood N, editors. Qualitative Methods for Health Research. 2nd Edition. London: SAGE Publications; 2009.
19. McMillan SS, King M, Tully MP. How to use the nominal group and Delphi techniques. Int J Clin Pharm. 2016;38(3):655-62.
20. Naranjo A, Rosas J, Ojeda S, Salas E, grupo CANAL. [Management of osteoporosis in primary care before and after the result of densitometry: Treatments in real practice versus the recommended by guidelines. CANAL study]. Reumatol Clin. 2013;9:269-73.
21. Francis RM, Baillie SP, Chuck AJ, Crook PR, Dunn N, Fordham JN, et al. Acute and long-term management of patients with vertebral fractures. QJM. 2004;97:63-74.
22. Kim DH, Vaccaro AR. Osteoporotic compression fractures of the spine; current options and considerations for treatment. Spine J. 2006;6:479-87.
23. Madridejos R, Tomás R, Llobet E, Gavagnach M, Martínez S, Moyà X. Elaboration, implementation and follow-up of a postmenopausal osteoporosis protocol: Collaboration between the Primary care and Rheumatology departments. Reumatol Clin. 2010;6:128-33.
24. Jódar E. Identifying and Managing Patients at High Risk for Fractures. Conclusions from the Second Spanish Multidisciplinary Forum-Parathyroid Hormone Use in Osteoporotic Patients at High Risk for Fractures. Drugs R D. 2012;12:199-206.
25. Wallace I, Callachand F, Elliott J, Gardiner P. An evaluation of an enhanced fracture liaison service as the optimal model for secondary prevention of osteoporosis. JRSM Short Rep. 2011;10;2(2):8.
26. Falchetti A, Amedei A, Masi L, Giusti F, Cavalli L, Casentini C, et al. Fracture unit: a (possible) model of implementation in Italy. Clin Cases Miner Bone Metab. 2011;8(1):9-12.
27. Huntjens KM, van Geel TA, van den Bergh JP, van Helden S, Willems P, Winkens B, et al. Fracture liaison service: impact on subsequent nonvertebral fracture incidence and mortality. J Bone Joint Surg Am. 2014;96(4):e29.
28. Naranjo A, Ojeda-Bruno S, Bilbao Cantarero A, Quevedo Abeledo JC, Henríquez-Hernández LA, Rodríguez-Lozano C. Results of a model of secondary prevention for osteoporotic fracture coordinated by rheumatology and focused on the nurse and primary care physicians. Reumatol Clin. 2014;10(5):299-303.
29. Peiró S, Portella E. Consensus doesn't always mean agreement: limitations of consensus methods in health services. Gac Sanit. 1993;7:294-300.
30. Black N, Murphy M, Lamping D, McKee M, Sanderson C, Askham J, et al. Consensus development methods: a review of best practice in creating clinical guidelines. J Health Serv Res Policy. 1999;4(4):236-48.
31. Meeta, Harinarayan CV, Marwah R, Sahay R, Kalra S, Babhulkar S. Clinical practice guidelines on postmenopausal osteoporosis: an executive summary and recommendations. J Midl Health. 2013;4(2):107-26.

Pineda-Moncusí M[1], Rodríguez-Sanz M[1], Díez-Pérez A[1,2], Aymar I[1,2], Martos T[3], Servitja S[3], Tusquets I[3], García-Giralt N[1], Nogués X[1,2]

1 IMIM (Instituto Hospital del Mar de Investigaciones Médicas) - Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF) - Instituto de Salud Carlos III FEDER - Barcelona (España)

2 Departamento de Medicina Interna - Parque de Salud Mar - Universidad Autónoma de Barcelona - Barcelona (España)

3 IMIM (Instituto Hospital del Mar de Investigaciones Médicas) - Departamento de Oncología Médica - Medical Oncology Department - Parque de Salud Mar - Barcelona (España)

Genetic analysis of steroid pathway enzymes associated with adverse musculoskeletal effects of aromatase inhibitors

DOI: http://dx.doi.org/10.4321/S1889-836X2017000200004

Correspondence: Natalia García-Giralt - Institut Hospital del Mar d'Investigacions Mèdiques (IMIM) - Carrer del Dr. Aiguader, 88 - 08003 Barcelona (Spain)
e-mail: ngarcia@imim.es

Date of receipt: 20/12/2016
Date of acceptance: 09/03/2017

Work submitted as presentation fellowship of basic research FEIOMM 2012.

Summary

Objetives: Identify putative functional variants in the *CYP11A1* and *CYP17A1* genes associated with musculoskeletal effects (accelerated bone mass loss and arthralgia) derived from treatment with aromatase inhibitors (AI).

Material and methods: The B-ABLE cohort is a prospective study of postmenopausal women with breast cancer undergoing AI treatment. Bone mineral density in the lumbar spine and femoral neck was measured by densitometry and joint pain using visual analogue scale. From single-nucleotide polymorphisms (SNPs) in genes *CYP11A1* (rs4077581, rs11632698 and rs900798) and *CYP17A1* (rs4919686, rs4919683, rs4919687, rs3781287, rs10786712, rs6163, rs743572), previously associated with musculoskeletal events, haplotypes were constructed for each pacient from the cohort, and those haplotypes that showed greatest phenotypic differences were chosen ($p<0.05$). Within each haplotype, patients with extreme phenotypes were chosen for the sequencing of respective genes and identifying functional genetic variants. Finally, a multiple linear regression analysis was carried out considering the models of dominant, recessive and additive genetic inheritance.

Results: No mutation was found in coding regions. A genetic variant (D15S520), in the basal promoter region of gene *CYP11A1*, was found associated with femoral neck bone loss at 24 month of AI treatment.

Conclusions: Variants in regulatory regions of the *CYP11A1* gene could modulate the expression of this gene, thus explaining part of the phenotypic variability found in bone loss of patients undergoing AI treatment.

Key words: *aromatase inhibitors, breast cancer, arthralgia, bone mineral density, CYP11A1, CYP17A1, genetic association study.*

Introduction

The use of aromatase inhibitors (AI) as adjuvant therapy after surgery, and/or radiotherapy, and/or chemotherapy, has achieved a significant increase in survival in postmenopausal women diagnosed with breast cancer with hormone receptors (estrogen and/or progesterone) positive (HR), in the initial stages[1,2].

The action of aromatase on testosterone and androstenedione produces estradiol and estrone[3]. These two components constitute the main source of estrogen in postmenopausal women. This aromatization process is performed in peripheral tissues, such as adipose tissue and muscle. Approximately two-thirds of breast tumors have been shown to have aromatase activity, locally producing estrogens in the tumor itself that stimulate the growth of breast tumor cells[4]. AI directly blocks estrogen production in the tumor and also causes a drastic reduction in circulating estrogen levels[5].

Sustained estrogen deprivation due to AI therapy causes an accelerated loss of bone mass, increasing the risk of osteoporotic fracture[6]. AIs may also produce other adverse musculoskeletal effects, such as arthralgia and muscle pain, which may hinder adherence to therapy during the years of prescribed treatment[7,8].

Furthermore, patients treated with AI reportedly present a large inter-individual variability in the appearance and intensity of musculoskeletal symptoms, suggesting that there are factors that may increase their appearance. In this sense, vitamin D levels (Vit D) have been linked to the appearance of arthralgias[9]. Likewise, there is probably also a genetic basis that modifies, in part, the effect of AI. Several studies have linked genetic variants associated with increased pain and loss of bone mass in women treated with AI of the B-ABLE cohort[10,11].

Specifically, single nucleotide polymorphisms (SNPs) in the *CYP11A1* gene: rs4077581, rs11632698 and rs900798 were associated with loss of bone mineral density (BMD) at the femoral neck (FN) at 2 years of treatment with IA[11]. The *CYP11A1* gene encodes the cholesterol side chain cleavage enzyme (alternative name: P450scc) that catalyzes the first and limiting step of steroidogenesis, converting cholesterol to pregnenolone. In addition, P450scc can also hydroxylate vitamin D2, D3 and its precursors[12,13], suggesting a broad spectrum of functions in cellular metabolism.

On the other hand, seven SNPs of the *CYP17A1* gene (rs4919686, rs4919683, rs4919687, rs3781287, rs10786712, rs6163, rs743572) were associated with increased pain at 1 year of treatment with IA[10]. *CYP17A1* (17α-hydroxylase/17,20 lyase) is a key enzyme in the steroidogenic pathway that produces progestins, mineralocorticoids, glucocorticoids, androgens, and estrogens.

None of the SNPs of the *CYP11A1* and *CYP17A1* genes, previously genotyped, cause non-synonymous changes in protein, nor are they known to have any regulatory function of gene expression.

It is possible that functional variants of the genes involved in both the coding region that would modify enzyme activity and in regulatory regions that would regulate gene expression levels could be implicated in AI side effects. Therefore, the aim of this study is to identify putatively functional variants in the *CYP11A1* and *CYP17A1* genes.

Material and methods

Study population

The B-ABLE cohort (Barcelona-Aromatase induced Bone Loss in Early breast cancer) is the population of a prospective study that includes postmenopausal patients with RH positive breast cancer and treated at the Hospital del Mar de Barcelona. Participants receive AI (letrozole, exemestane or anastrozole) over 5 years, or alternatively after 2 or 3 years of treatment with tamoxifen (3 and 2 years of AI, respectively), according to the American Society of Clinical Oncology's recommendations, starting within 6 weeks post op or 1 month after the last cycle of chemotherapy[14].

Exclusion criteria were: alcoholism, grade 3b renal insufficiency, rheumatoid arthritis, bone metabolic diseases other than osteoporosis, Paget's disease, osteomalacia, primary hyperparathyroidism, hyperthyroidism, insulin-dependent diabetes mellitus, previous or ongoing treatment with antiresorptive agents, oral corticosteroids or any other drug that could affect bone metabolism except tamoxifen.

Measurements

Bone mineral density

At the outset and every 12 months until the end of treatment, levels of BMD at the lumbar (LS L1-L4), femoral neck (FN) and total hip (TH) were measured using the dual X-ray energy densitometer (DXA) QDR 4500 SL® (Hologic, Waltham, Massachusetts, USA). The variation coefficient for this technique in our center is 1% in LS and 1.65% in FN. Densitometries with artifacts, degenerative disc disease with osteophytes, osteoarthritis with hyperostosis of the facet joints, vertebral fractures and/or aortic calcifications, and all those that could cause a false increase in BMD, were excluded as in the description of Blake et al.[15]. It was then analyzed by the relative loss of bone mass.

Visual Analogue Scale

Joint pain was measured using the visual analogue scale (VAS), at baseline, at 3 months and then every 12 months until the end of the study. Joint pain was assessed: hands, shoulders, knees, hips, ankles and feet, on a scale of 1 to 10 with decimals. Subsequently it was analyzed by means of the VAS absolute change.

Demographic variables

Data were collected from a large number of clinical variables at the time of recruitment, including age, menarche and menopause ages, lactation time, number of deliveries, previous chemotherapy and

radiotherapy, adjuvant treatments, weight, smoking habits and calcium intake through the INDI-CAD survey[16].

Construction of haplotypes

Previous studies in the B-ABLE cohort genotyped SNPs located in the *CYP11A1* and *CYP17A1*[10,11] genes. SNPs that showed a statistically significant association with the evaluated phenotypes were chosen for the construction of haplotypes (Figure 1).

To establish the relationship of the haplotypes of the *CYP11A1* gene to the SNPs rs4077581, rs11632698 and rs900798 in the B-ABLE cohort, the haplotype frequencies were calculated with the haplo.em analysis and the most common haplotypes (frequency >0.01).

The *CYP17A1* gene haplotypes were constructed in the same manner with the SNPs rs743572, rs6163, rs10786712, rs3781287, rs4919687, rs4919686 and rs4919683. Each haplotype was assigned a code to facilitate its nomenclature during the study.

DNA Extraction and Sanger Sequencing

DNA extraction was performed from peripheral blood using the Wizard® Genomic DNA Purification Kit (PROMEGA). The coding regions, 5'UTR, 3'UTR and proximal promoter (up to -601 bp for *CYP11A1* and -589 bp for *CYP17A1)* were amplified with the primers described in Table 1.

Sequencing was performed using the Sanger method. The sequences were analyzed with the Sequence Scanner program (v1.0) and alignment with the reference sequence (NCBI Reference Sequence: *CYP11A1* NG_007973.1 and *CYP17A1* NG_007955.1) was carried out through the Multiple Sequence Alignment (EMBL-EBI).

Statistical analysis

The frequency of the *CYP11A1* and *CYP17A1* SNPs was estimated using the expectation-maximization algorithm. The association between haplotypes and phenotypes (change in BMD in CF and increased pain) was analyzed using the haplo.glm, based on glm regression analysis, adjusting for age, body mass index (BMI), previous tamoxifen therapy And chemotherapy. The most common haplotype was used as the reference haplotype and the additive model was assumed to obtain a p-value and the β-coefficient relative to the reference haplotype.

The potential differences between the characteristics of the patients selected according to their haplotype and with extreme phenotypes were evaluated with Student's t-test for independent samples.

The association between the genetic variants found in the sequencing and the extreme phenotypes were analyzed by multiple linear regression, contemplating dominant, recessive and additive genetic inheritance models.

All statistical analyzes were defined as significant with $P<0.05$. These were performed using the SPSS (version 22) and R for Windows (version 2.15.2) statistical programs using packages, foreign, rms, multtest, plyr, boot, haplo.stats and SNPassoc.

Ethics statement

The study protocols have been approved by the Ethical Committee for Clinical Research of the Marine Health Park (2013/5283/I). Approved protocols for obtaining DNA from blood samples were explained to potential participants, who signed an informed consent before being included in the study.

Results

Baseline characteristics of patients in the B-ABLE cohort

Table 2 shows the demographic characteristics, BMD values and the evolution of the musculoskeletal symptomatology by VAS, for the *CYP11A1* and *CYP17A1* genes, in which the haplotypes were constructed.

The scheme of the procedure to reach the final analysis of genetic association with extreme phenotypes of BMD and musculoskeletal symptomatology by VAS is shown in figure 2.

Construction of the haplotypes of the *CYP11A1* gene and the *CYP17A1* gene

Table 3 shows the constructed haplotypes and the association analysis of the *CYP11A1* and *CYP17A1* genes with the BM change in CF at 2 years and increased pain at 12 months of AI treatment, respectively.

In the *CYP11A1* gene, the haplotype that showed a major phenotypic difference with respect to the reference haplotype (11.1) was 11.2, where patients carrying haplotype 11.1 in homozygosity had a loss of BMD 4.41 times greater than haplotype carriers 11.2 in homozygosis (Table 4).

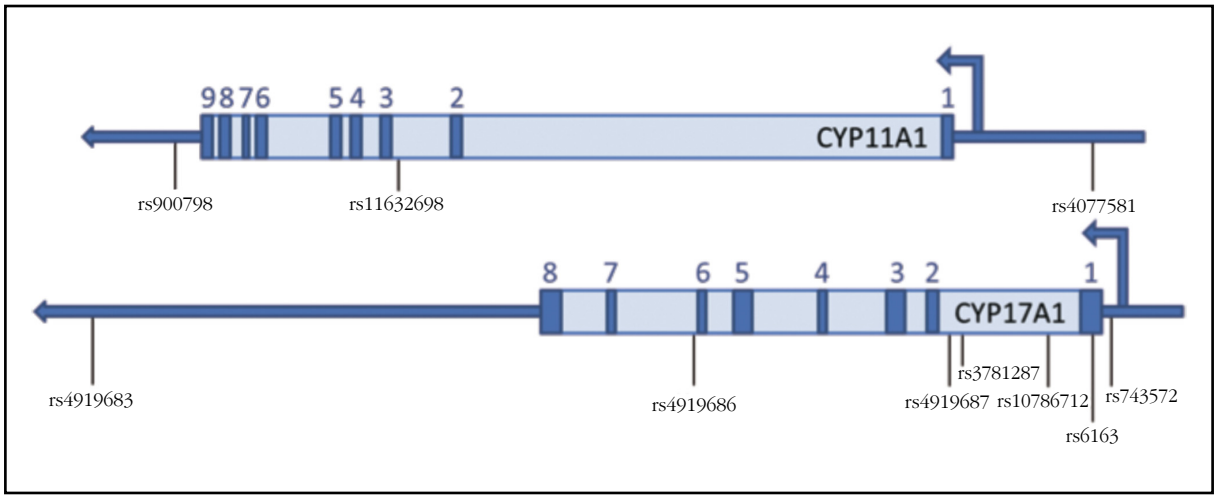
In the case of the *CYP17A1* gene, haplotypes 17.3 and 17.4 showed statistically significant differences with respect to the reference haplotype (17.1). Patients homozygous for haplotype 17.1 showed an increase in pain 3.26 times more than patients homozygous for haplotype 17.4 (Table 4).

Selection of patients for the genetic study by Sanger sequencing

Based on the results of the haplotype-association analysis, we selected patients from the B-ABLE cohort who had haplotypes (with a 99% probability) showing greater phenotypic differences: for the *CYP11A1* gene, The haplotypes 11.1 and 11.2 in homozygosis. For the *CYP17A1* gene, we selected patients with haplotypes 17.3 and 17.4, both in homozygosis and in heterozygosity. In addition, patients with haplotype 17.1 and any other haplotype (with the exception of 17.3 and 17.4) were selected (Figure 2 and Table 3).

Later, within each *CYP11A1* gene haplotype group, patients who showed an extreme phenotype in CF BMD (greater or less loss of BMD at 24 months of treatment) (n=40) were selected. The same procedure was performed for the haplotype groups of the *CYP17A1* gene in which patients with the extreme phenotype for arthralgia (greater or lesser pain increase at 12 months of treatment) (n=39) were selected (Table 5).

Figure 1. SNPs selected for the construction of haplotypes



Identification of genetic variants and analysis of association with extreme phenotypes

Following sequencing of the *CYP11A1* and *CYP17A1* genes, several SNPs were found in both genes. None of them corresponded to a non-synonymous change, or in splicing sites and, therefore, a change in the protein sequence was ruled out.

However, in the basal promoter region of the *CYP11A1* gene, a genetic variant (D15S520) associated with the BMD variation in CF at 24 months was found (Coefficient β=-6.32; 95% confidence interval (CI): [-8.55, -4.09], p=3.71e-06).

The D15S520 polymorphism is a microsatellite in the -373 bp position that is used as a genetic marker (Sequence Tagged Sites, STS) and consists of the tandem repeat of pentanucleotide (TAAAA) n. In our patients, the number of repetitions observed was 4, 6, 8 and 9.

The haplotype 11.1 was found to correlate with the allele of 4 replicates of the pentanucleotide. In contrast, patients carrying haplotype 11.2 had different alleles of the microsatellite that could be homozygosis or heterozygous, but never the allele with 4 replicates.

Discussion

AIs have a number of side effects, including the onset or increase of arthralgias and loss of bone mass, thus increasing the risk of fractures. All this can affect compliance with therapy, decrease the quality of life of patients and increase the risk of breast tumor recurrence.

In previous studies, genetic variants of the *CYP11A1* and *CYP17A1* genes were associated with loss of BMD in FN[11] and increased joint pain[10], respectively. None of the SNPs associated with these events produced a change in the protein structure and, therefore, a possible functionality of these SNPs in the determination of the event was discarded.

In order to identify putative functional genetic variants that explain the association of these genes with musculoskeletal effects, the coding and regulatory regions of the *CYP11A1* and *CYP17A1* genes were sequenced.

No variant was found in the coding region that would cause a change in the amino acid sequence of the protein and, therefore, could involve a structural change of the enzyme. However, a genetic variant, D15S520, located in the regulatory region of *CYP11A1*, was found to be associated with loss of bone mass.

The D15S520 is a microsatellite based repeating pentanucleotide (TAAAA) n located in the *CYP11A1* promoter, at 528 bp upstream from the start of gene translation. In our study, this polymorphism was found to be significantly associated with loss of bone mass at 24 months of AI treatment. It has been observed that all patients carrying the 11.1/11.1 haplotype were also carriers of the 4/4 genotype. In the B-ABLE cohort, these patients had a greater predisposition to lose bone mass (-3.014%) than those with haplotypes 11.2/11.2 (-0.683%).

This microsatellite was previously associated with the risk of breast cancer[17,18], although there is some controversy concerning the results[19,20]. The study by Sakoda et al.[18] suggested that women with 4 repetitions in homozygosis would have a lower risk of breast cancer. One hypothesis would be that the allele of 4 replicates would affect the expression of the *CYP11A1* gene by decreasing estrogen production. As a consequence, lower estrogen exposure would reduce the risk of breast cancer[21], but during treatment with AI, the remaining estrogen levels may be lower than those of the carriers of the other alleles, thus increasing the loss of bone mass.

The detection of genetic variants that partly explain the action of AIs on the musculoskeletal system would allow for the development of personalized therapies in order to avoid, or at least anticipate, the side effects of AI. This could improve adherence to the treatment of these patients, which currently stands between 75.5-78.5%, thus avoiding relapses and a new contralateral breast cancer[22].

Table 1. Pairs of primers used

CYP11A1	Promoter	F'	5'-CAACCAGATTTGCCAAGGTC-3'
		R'	5'-GGGCCAAGATTATAACTACCAGC-3'
	5'UTR y EXÓN 1	F'	5'-GCACAGGCAGATATTTCAGGA-3'
		R'	5'-GGGGACTACAGCAGGGCTAC-3'
	EXÓN 2	F'	5'-CCTATTGTCTTGTCCTTCAGCA-3'
		R'	5'-AGGTGGGACTCAGTGAGCAA-3'
	EXÓN 3	F'	5'-GTGAGAGGCAGAGGGTGCT-3'
		R'	5'-CAGAGCAAGGGGTCTCACTC-3'
	EXÓN 4	F'	5'-GTTGCCAGAGGTCAGCTTTC-3'
		R'	5'-CAACAGCCAGCCTTCCAT-3'
	EXÓN 5	F'	5'-CCCCAAGAATTCGATGAAAA-3'
		R'	5'-TGACCCCACCATCTTAGGAG-3'
	EXÓN 6	F'	5'-CAAGTGCTGCCCTGAATGTT-3'
		R'	5'-TGTGTGGCATCTCAGCCCTA-3'
	EXÓN 7	F'	5'-GAGGTTGGAAGCAGGAAGTG-3'
		R'	5'-CTCAGACCCAGGCAAATCAT-3'
	EXÓN 8	F'	5'-AAGGGTGGGACAATCATCCT-3'
		R'	5'-AACTGTGGGAGAGAGCGAGA-3'
	EXÓN 9 y 3'UTR	F'	5'-CAACCACTCATCACCCACTG-3'
		R'	5'-GATTCTGCTGGCTCCTGAAC-3'
CYP17A1	Promoter 1.1	F'	5'-GGTTCCCCCAGTACGCTAGT-3'
		R'	5'-GCCTTGTGGAAAGATTCTCCT-3'
	Promoter 1.2	F'	5'-TGACCCTCCTGAATCTGTCA-3'
		R'	5'-TTGGGCCAAAACAAATAAGC-3'
	5'UTR y EXÓN 1	F'	5'-GTTTGCCCTGGAGTTGAGC-3'
		R'	5'-TCTGAAGACCTGAACAATCCCA-3'
	EXÓN 1.1	F'	5'-AAGGGCAAGGACTTCTCTGG-3'
		R'	5'-TGTGAGCCTGAGTAGCTGGA-3'
	EXÓN 1.2	F'	5'-GAAAATGGGGGCAGTGACTA-3'
		R'	5'-GAGCCGCCTCCTCCTAGA-3'
	EXÓN 1.3	F'	5'-CAGGGTCAGGAAATGGAAAA-3'
		R'	5'-GCGATACCCTTACGGTTGTT-3'
	EXÓN 2 y 3	F'	5'-CCAGAGGTGTAAGGGCAAGA-3'
		R'	5'-AAAGGAAGGAAGATTGGGGAC-3'
	EXÓN 3	F'	5'-GTGGACCTAGTCCCCTGGTT-3'
		R'	5'-AGGGTTTTGTTGGGGAAAAT-3'
	EXÓN 4 y INTRÓN 4	F'	5'-CCGCCTCCAGGAGAGACT-3'
		R'	5'-GTGCAATGGCATGATCTCAG-3'
	INTRÓN 4.2 y EXÓN 5	F'	5'-CCTGCCCAGACTTGCTCTAC-3'
		R'	5'-GGGTCAAAGCCAACTACTGC-3'
	INTRÓN 5, EXÓN 6 y INTRÓN 6.1	F'	5'-CACAATCCTCAGGTGTGCTT-3'
		R'	5'-TCTTGAACCCCTGACCTCAT-3'
	INTRÓN 6.2	F'	5'-GCTGGCCAACCTAAAGTCAG-3'
		R'	5'-GCCCTTTACTCCCTCATTCC-3'
	EXÓN 7 y INTRON 7.1	F'	5'-ACAGAAGCGCCTGTTAGGAG-3'
		R'	5'-AGCCCTTAACGACACAGAGG-3'
	EXÓN 8 y 3'UTR	F'	5'-TCTCTTTTCCATCCTTCCTGA-3'
		R'	5'-CGGTGTTGAAAGAATGAGTGAG-3'

F: forward; R: reverse.

Table 2. Baseline characteristics of patients genotyped for the *CYP11A1* and *CYP17A1* genes

	Patients *CYP11A1* (n=391)	**Patients *CYP17A1* (n=532)**
Age (years), mean ± SD	61.3 ± 8.5	61.9 ± 8.5
BMI, mean ± SD	29.5 ± 5.4	28.9 ± 5.2
Age at onset of menopause (years), mean ± SD	49.3 ± 4.5	49.4 ± 4.3
Age of menarche (years), median (IR)	12 (3)	12 (3)
Lactation (months), median (IR)	3 (11)	3 (10)
Number of children, median (IR)	2 (2)	2 (2)
Previous therapy with tamoxifen, n (%)	159 (40.7%)	227 (42.7%)
Previous chemotherapy, n (%)	235 (60.1%)	319 (60.0%)
Aromatase inhibitor, n (%)		
Letrozole	262 (67.0%)	348 (65.4%)
Exemestane	124 (31.7%)	173 (32.5%)
Anastrozole	5 (1.3%)	11 (2.1%)
BMD LS (g/cm^2), mean ± SD	0.961 ± 0.109	0.916 ± 0.132
BMD FN (g/cm^2), mean ± SD	0.747 ± 0.085	0.718 ± 0.100
BMD TH (g/cm^2), mean ± SD	0.895 ± 0.096	0.850 ± 0.112
VAS, mean ± SD	2.435 ± 2.525	2.434 ± 2.469

SD: standard deviation; BMI: body mass index; IR: interquartile range, BMD LS, FN and TH: bone mineral density of the lumbar spine, femoral neck and total hip; VAS: visual analogue scale.

The main limitation of this study is that it does not prove that this microsatellite is really a functional variant, since there are no functional studies of the *CYP11A1* promoter that validate this hypothesis. However, the fact that no functional variable was found in the coding regions of any of the genes studied seems to indicate that the observed association between these genes and the phenotypes has to be caused by genetic variants located in regulatory regions. Another limitation of the study is the use of the EVA parameter for the evaluation of the musculoskeletal symptomatology. EVA assumes that pain is a one-dimensional experience that can be measured on a single-point intensity scale. However, the toxicity reported by the patient more comprehensively captures the side effects of therapies (ie, pain) in daily experience and is more consistent with the patient's quality of life than the clinician-verified toxicity, Thus, being appropriate for the investigation of the musculoskeletal symptomatology. Likewise, the VAS scale ratio allows detecting the percentage differences between the VAS measurements obtained at multiple points in time. Other advantages of the VAS are its ease and brevity of punctuation, minimal intrusiveness and conceptual simplicity.

In conclusion, the D15S520 variant of the *CYP11A1* gene promoter could modulate the expression of this gene, thus explaining some of the phenotypic variability found in the loss of bone mass of patients under treatment with AI. Furthermore, no variant has been found in *CYP17A1* to explain the increase or decrease in joint pain observed in patients receiving AI. The promoter regions of these genes should be further studied to detect possible genetic variants that could be involved in the regulation of their expression.

Conflict of interest: The authors declare that they have no conflicts of interest in relation to this work.

Funding: This work has been funded by the FEIOMM 2010 and 2012 grants, the Thematic Network for Cooperative Research in Aging and Fragility (RETICEF, RD12/0043/0022), and the support of PI13/00444 Of Science and Innovation). The Generalitat of Catalonia (DIUE 2014 SGR 775) and ERDF funds have also contributed to its financing.

Bibliography

1. Nabholtz JM. Long-term safety of aromatase inhibitors in the treatment of breast cancer. Ther Clin Risk Manag. 2008;4(1):189-204.
2. Gonnelli S, Petrioli R. Aromatase inhibitors, efficacy and metabolic risk in the treatment of postmenopausal

Figure 2. General outline of the association analysis process performed in the study

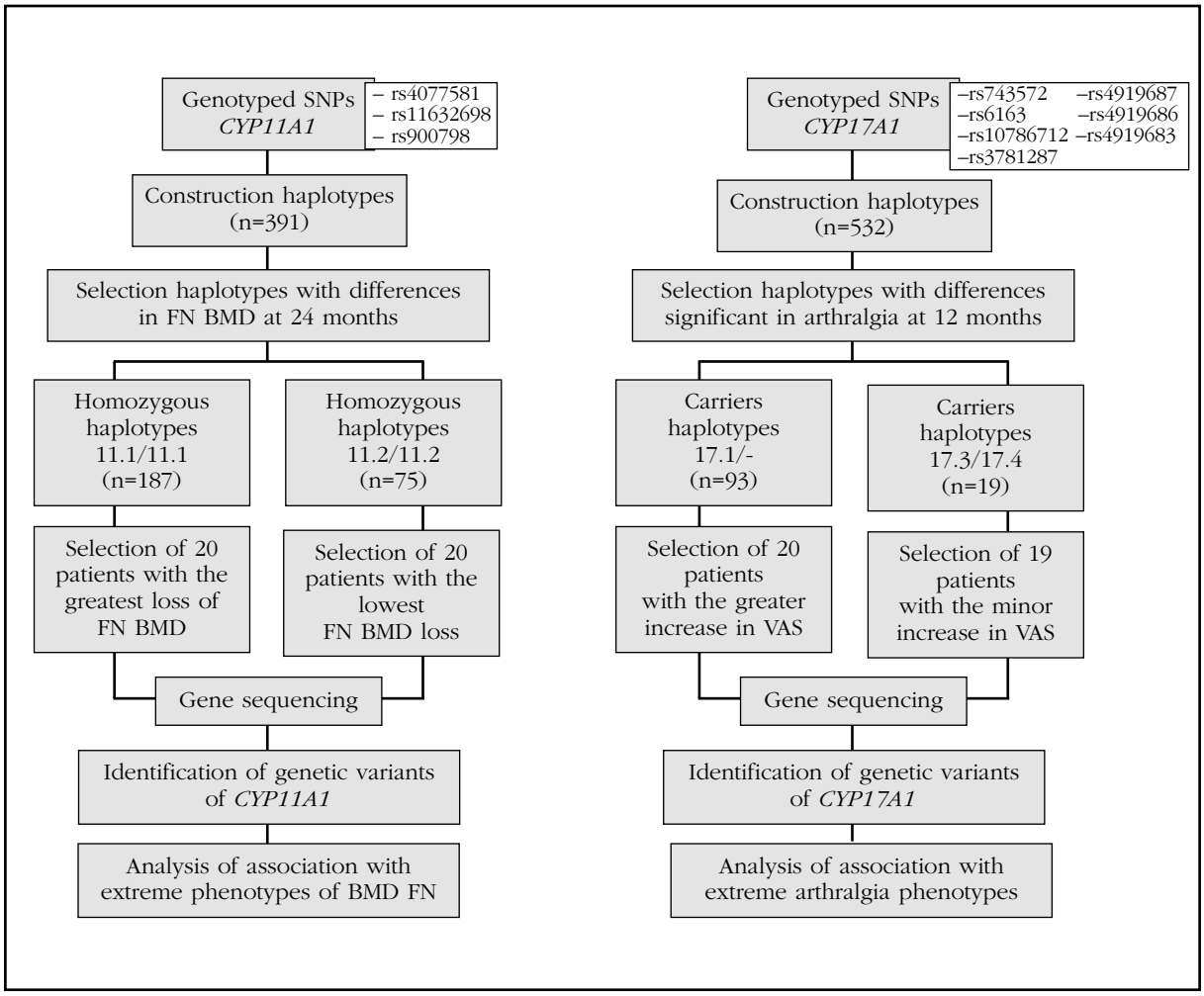


Table 3. Association between haplotypes of *CYP11A1* and *CYP17A1* genes, with loss of BMD in FN at 2 years and changes in pain at 12 months of treatment with AI, respectively

Gen	Haplotypes	Haplotype code	Frequency	Coefficient[c] [95% CI] each copy of the haplotype	P value
CYP11A1[a]	TGG*	11.1	0.517	Ref.	Ref.
	CAT	11.2	0.368	0.99 [0.29 ; 1.69]	0.006
	TAG	11.3	0.087	0.26 [-0.96 ; 1.47]	0.676
	TAT	11.4	0.026	1.03 [-0.96 ; 1.47]	0.342
CYP17A1[b]	ACCTGAC*	17.1	0.555	Ref.	Ref.
	ACCGGAA	17.2	0.014	-0.32 [-1.45 ; 2.09]	0.723
	GATGAAA	17.3	0.014	-1.67 [-3.24 ; -0.10]	0.037
	GATGACA	17.4	0.278	-0.61 [-1.03 ; -0.19]	0.005
	GATGGAA	17.5	0.123	-0.30 [-0.82 ; 0.22]	0.26

*Reference haplotype; [a]Haplotypes built by: rs4077581, rs11632698 and rs900798; [b]Haplotypes constructed by: rs743572, rs6163, rs10786712, rs3781287, rs4919687, rs4919686 and rs4919683; [c]Adjusted for: age, body mass index, chemotherapy, and previous tamoxifen.
BMD: bone mineral density; FN: femoral neck; CI: confidence interval.

Table 4. Mean of phenotypes (loss of BMD in FN in CYP11A1 and increase in pain in CYP17A1) of patients in the cohort B-ABLE carrying the haplotypes in homozygosis

Gen	Haplotype code	N patients homozygotes	Mean phenotype homozygotes patients
CYP11A1	11.1	187	-3.01%
	11.2	75	-0.683%
	11.3	3	-2.42%
	11.4	1	-
CYP17A1	17.1	93	1.76
	17.2	0	-
	17.3	1	-
	17.4	18	0.54
	17.5	0	-

BMD: bone mineral density; FN: femoral neck.

women with early breast cancer. Clin Interv Aging. 2008;3(4):647-57.

3. Ma CX, Reinert T, Chmielewska I, Ellis MJ. Mechanisms of aromatase inhibitor resistance. Nat Rev Cancer. 2015;15(5):261-75.
4. Bolufer P, Ricart E, Lluch A, Vazquez C, Rodriguez A, Ruiz A, et al. Aromatase activity and estradiol in human breast cancer: its relationship to estradiol and epidermal growth factor receptors and to tumor-node-metastasis staging. J Clin Oncol. 1992;10(3):438-46.
5. Fabian CJ. The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. Int J Clin Pract. 2007;61(12):2051-63.
6. Amir E, Seruga B, Niraula S, Carlsson L, Ocana A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. J Natl Cancer Inst. 2011;103(17):1299-309.
7. Dent S, Di Valentin T, Vandermeer L, Spaans J, Verma S. Long term toxicities in women with early stage breast cancer treated with aromatase inhibitors: data from a tertiary care center. Breast Cancer Res Treat. 2006;100S1(4057):S190-1.
8. Henry NL, Giles JT, Ang D, Mohan M, Dadabhoy D, Robarge J, et al. Prospective characterization of musculoskeletal symptoms in early stage breast cancer patients treated with aromatase inhibitors. Breast Cancer Res Treat. 2008;111(2):365-72.
9. Prieto-Alhambra D, Javaid MK, Servitja S, Arden NK, Martinez-Garcia M, Diez-Perez A, et al. Vitamin D threshold to prevent aromatase inhibitor-induced arthralgia: a prospective cohort study. Breast Cancer Res Treat. 2011;125(3):869-78.
10. Garcia-Giralt N, Rodriguez-Sanz M, Prieto-Alhambra D, Servitja S, Torres-Del Pliego E, Balcells S, et al. Genetic determinants of aromatase inhibitor-related arthralgia: the B-ABLE cohort study. Breast Cancer Res Treat. 2013;140(2):385-95.
11. Rodriguez-Sanz M, Garcia-Giralt N, Prieto-Alhambra D, Servitja S, Balcells S, Pecorelli R, et al. CYP11A1 expression in bone is associated with aromatase inhibitor-related bone loss. J Mol Endocrinol. 2015;55(1):69-79.
12. Nguyen MN, Slominski A, Li W, Ng YR, Tuckey RC. Metabolism of vitamin d2 to 17,20,24-trihydroxyvitamin d2 by cytochrome p450scc (CYP11A1). Drug Metab Dispos. 2009;37(4):761-7.
13. Tuckey RC, Janjetovic Z, Li W, Nguyen MN, Zmijewski MA, Zjawiony J, et al. Metabolism of 1alpha-hydroxyvitamin D3 by cytochrome P450scc to biologically active 1alpha,20-dihydroxyvitamin D3. J Steroid Biochem Mol Biol. 2008;112(4-5):213-9.
14. Servitja S, Nogues X, Prieto-Alhambra D, Martinez-Garcia M, Garrigos L, Pena MJ, et al. Bone health in a prospective cohort of postmenopausal women receiving aromatase inhibitors for early breast cancer. Breast. 2012;21(1):95-101.
15. Blake G, E. Adams J, Bishop N. DXA in Adults and Children. In: Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. Eighth Edition ed: John Wiley & Sons, Inc.; 2013. p. 249-63.
16. Orozco López P, Zwart Salmerón M, Vilert Garrofa E, Olmos Domínguez C. Predicción de la ingesta total de calcio a través del consumo de lácteos en la población adulta de España. Estudio INDICAD 2001. Aten Primaria. 2004;33(5):237-43.
17. Zheng W, Gao Y-T, Shu X-O, Wen W, Cai Q, Dai Q, et al. Population-Based Case-Control Study of CYP11A Gene Polymorphism and Breast Cancer Risk. Cancer Epidemiol Biomarkers Prev. 2004;13(5):709-14.
18. Sakoda LC, Blackston C, Doherty JA, Ray RM, Lin MG, Stalsberg H, et al. Polymorphisms in steroid hormone biosynthesis genes and risk of breast cancer and fibrocystic breast conditions in Chinese women. Cancer Epidemiol Biomarkers Prev. 2008;17(5):1066-73.
19. Setiawan VW, Cheng I, Stram DO, Giorgi E, Pike MC, Van Den Berg D, et al. A systematic assessment of common genetic variation in CYP11A and risk of breast cancer. Cancer Res. 2006;66(24):12019-25.
20. Yaspan BL, Breyer JP, Cai Q, Dai Q, Elmore JB, Amundson I, et al. Haplotype analysis of CYP11A1 identifies promoter variants associated with breast cancer risk. Cancer Res. 2007;67(12):5673-82.
21. Dumitrescu RG, Cotarla I. Understanding breast cancer risk -- where do we stand in 2005? J Cell Mol Med. 2005;9(1):208-21.
22. Font R, Espinas JA, Gil-Gil M, Barnadas A, Ojeda B, Tusquets I, et al. Prescription refill, patient self-report and physician report in assessing adherence to oral endocrine therapy in early breast cancer patients: a retrospective cohort study in Catalonia, Spain. Br J Cancer. 2012;107(8):1249-56.

Table 5. Characteristics of patients with selected extreme phenotypes for genetic analysis

Patients *CYP11A1*	**11.2/11.2 (n=20)**	**11.1/11.1 (n=20)**
Age (years), mean ± SD	60.6 ± 10.8	59.1 ± 9.9
BMI, mean ± SD	27.31 ± 4.6	27.91 ± 4.6
Age at onset of menopause (years), mean ± SD	47.8 ± 3.6	48.2 ± 4.9
Age of menarche (years), median (IR)	13 (2)	12 (2)
Lactation (months), median (IR)	5 (15)	2,5 (9)
Number of children, median (IR)	2 (2)	2 (2)
Previous therapy with tamoxifen, n (%)	0 (0%)	0 (0%)
Previous chemotherapy, n (%)	13 (65.0%)	13 (65.0%)
Aromatase inhibitor, n (%)		
Letrozole	10 (50.0%)	9 (45.0%)
Exemestane	10 (50.0%)	11 (55.0%)
BMD FN (g/cm^2) (basal), mean ± SD	0.763 ± 0.104	0.777 ± 0.073*
Change in BMD FN (2 years), relative mean (%) ± SD	2.330 ± 3.203	-7.858 ± 3.684**
Patients *CYP17A1*	**17.3/17.4 (n=19)**	**17.1/- (n=20)**
Age (years), mean ± SD	61.79 ± 9.13	61.15 ± 7.85
BMI, mean ± SD	29.22 ± 7.29	31.01 ± 6.23
Age at onset of menopause (years), mean ± SD	48.63 ± 3.99	48.65 ± 5.02
Age of menarche (years), median (IR)	12 (3)	12 (3)
Lactation (months), median (IR)	3 (12)	6 (14)
Number of children, median (IR)	2 (1)	2 (1)
Previous therapy with tamoxifen, n (%)	14 (73.7%)	15 (75.0%)
Previous chemotherapy, n (%)	12 (63.2%)	14 (70.0%)
Aromatase inhibitor, n (%)		
Letrozole	11 (57.9%)	8 (40.0%)
Exemestano	7 (36.8%)	11 (55.0%)
Anastrozole	1 (5,3%)	1 (5,0%)
VAS (basal), mean ± SD	2.750 ± 0.097	0.825 ± 1.270*
Change in VAS (1 year), mean ± SD	-0.078 ± 2.264	6.290 ± 1.032**

SD: standard deviation; BMI: body mass index; IR: interquartile range, BMD FN: bone mineral density of the femoral neck; VAS: visual analogue scale; *p<0.01; **p<0.001.

López Gavilanez E[1], Guerrero Franco K[2], Segale Bajaña A[2], Solórzano Romero N[3], Navarro Chavez M[3]

1 Servicio Endocrinología - Hospital Docente Policía Nacional Guayaquil Nº 2 - Guayaquil (Ecuador)

2 Servicio Medicina Interna - Hospital Docente Policía Nacional Guayaquil Nº 2 - Guayaquil (Ecuador)

3 Servicio Endocrinología - Hospital Luis Vernaza - Guayaquil (Ecuador)

Screening and biochemical characterization of primary hyperparathyroidism in Guayaquil (Ecuador)

DOI: http://dx.doi.org/10.4321/S1889-836X2017000200005

Correspondence: Enrique López Gavilanez - Hospital Docente Policía Nacional Guayaquil Nº 2 - Avenida de la Américas, s/n - Guayaquil (Ecuador)
e-mail: enrique_lopezg57@hotmail.com

Date of receipt: 02/01/2017
Date of acceptance: 07/03/2017

Summary

Objectives: To determine the prevalence of primary hyperparathyroidism (HPTP) using PTH and Ionic calcium screening in a population sample of Guayaquil (Ecuador).

Materials and methods: Prospective, cross-sectional study carried out between January 1, 2009 and November 30, 2014 of 13,860 people who attended routine control tests. All were tested in serum parathyroid hormone (PTH), ionic calcium, serum creatinine and the 25 (OH) total vitamin D (total VD). The diagnosis of HPTP was confirmed if PTH or Ionic calcium levels remained high at least in two different occasions. We excluded patients with raised serum creatinine, vitamin D insufficiency, malabsorption, chronic liver disease, or those receiving treatments that alter phosphocalcic metabolism.

Results: 61 cases were found with raised PTH on at least two different occasions. Among these, 34 presented vitamin D insufficiency and were excluded from the analysis. In 27 cases (4 men and 23 women) the diagnosis of HPTP was confirmed. The average age for women was 64.5±15.4 years and men of 71.3±12.8 years; average PTH values were 115±24.2 pg/ml; Ionic calcium, 5.15±0.4 mg/dl; total VD, 47.1±20.2 ng/ml; and serum creatinine 0.84±0.2 mg/ml; prevalence of HPTP corresponds to 2 cases per thousand adults (95% CI: 1.71-2.18). The greatest increase in prevalence occurred in women aged 60 years.

Conclusion: PTH prevalence in this sample is low compared to that reported in international series, being higher in advanced ages and in women. With the proposed screening for PTH and ionic calcium, we detected the normocalcemic form of HPTP in most cases.

Key words: *primary hyperparathyroidism, screening, ionic calcium, prevalence, normocalcemia, epidemiology, vitamin D.*

Introduction

Primary hyperparathyroidism (HPTP) is a relatively common endocrine disorder. Among endocrine diseases, it ranks third in frequency of diagnosis[1]. HPTP is usually diagnosed in the sixth decade of life and is more common in women[1]. Its clinical presentation has changed in recent decades, evolving from a classical form with significant bone and renal involvement[2], to the asymptomatic form that we are currently seeing[3].

The epidemiology of HPTP has been difficult to establish, since the international literature contains different figures on incidence and prevalence in different populations.

The prevalence of HPTP depends on the populations studied and the detection methods used. In studies of Caucasian populations, it ranges from 1 to 7 per 1,000 adults[4-6]. A biochemical screening study has established a prevalence of 1 to 21 per 1,000 adults[7-9]. Incidence also varies according to the sources. Incidence studies with PTH and total calcium determinations have been described, in which both high and low rates are reported[5,7,9-15].

In Latin America, there are few studies on the epidemiology of the disease, with the exception of Eufrazino et al.[16] in Recife (Brazil), Mautalen et al. in Argentina[17], and in Chile by López et al.[18]. In Ecuador, PTH prevalence of 7.1% was found in a selected sample of postmenopausal women with low bone mass[19].

The present study would be the first of its kind to evaluate HPTP prevalence in the city of Guayaquil (Ecuador), applying a uniform biochemical screening, by means of the simultaneous measurement of parathyroid hormone (PTH) and serum ionic calcium, and compare our results with those reported in the literature.

Material and methods

This descriptive, prospective and cross-sectional epidemiological study aims to determine the prevalence of PTH during the period between January 1, 2009 and November 30, 2014 in two reference centers in the city of Guayaquil. According to data from the last Population and Housing Census of 2010, provided by the National Institute of Statistics and Censuses of Ecuador (INEC)[20], the population of Guayaquil grew from 2,440,553 to 2,560,505 inhabitants from 2010 to 2014 (Table 1).

The study was approved by the Ethics and Research Committee at the Guayaquil National Police Teaching Hospital Nº 2.

The diagnosis of PTHP was defined when PTH levels >72 pg/ml (normal values: 12-72 pg/ml) and/or ionic calcium >5.6 mg/dl (normal values: 4.5-5,6 mg/dl) remained elevated on at least two or more different occasions. Serum creatinine, total 25 (OH) vitamin D (D2+D3), and a basic biochemical study (complete blood count, glycemia, liver enzymes, serum lipids, and nitrogen products) were also measured in serum.

Biochemical screening with serum PTH and ionic calcium measurement was carried out in 13,860 people living in the city of Guayaquil (Figure 1), who underwent routine check-ups at the hospitals participating in the study. Patients were treated in primary care units, where they underwent screening tests. Those who returned for routine monitoring and presented serum PTH and/or calcium levels higher than the reference ranges were required to perform an additional assessment of PTH and serum calcium levels.

Serum ionic calcium was measured after 12 hours of fasting and without tourniquet use, under anaerobic conditions (taking the sample in a vacuum tube and uncovering the tube just before the test), and was reported without correction for pH, by direct measurement with selective ion electrode (NOVA-8 equipment), with reference values of 4.5 to 5.6 mg/dl.

Serum PTH (intact molecule) was measured with SIEMENS Immulite 2000 equipment (enzyme-labeled, two-site solid-phase chemiluminescent immunometric assay), with reference values ranging from 12 to 72 pg/ml. The intra-assay precision presented a coefficient of variation of 5.7, 4.3 and 4.2% for concentrations of 72, 258 and 662 pg/ml, respectively, and an interassay coefficient of variation of 6.3 and 8.8% For concentrations of 54 and 387 pg/ml, respectively. The limit of detection was 3.0 pg/ml and linearity up to 500,000 without Hook effect. PTH levels were considered inadequately "normal" when they were above the 75th percentile of the reference value (PTH ≤57 pg/ml) in the presence of hypercalcemia on 2 different occasions.

Serum level of 25 (OH) total vitamin D (total VD=D3+D2) was measured by chemiluminescence, with normal values: 30-70 ng/ml (Centauro kit; competitive 1-step assay with anti-Flurocein). Total precision presented a coefficient of variation of 11.1, 9.6, 9.8, 8.2, 7.8, 4.8% for concentrations of 11.7, 18.0, 32.4, 49, 9, 55.8, 132.1 ng/ml, respectively, with detection limit of 3.2 and linearity up to 150 ng/ml. values ≥30 ng/ml were considered sufficient; mild insufficiency, between 20-29 ng/ml; moderate insufficiency, between 10-19 ng/ml; and severe deficiency, <10 ng/ml[21,22].

Renal function integrity was documented in all cases by measuring serum creatinine levels and calculating endogenous creatinine clearance expressed in ml/min (formula corrected for age, sex, weight and serum creatinine:

[140 – age (years) x weight (kg)] / [72 x creatinine Serum (mg/dl)] x 0.85 (correction factor alone in women).

Cases with a high level of calcium and/or PTH on one occasion were considered spurious and excluded. Cases with raised serum creatinine, malabsorption, chronic liver disease, vitamin D insufficiency or those receiving treatment that could alter phosphocalcic metabolism and/or PTH levels (glucocorticoids, estrogens, bisphosphonates, thiazides, lithium , calcium). All biochemical measurements were carried out in a single reference laboratory. All women and men aged 20 years or older were included in the screening sample. Ages ran-

Table 1. Projection of the Ecuadorian population by calendar years, according to cantons

Code	Name of the canton	2010	2014
901	Guayaquil	2,440,553	2,560,505

Source: Population and housing census of the year 2010. National Institute of Statistics and Censuses of Ecuador (INEC)[21].

ged from 20 to 89 years. We stratified the cases by sex and age groups: 24-50, 51-60, 61-70 and 71-89 (Figure 2). Data are expressed as mean and standard deviations with the corresponding confidence intervals, 95% confidence level. For the comparison of groups, we used Student's t-test for independent means. A value of $p<0.05$ was considered statistically significant. Prevalence of the disease was calculated as the number of existing cases divided by the population screened and expressed as a proportion of each population of 1,000 adults. Epidat 3.1 software was used to analyze the data.

Results

Of the 13,860 people evaluated in the screening period, HPTP was found in 27 cases. Patients (n=13,378) with elevated serum creatinine and/or receiving treatments affecting phospocalcic metabolism (estrogens, bisphosphonates, lithium, calcium, thiazides) were excluded. Patients with normal and untreated serum creatinine but who had normal levels of ionic calcium and serum PTH were also excluded. In 482 cases PTH was elevated on at least one occasion of several successive ones; 61 cases had elevated PTH on two or more occasions, but 34 of them had total VD values in the range of insufficiency and were excluded. In the remaining 27 cases, the biochemical diagnosis of PTH was confirmed by raised PTH on two or more occasions (95% CI: 105.01-124.18), preserved renal function (95% CI: 0.765-0.915), and sufficiency of total RV (≥30 ng/ml) (95% CI: 39.12-55.09). In 25 cases (93%), the ionic calcium was in normal ranges (95% CI: 5.0-5.29) and only 2 (women) had minimally elevated values (5.89 and 5.95 mg/dl, respectively).

HPTP was diagnosed more frequently in women than in men (4 men and 23 women), with a 6:1 ratio; the majority of women (87%) were menopausal (n=20).

Table 2 presents the results of the biochemical variables of all patients with confirmed diagnosis of PTHP.

In our series, PTHP was diagnosed more frequently from the sixth decade of life in women, with a mean age of cases around 65 years (95% CI: 57.82-71.12; Range between 24 and 88) and a little later in men, at age 71 (95% CI: 50.95-91.55).

PTH levels far exceeded the upper limit of normal and were similar between women and men (p=ns). In spite of the large increase in PTH levels, in almost all cases serum calcium levels were in the normal range or were slightly higher (range 4.52 to 5.95), and were not different between Men and women (p=ns). Serum levels of total vitamin D were found in the normal range (>30 ng/ml) in 27 cases, and were not different between males and females (p=ns).

In 34 cases vitamin D was in the insufficiency range 21.62±4.7 ng/ml (95% CI: 19.98-23.26) and were excluded from the analysis. In these cases, vitamin D replacement was not performed.

Serum creatinine was within the normal ranges in all cases (95% CI: 0.76-0.91), as well as the endogenous creatinine clearance calculated by the corrected formula.

HPTP prevalence in this sample of the population of Guayaquil corresponds to 2 cases per thousand adults (95% CI: 1.714-2.182). The highest increase in the prevalence of PPH was seen in women ≥60 years and in men ≥70 years (Figure 2).

Discussion

Prevalence studies of HPTP have been carried out mostly in Caucasian populations[4,6,7,10], so that there is no exhaustive information available in other ethnicities and races of our Latin America. Only recently, Yeh MW et al. reported an age-adjusted prevalence of 169.4 and 54.8 per 100,000 women and men in a sub-group of Hispanic race, respectively[11]. The population of Ecuador is multiethnic and the mestizo group is the majority, with an estimated 72% of the total population[20].

Data from epidemiological studies show that, in certain populations at risk, for example, in postmenopausal women and with decreased bone mass, the prevalence ranges from 2.1 to 11.5%[19,23-25].

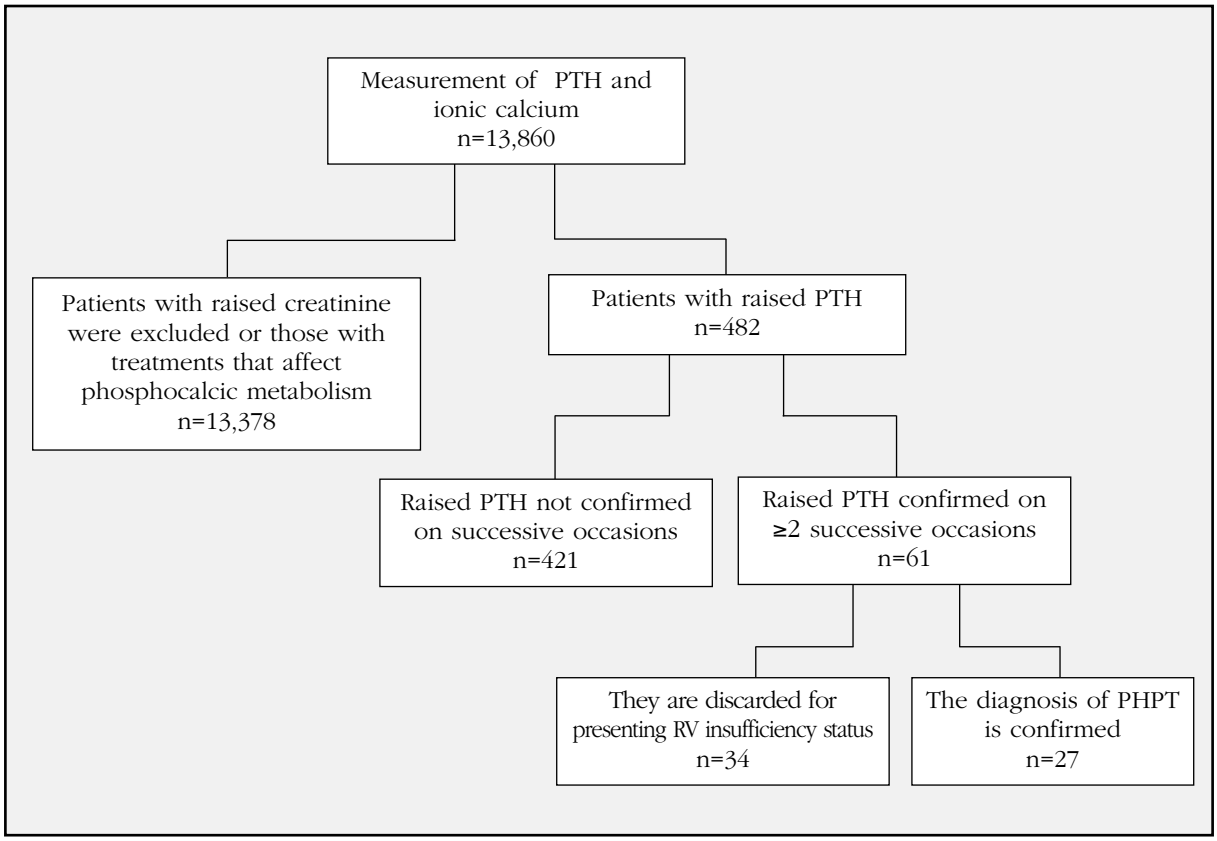
HPTP is much more common among women, with a ratio of women to men over the age of 60 in the range of 5 to 7:1[1,17], which is in agreement with our results.

PTHP is recognized as the most common cause of hypercalcemia in outpatient care[26], and in its classic form it has raised levels of PTH, renal lithiasis, and severe bone involvement[1,9,27]. This classic form is still frequently found in developing countries, probably due to the time of delay in diagnosis and the lack of accessibility to measurements of calciotropic hormones and ionic calcium[27].

Another form of presentation of PTHP was identified formally in 2008, identified as normocalcemic HPTP[28], but its description is still incomplete, particularly with respect to its epidemiology, natural history and treatment. Patients with this condition lack the classic HPTP characteristics, and have high levels of PTH with normal serum calcium, which are considered an early sign of the disease[29,30]. The diagnosis should focus on the exclusion of all causes of secondary hyperparathyroidism, particularly vitamin D deficiency (<30 ng/ml) and decreased renal function (endogenous creatinine clearance <60 ml/min)[30].

Normocalcemic HPTP prevalence varies from 0.7 to 16.7%[31-33] according to the design of the stu-

Figure 1. Design of PHPT screening in Guayaquil (Ecuador)



HPTP: primary hyperparathyroidism; PTH: parathyroid hormone; VD: vitamin D.

dies, populations studied, age, sex and methods used. In our series, most cases (93%) had high levels of PTH with ionic calcium in the range of normal or minimally elevated. This would show that the detection was carried out in the early stages of the disease[34] and/or that the predominant form of HPTP presentation in our population is normocalcemic.

The detection of HPTP cases in epidemiological studies has been carried out using a combination of biochemical, histopathological, radiological and clinical data sources[5,6,13,14,35]. However, it should be noted that all data sources have a considerable bias in the results. Taking this into account, our study reveals some findings that are worth highlighting.

Although some controversy persists regarding the usefulness of serum ionic calcium determination, this is a method that reliably allows the diagnostic approach in HPTP. The total calcium concentration does not reliably reflect the predicted increase in the free fraction, especially in cases with minimal or no increase in the total serum calcium level[29,36,37].

Population screening with the simultaneous measurement of serum PTH and ionic calcium at least twice allows us to effectively and safely identify cases. Measuring vitamin D levels and assessing the integrity of renal function allows us to separate the secondary causes of parathyroid hyperfunction. In general terms, the use of our biochemical screening of HPTP would solve the possible research bias obtained in the results of other studies. For example, if only histopathological data were used, there would be a higher detection rate for the minority of patients who are treated surgically. Another bias may also be found in patients with thyroidectomies, where parathyroid adenomas may be found coincidentally in normocalcemic individuals, but these patients cannot be considered cases of PTH[38]. As for radiological studies, they are not an appropriate method for HPTP screening because of their reduced sensitivity and specificity[39].

Among the weaknesses of our study, we pointed out that we did not measure calcium in urine, so we recognize that in our series, the presence of cases with idiopathic hypercalciuria or familial hypocalciuric hypercalcemia cannot be ruled out, although the latter is a rare disease with an estimated prevalence in 1 out of 78,000 people[40]. We also note the inherent limitations of the formula used to calculate endogenous creatinine clearance.

In conclusion, we have characterized the largest series of patients with PHTP described to date in our country and documented the prevalence of HPTP for the first time in our population. Compared to the international series, the prevalence of HPTP is low in this sample and is higher in women and in advanced ages. The biochemical presentation corresponds mostly to the normocalcemic form of the disease.

Figure 2. Cases of primary hyperparathyroidism stratified by age and sex

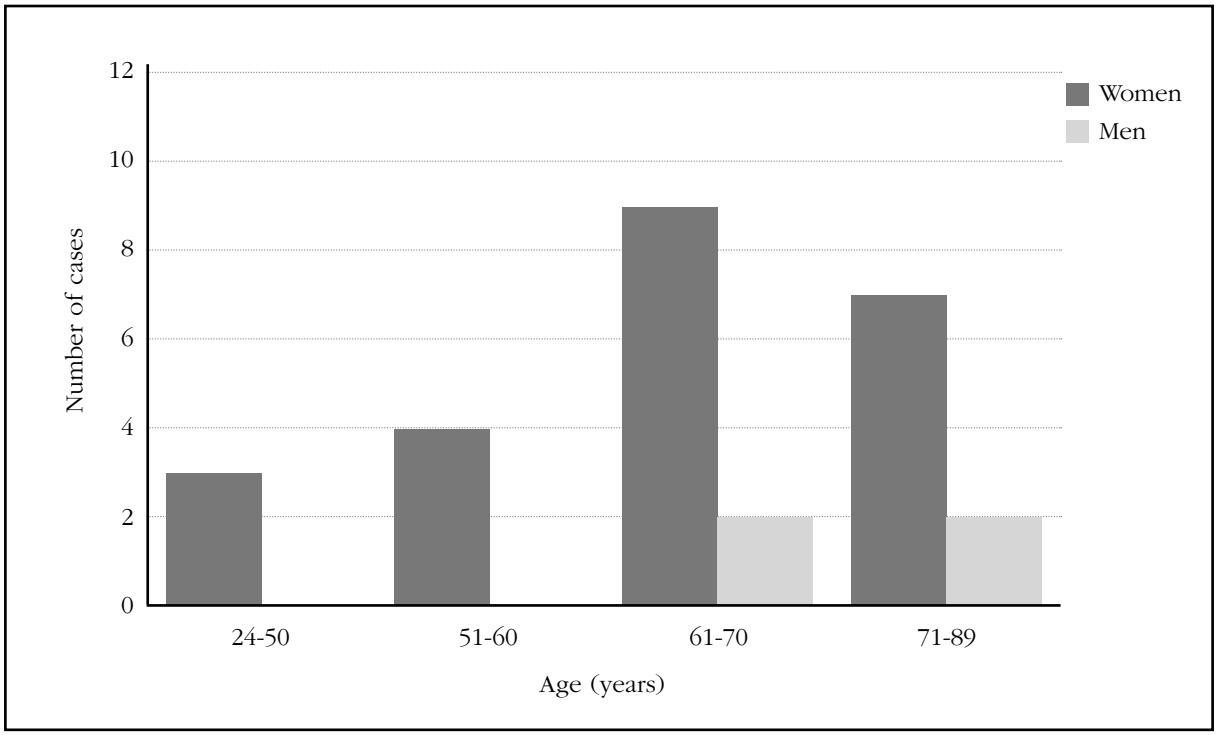


Table 2. Biochemical characteristics of cases with diagnosis of confirmed primary hyperparathyroidism

	Men (n=4)	Women (n=23)	Total (n=27)
Age, years	71.25 ± 12.76	64.2 ± 15.4	65 ±1 5
PTH, pg/ml	113 ± 15.12	114.9 ± 25.8	114.6 ± 24.2
Ionic calcium, mg/dl	5.15 ± 0.36	5.15 ± 0.38	5.15 ± 0.37
Total vitamin D, ng/ml	44.2 ± 6.85	47.6 ± 21.8	47.3 ± 20.18
Serum creatinine, mg/ml	0.85 ± 0.16	0.84 ± 0.19	0.84 ± 0.19

PTH: parathyroid hormone. Values are expressed as mean ± standard deviation.

Our data may help health authorities develop effective strategies for prevention and treatment of skeletal (and non-skeletal) complications of HPTP in our population.

Conflict of interest: The authors declare that they have no conflicts of interest in relation to this work.

Bibliography

1. Fraser WD. Hyperparathyroidism. Lancet. 2009;374 (9684):145-58.
2. Díaz Curiel M, Rapado A, López Gavilanez E, Peramo B, Díez A. Type of bone loss involved in the follow up of surgical or medical treatment of primary hyperparathyroidism. In: Christiansen C, Overgaard K, eds. Proceedings of the third International Symposium on Osteoporosis, vol. 3;1990; Denmark. Copenhagen: Osteopress; 1990.1560-2 p.
3. Bilezikian JP, Potts JT Jr. Asymptomatic primary hyperparathyroidism: new issues and new questions–bridging the past with the future. J Bone Miner Res. 2002;17(suppl 2):N57-67.
4. Christensson T, Hellström K, Wengle B, Alveryd A, Wikland B. Prevalence of hypercalcaemia in a health screening in Stockholm. Acta Med Scand. 1976;200:131-7.
5. Wermers RA, Khosla S, Atkinson EJ, Hodgson SF, O'Fallon WM, Melton LJ III. The rise and fall of primary hyperparathyroidism:a population-based study in Rochester, Minnesota, 1965-1992. Ann Intern Med. 1997;126:433-40.
6. Adami S, Marcocci C, Gatti D. Epidemiology of primary hyperparathyroidism in Europe. J Bone Miner Res. 2002;17(Suppl 2):N18-23.
7. Jorde R, Bønaa KH, Sundsfjord J. Primary hyperparathyroidism detected in a health screening: The Tromsø Study. J Clin Epidemiol. 2000;53(11):1164-9.
8. Stenstrom G, Heedman PA. Clinical findings in patients with hypercalcaemia. A final investigation based on biochemical screening. Acta Med Scand. 1974;195:473-7.
9. Mundy GR, Cove DH, Fisken R. Primary hyperparathyroidism: changes in the pattern of clinical presentation. Lancet. 1980;1:1317-20.
10. Melton III L.J. The Epidemiology of Primary Hyperparathyroidism in North America. J. Bone and Mineral Research. 2002;17(Suppl 2):N12-7.

11. Yeh MW, Ituarte PH, Zhou HC, Nishimoto S, Liu IL, Harari A, et al. Incidence and Prevalence of Primary Hyperparathyroidism in a Racially Mixed Population. J Clin Endocrinol Metab. 2013;98(3):1122-9.
12. Wermers RA, Khosla S, Atkinson EJ, Achenbach SJ, Oberg AL, Grant CS, et al. Incidence of primary hyperparathyroidism in Rochester, Minnesota, 1993–2001: An update on the changing epidemiology of the disease. J Bone Miner Res. 2006;21(1):171-7.
13. Muñoz Torres M, Jodar Gimeno E, Reyes Garcia R, Martínez Díaz Guerra G, Amado JA, Gaztambide S. et al. Results from a national survey on the management of primary hyperparathyroidism. J Endocrinol Invest. 2012;35:957-63.
14. Dedov II, Mokrysheva NG, Mirnaia SS, Rostomian LG, Pigarova EA, Rozhinskaia LI. Epidemiology of primary hyperparathyroidism in Russia (the first results from the database of federal state institution 'Endocrinological Research Centre'. Problemy Endokrinologii. 2011; 57(3):3-10.
15. Griebeler ML, Kearns AE, Ryu E, Hathcock MA, Melton LJ 3rd, Wermers RA. Secular trends in the incidence of primary hyperparathyroidism over five decades (1965-2010). Bone. 2015;73:1-7.
16. Eufrazino C, Veras A, Bandeira F. Epidemiology of primary hyperparathyroidism and its nonclassical manifestations in the city of Recife, Brazil. Clin Med Insights Endocrinol Diabetes. 2013;6:69-74.
17. Mautalen CA, Gallo Morando C, Torres Agüero M, Barcat JA, Arata RO, Molins M. Tratamiento quirúrgico del hiperparatiroidismo. Medicina (B Aires). 1972;32:150-8.
18. López JM, Sapunar J, Campusano C, Arteaga E, Rodríguez JA, León A, et al. Changes in the clinical presentation of primary hyperparathyroidism. Analysis of 84 cases. Rev Med Chil. 1993;121(3):265-72.
19. López Gavilanez E, Huamán Garaycoa F, Segale Bajaña A, Castillo Delvalle M, Macías Briones G. Screening for primary hyperparathyroidism in women with low bone mass. Osteoporosis Int. 2006;17(Suppl 2):S191.
20. http://www.ecuadorencifras.gob.ec/censo-de-poblacion-y-vivienda (accedido el 1 diciembre 2015).
21. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. IOF Committee of Scientific Advisors (CSA) Nutrition Working Group. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009;20(11):1807-20.
22. Mori H, Okada Y, Tanaka Y. Incidence of vitamin D deficiency and its relevance to bone metabolism in Japanese postmenopausal women with type 2 diabetes mellitus. Intern Med. 2015;54(13):1599-604.
23. Lundgren E, Hagström EG, Lundin J, Winnerbäck K, Roos J, Ljunghall S, et al. Primary hyperparathyroidism revisited in menopausal women with serum calcium in the upper normal range at population-based screening 8 years ago. World J Surg. 2002;26(8):931-6.
24. Lundgren E, Rastad J, Thrufjell E, Akerström G, Ljunghall S. Population-based screening for primary hyperparathyroidism with serum calcium and parathyroid hormone values in menopausal women. Surgery. 1997;121(3):287-94.
25. Misiorowski W, Zgliczyński W. Prevalence of primary hyperparathyroidism among patients with low bone mass. Adv Med Sci. 2012;57(2):308-13.
26. Lafferty FW. Differential diagnosis of hypercalcemia. J Bone Miner Res. 1991;6(S2):S51-8.
27. González L, López Gavilanez E, Tagle M, Morán B. Síndrome Neuromuscular Severo, hipercalcemia y tumor óseo. Rev Esp Enf Metab Oseas. 1997;6(5)181-4.
28. Bilezikian JP, Khan AA, Potts JT Jr. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the third international workshop. J Clin Endocrinol Metab. 2009;94(2):335-8.
29. López Gavilanez E, Huaman Garaycoa F, Garcés Santos JC, Marengo Baquerizo C, Reyes Aguirre G. Osteoporosis, elevated PTH and inadequately "Normal" Ionic Calcium. Rev Esp Enf Metab Oseas. 2005;14(3):52-4.
30. Martínez Díaz-Guerra G; Jódar Gimeno E; Reyes García R; Gómez Sáez JM; Muñoz-Torres M. Normocalcemic primary hyperparathyroidism Recommendations for management and follow up 2013. Endocrinol Nutr. 2013;60(8):456.e1-5
31. Cusano N, Wang P, Cremers S, Haney E, Bauer D, Orwoll E, et al. Asymptomatic normocalcemic primary hyperparathyroidism: characterization of a new phenotype of normocalcemic primary hyperparathyroidism. Poster session presented at:"33rd Annual Meeting of the American Society of Bone and Mineral Research"; September 16–20, 2011.San Diego, California. SU0167.
32. Garcia-Martin A, Reyes-Garcia R, Munoz-Torres M. Normocalcemic primary hyperparathyroidism: one-year follow-up in one hundred postmenopausal women. Endocrine. 2012;42(3):764-5.
33. Berger C, Langsetmo L, Hanley D, et al. Relative prevalence of normocalcemic and hypercalcemic hyperparathyroidism in a community-dwelling cohort. Poster session presented at:"33rd Annual Meeting of the American Society of Bone and Mineral Research"; September 16-20, 2011. San Diego, California. SU0173.
34. Maruani G, Hertig A, Paillard M, Houillier P. Normocalcemic primary hyperparathyroidism: evidence for a generalized target-tissue resistance to parathyroid hormone. J Clin Endocrinol Metab. 2003;88 (10):4641-8.
35. Yu N, Donnan PT, Murphy MJ, Leese GP. Epidemiology of primary hyperparathyroidism in Tayside, Scotland, UK. Clin Endocrinol. 2009;71(4):485-93.
36. Ladeson JH. Calcium Determination in Primary Hyperparathyroidism. J Bone Miner Res. 1991;6(Suppl 2):S33-41.
37. Tee MC, Holmes DT, Wiseman SM. Ionized vs serum calcium in the diagnosis and management of primary hyperparathyroidism: Which is superior?. Am J Surg. 2013;205(5):591-6.
38. Carnaille BM, Pattou FN, Oudar C, Lecomte-Houcke MC, Rocha JE, Proye CA. Parathyroid incidentalomas in normocalcemic patients during thyroid surgery. World J Surg. 1996;20(7):830-4.
39. Udelsman R, Pasieka JL, Sturgeon C, Young JEM, Clark OH. Surgery for asymptomatic primary hyperparathyroidism: proceedings of the Third International Workshop. J Clin Endocrinol Metab. 2009;94(2):366-72.
40. Hinnie J, Bell E, McKillop E, Gallacher S. The prevalence of familial hypocalciuric hypercalcemia (FHH). Calcif Tissue Int. 2001;68(4):216-8.

Hawkins Carranza F, Guadalix Iglesias S, Martínez Díaz-Guerra G, López Álvarez B, De Mingo Domínguez ML
Instituto de Investigación Hospital 12 de Octubre (i+12) - Facultad de Medicina - Universidad Complutense de Madrid (España)

Thyroid hormones, TSH, thyroid cancer and bones in pre- and postmenopausal women

DOI: http://dx.doi.org/10.4321/S1889-836X2017000200006

Correspondence: Federico Hawkins - Servicio de Endocrinología - Hospital Universitario 12 de Octubre - Avda. de Córdoba, s/n - 28041 Madrid (Spain)
e-mail: Federico.hawkins@salud.madrid.org

Summary

In recent years, progress has been made in regulating skeletal development and maintenance of bone mass of the adult by the hypothalamus-pituitary-thyroid axis. Studies have been carried out into the effect of thyroid hormones on the osteoblasts, osteoclast and the chondrocyte. This research has led to better genetic knowledge into the physiology of the cellular action of these hormones. Recently, possible D2 deodinase interventions in osteoporosis have been proposed. The link between bone mineral dignity, bone quality and the risk of fractures with thyroid hormones in normal postmenopausal women suggest a role for these hormones, even within the range of normal thyroid, in these diseases.

On the other hand, the incidence of differentiated thyroid cancer, experimental *in vivo* thyroid hormone suppression by therapy, recurrent disease, has increased significantly. There are management guides, but it is clear that the secondary derivatives require a precise balance-adjusted indication, risk-benefit ratio of thyroid hormone dosage, prescribed long term, especially in cases of low tumor aggressiveness, advanced age and even in fragile patients. High risk patients should be referred for a bone densitometry, to consider treating future fractures. Prevention of osteoporosis, particularly in postmenopausal women, is highly desirable and should include adequate diet in calcium and vitamin D supplementation if necessary. There is still no consensus on osteoporosis treatment in the patient with thyroid cancer and suppressive treatment, but the indicated criteria for postmenopausal osteoporosis seem to be applicable in general.

Key words: *thyroid cancer, dual-photon densitometry, bone mineral density, trabecular bone score, hyperthyroidism and subclinical hypothyroidism, thyrotrophic hormone.*

Introduction

Thyroid hormones (HT) are involved in skeletal development, peak bone mass acquisition, and maintenance of bone remodeling. Clinical-epidemiological studies indicate that both deficiency and excess of HT are associated with risk of fractures, with euthyroidism being considered as fundamental for the normal functioning of bone remodeling[1].

This "homeostatic" response to HT is regulated at different levels, but in particular by the conversion of thyroxine (T_4) to triiodothyronine (T_3) by iodothyronine deiodinases, responsible for the latter acting on its peripheral receptors.

In this paper, we will review the cellular actions of HT on bone, and especially the *in vivo* experimental model of thyroid stimulating hormone excess and suppression (TSH) in patients with differentiated thyroid carcinoma (CDT) in women Pre and postmenopausal. In men with CDT there are no longitudinal quality studies for analysis.

Thyroid hormones and bone

HT and bone are closely related, since HT are key regulators of bone remodeling. HT plays a key role in the growth and development of vertebrates. HT are iodothyronines synthesized in the thyroid gland, whose constant secretion is ensured by two mechanisms: 1) secretion of HT controlled by a retroactive mechanism, hypothalamic-pituitary-thyroid gland axis (Figure 1), and 2) by regulated intracellular activation by iodothyronine-deiodinases[2].

Thyroid stimulating hormone (TSH) produced by the thyrotrophic cells of the pituitary gland, promotes the synthesis and secretion of HT, mainly 3, 5,3',5'-tetraiodothyronine (T_4), or thyroxine. It is considered that T_4 behaves as a prohormone that needs to be converted to the 3,3',5 triiodothyronine (T_3), which is more potent and is considered biologically active, which is carried out through a 5'-monodeiodination Present in tissues. If iodine cleavage is position 5, this molecule results in the inactive metabolite 3,3',5'-triiodothyronine, or reverse T_3 (rT_3), with weak agonist activity on the same receptors as T_3.

The thyroid gland secretes T_4 and also small amounts of T_3, the active hormone. The majority of circulating T_3 originates from the deiodination of T_4 in peripheral tissues. To perform genomic action, T_4 must be converted to T_3 (Figure 2). Of the three deiodinases involved in the metabolism of HT, deiodinase type 1 (D1), which is expressed mainly in the thyroid gland, is the main responsible for the transformation of T_4 to T_3. It is estimated that D2 intervenes in the control of its concentrations, contributing to limit the access of the HT to the tissues, during the processes of tissue development and repair. The joint action of D2 and D3 would be responsible for the intracellular control of the availability of T_3[3].

The uptake of thyroid hormones by tissues is carried out by specific transporter proteins. Both T_4 and T_3 enter the target cells through membrane-specific transporters, including monocarboxylate transporters 8 and 10 (MCT8 and MCT10) and OATP1c1[4]. The best studied was the MCT8 monocarboxylated transporter, with inactivating mutations in gene 8 located on the X chromosome of this protein that cause Allan-Herndon-Dudley syndrome, with high concentrations of HT and neurological abnormalities, as well as hearing disorders[5].

Receptors for HT

Once in the cellular interior, deiodinase D2 converts T_4 to T_3 and deiodinase D3 inactivates both T_3 and T_4, converting them to T_2 and T_3 reverse. T_3 enters the nucleus where 3 types of thyroid hormone (TR) receptors are found: TRα1, TRβ1 and TRβ2, to which it binds by forming a heterodimer with the retinoid X receptor (RXR), which binds in turn to The DNA sequence termed the "HT response element" (TRE) of the T_3 target gene, controlling its expression[1].

These three functional receptors for HT (TRα1, TRβ1 and TRβ2) are encoded by the THR and THR genes, which regulate their expression and transcriptional responses to TR. The expression of TRα1 and TRβ1 has been described in the bone, the former being in predominant concentrations of 10: 1. It is considered, therefore, that TRα is the fundamental mediator of T_3 action on the bone[6].

HT, TSH and bone development

Eutyroidism is essential for the normal development of the skeleton. This is carried out through the process of intramembranous ossification (differentiation of mesenchymal progenitors into cells forming osteoblasts) and endochondral ossification, through which the long bones form a cartilage mold. Chondrocytes are formed from the mesenchymal precursors to form this cartilage mold; In the primary ossification center of this occurs the progressive mineralization. Vascular invasion and emigration of osteoblasts transform this area into trabecular bone; The precursors located in the most peripheral mesenchyme in the perichondrium are differentiated into osteoblasts and form cortical bone. This proliferation and longitudinal growth continues to maturity[1-3].

Both the TRα1 receptor and the TRβ1 are expressed in the chondrocytes of the growth plates, suggesting that they are targets for the action of T_3. Chondrocyte proliferation and differentiation is controlled by Indian hedgehog, PTHrp, BMP-R1A, IGF1, Wnt, and FGFs. The first three by a negative feedback that induces plaque growth and inhibits its differentiation by controlling its linear growth. The HT intervene in this regulation, sensitive to the availability of T_3, which stimulates gene expression for the synthesis of cartilage matrix and its subsequent mineralization.

In osteoclasts it has not been possible to establish that T_3 has effects through the functional receptors expressed in these cells, being possible that they are indirect mediated through the osteoblasts. In states of excess of HT an increase in the number and activity of osteoclasts, as well as bone

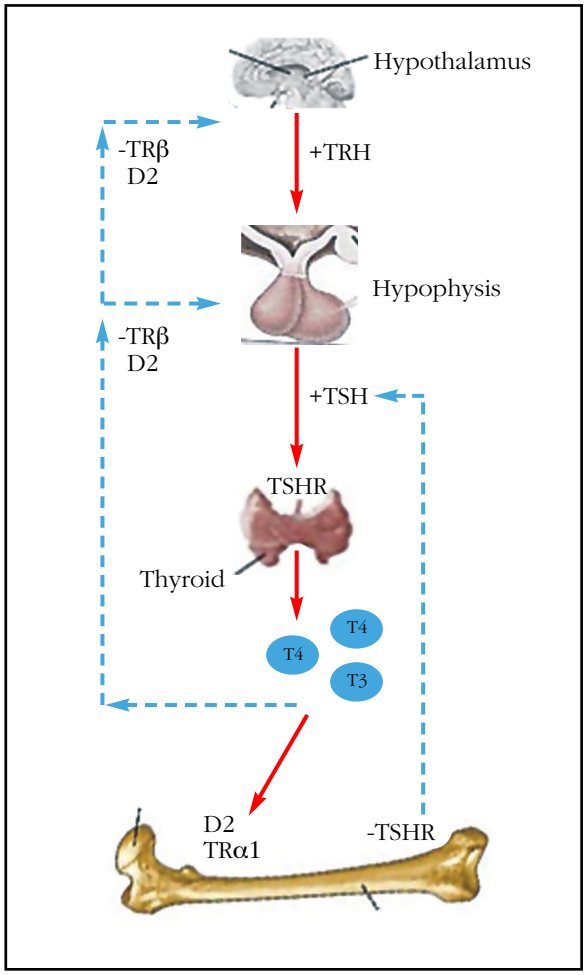
loss, is detected. T_3 also stimulates the differentiation of osteoblasts, the synthesis and mineralization of the bone matrix; These effects are carried out through the regulation of procollagen enzymes, including bone alkaline phosphatase, and metalloproteinases 9 and 13[7]. It is not yet clear whether these effects are mediated via the activator receptor ligand pathway For nuclear factor B (RANKL)[8], although studies with cell cultures of osteoblasts or precursors, demonstrate that T_3 increases the expression of RANKL and interleukins 6 and 8[9].

It is possible that the action of T_3 on osteoblasts is mediated by the expression of osteoprotegerin, which would act by inhibiting RANKL, which in turn stimulates osteoclastogenesis. What has been demonstrated is that T_3 induces the transcription of IGF1, while stimulating its IGF1BP-2 and IGFBP-4 transport proteins, which, together with the increased activity of alkaline phosphatase (and, therefore, Better quality of mineralization) and the other effects already described, behaves as a stimulator of osteoblastic activity at different levels[1]. The TSH-thyroid axis is necessary for this normal skeletal development; TSH has a direct effect on bone, as demonstrated by *in vitro* studies in which it behaves as a direct inhibitor of bone remodeling, through acting on TSHR expressed in osteoblasts and osteoclasts. In relation to the skeletal development phase, TSH alterations are implicated in three diseases: 1) in congenital and acquired hypothyroidism that can cause decreased bone remodeling and increased risk of fractures; 2) in hyperthyroidism, with actions contrary to the previous one, greater remodeling, but also greater risk of fractures; and 3) in craniosynostosis with premature closure of cranial sutures, osteoporosis and fractures. However, since there are circulating levels of HT in these diseases, their effects can not be separated from the action of TSH on bone; The description that isolated TSH deficiency with a mutation affecting the beta-subunit TSH is characterized by a shortened metacarpal and metatarsal phenotype but with normal bone mineral density (BMD) response after treatment with HT in the absence of TSH has led to suggest that the predominant role on bone development corresponds to T_3.

Recently a heterozygous mutation in the THRα gene has been described in a 6-year-old girl, who had HT at the low or normal limit and normal TSH, had growth retardation and histological bone involvement similar to hypothyroidism, which implies a Important role for these TR receptors in human bone development[10].

In adults, hypothyroidism is characterized by decreased bone remodeling with less osteoclastic resorption and less bone formation. This implies a longer duration of the bone remodeling cycle, with an increase in the secondary period of mineralization. This could lead to an increased risk of fractures in these individuals. In contrast, in adult hyperthyroidism, there is a high bone remodeling with osteoporosis characterized by an increase in net bone resorption. There are also more fractures and lower bone mineral density.

Figure 1. Circulating thyroid hormones are under the control of the hypothalamic-pituitary-thyroid axis. TRH stimulates TSH release from the anterior pituitary, which in turn stimulates the synthesis and secretion of T_4 and T_3, which bind and activate TR, resulting in a retroactive inhibition of TRH production and TSH secretion. D2 converts T_4 into T_3 in the peripheral organs, contributing significantly to the circulating deposition of T_3

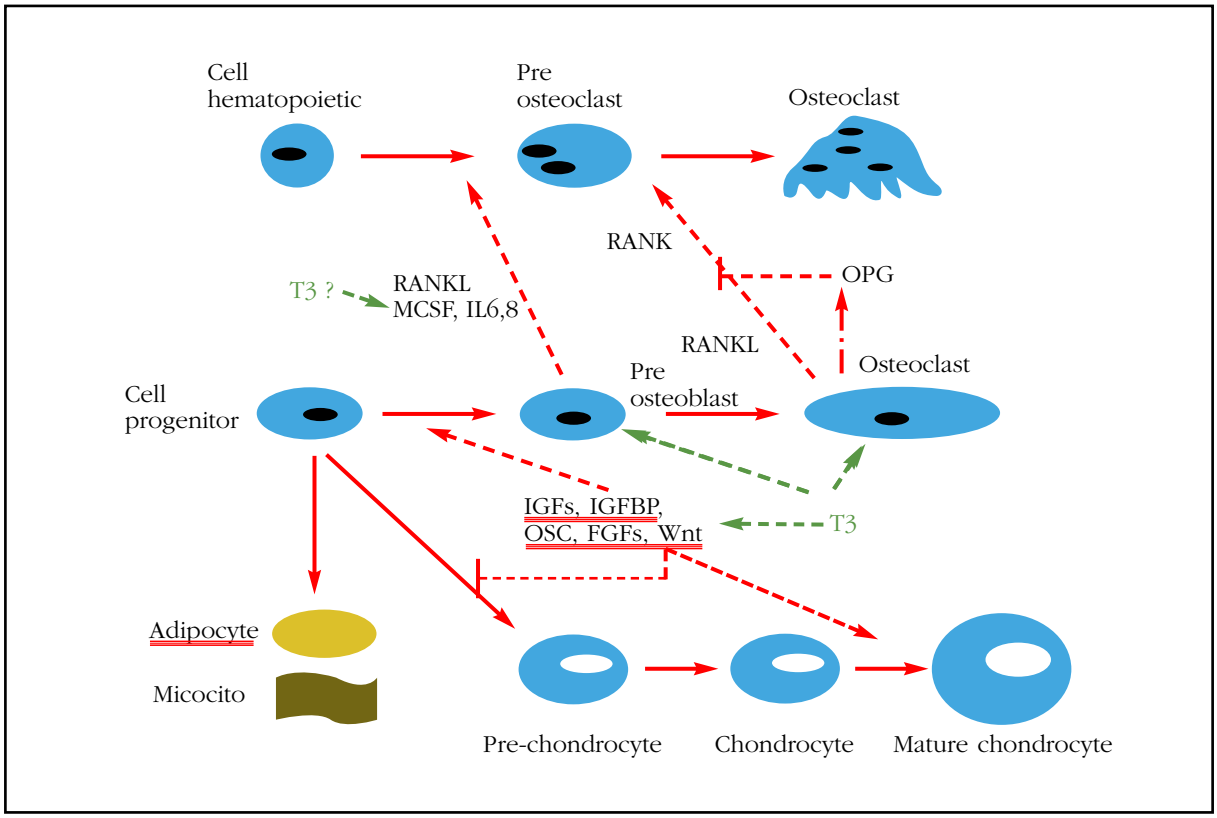


HT and TSH in relation to bone mineral density and fractures in normal population

There are prospective studies in premenopausal and postmenopausal women assessing the effect of TSH and HT levels on BMD in the normal population. Kim et al. Studied the relationship between circulating T_3 and TSH and its effect on bone mass in healthy subjects[11]. In a population of 37,431 adults performed BMD measurement and thyroid function test, excluding diseases that may affect these parameters. Low levels of TSH and elevated T_3 were associated with lower BMD values at all skeletal sites, and confirmed a protective effect of TSH on bone loss independent of the effect of T_3. The negative impact of T_3 on BMD could be offset by an increase in TSH only in those with T_3 levels in the normal-high range.

Studies in relation to fracture risk and bone loss and TSH levels have been conflicting. TSH levels in the low-normal range were associated with hip frac-

Figure 2. The T_3 would act indirectly on the osteoclast by an action mediated by the osteoblast, possibly inducing the release of RANKL and interleukins 6 and 8, and PGE2, in early stages on precursors or favoring the differentiation of the preosteoclast. The T_3 would act favoring the differentiation of the osteoblast and the phases of the mineralization of the matrix. It is possible that induction of IGF-1 transcription and its carrier proteins, and other factors stimulate the proliferation and differentiation of the osteoblast. On the chondrocyte, the availability of TRα1 and TRβ1 in this cell lineage, allows T_3 to stimulate its maturation and therefore the process of endochondral ossification. (Modified by Wojcika et al.[4])



tures in elderly women[12]; While, in the same vein, a study of younger postmenopausal women showed that levels above the normal range were associated with a 35% reduction in the risk of non-vertebral fractures[13]. Finally, a meta-analysis performed with 70,298 participants described a risk of hip fractures of 1.61 (95% CI: 1.21-1.15) and for other fractures of 1.98 (95% CI: 1.41 -2.78) in patients with subclinical hyperthyroidism with TSH levels <0.10 mIU/L[14].

The history of hyperthyroidism appears to be a risk factor. In the SOF (Study of Osteoporotic Fractures) study of 192 elderly women with a follow-up of 4.1 years, the highest incidence of osteoporotic fracture was recorded in patients with a history of fractures and/or hyperthyroidism[15]. In this study, no evidence was found to correlate low TSH levels with low BMD. The authors concluded that hyperthyroidism may or may not reduce bone mass, but that in their study the decline in BMD was not responsible for the strong association between prior hyperthyroidism and the risk of hip fracture.

HT and TSH: relationship with bone mineral density and fractures in women with thyroid dysfunction

Clinical hyperthyroidism is recognized as a risk factor for bone loss, promoting bone turnover and trabecular perforation. In relation to endogenous hyperthyroidism (Graves' disease, multilocular toxic goiter), the data indicate that it may also increase the risk of fractures in general and/or vertebral fractures in postmenopausal women. The prospective study by Bauer et al.[16] showed that hyperthyroid women with TSH levels <0.1 mU/L, compared to euthyroid controls, had a three-fold increased risk of hip fracture (OR: 3.6, CI 95%: 1.0-12.9) and four times of vertebral fracture (OR: 4.5; 95% CI: 1.3-15.6).

In a study by Baqi et al. In premenopausal women receiving oral levothyroxine (LT4), there was a significant correlation between BMD at the lumbar spine (CL) level and hip and TSH levels, as well as a negative correlation between TSH levels and markers Osteocalcin and N-terminal telopeptide of type I collagen (NTX)[17]. The results were more favorable for BMD and levels of bone remodeling markers (MRO) in patients with TSH >0.3 mU/l than those with values <0.3 mU/l.

However, at the level of subclinical hyperthyroidism (TSH suppressed with thyroid hormones in normal range), the effects of HT on bone are more controversial. A prospective study of 2,004 patients with subclinical hyperthyroidism reported a 1.25-fold increase in fracture in these, similar to

the 1.9-fold increase in fracture risk found in patients treated with T_4[18,19]. However, a recent study by Garin et al., Conducted in 4,936 subjects over 65 years of age for 12 years, found no relation between the risk of hip fracture and subclinical hyperthyroidism[20].

Two meta-analyzes of postmenopausal studies with subclinical hyperthyroidism due to exogenous substitution have found a decrease in BMD with an annual loss of 0.91% of bone mass[21,22]. The meta-analysis of Wirth et al., Which includes only 5 published studies with a high quality index, concludes that subclinical hyperthyroidism may be associated with a risk of 2.16 (95% CI: 0.87-5.37) For hip fractures and 1.43 (95% CI: 0.73-2.78) for non-vertebral fractures[23].

Most studies in postmenopausal women show an association between high-normal levels of HT and lower BMD values, with an increased risk of non-vertebral fracture. Kim et al.[24] studied the results of BMD in a group of 959 women with subclinical hyperthyroidism (TSH <0.5 mIU/L) vs A group with TSH >0.5 mU/L. Women with TSH values in the normal-low limit maintained lower BMD values in the spine and femoral neck than those with TSH in the normal-high limit. The former also had a 2.2-fold increased risk of osteoporosis. Similarly, Morris et al.[25], in a sample of 581 healthy American women, describe a higher risk of osteoporosis in women with TSH values at the low-normal limit (0.39-1.8 mIU/L) with (OR: 3.4 [95% CI: 1.3-9.2] and 2.2 [95% CI: 1.2-3.8], respectively).

In summary, published data indicate that to demonstrate clear causality, randomized and controlled trials with a large number of patients are necessary, and to assess whether normalization of TSH levels in subclinical hyperthyroidism is associated with fracture risk. The data suggest that subclinical hyperthyroidism is associated with an increased risk of hip and non-vertebral fractures, but other factors should be analyzed and studies of higher quality should be performed.

In clinical hypothyroidism there is a decrease in bone formation that usually exceeds the decrease in resorption, as confirmed by histomorphometry data. In general, the existence of a normal BMD has been described, contrasting with an increase of 2 to 3 times the frequency of fractures, particularly of forearm in some series. In postmenopausal women with subclinical hypothyroidism, a similar risk of fractures has also been reported, especially those with autoimmune origin[26].

HT and bone trabecular microarchitecture

It has been commented on the possibility that the bone quality, determined by the trabecular microstructure, could also be influenced by the thyroid state. In this sense, Basset et al. Have shown thinning and decreased trabecular connectivity in a mouse model with thyrotoxicosis[27].

More recently, Hwangbo et al. Have studied 1,376 euthyroid subjects (648 postmenopausal) in which they determine HT, free T_4 and trabecular bone score (TBS)[28]. TBS is the technique by which, based on lumbar DXA scanning, it establishes textural gray levels as indirect indices of microarchitecture. They conclude that elevated levels of free T_4 were associated with impairment of trabecular microarchitecture, whereas TSH levels were not associated with lumbar TBS. This would support the results described in mice resistant to HT, in which it has been shown that elevated HT rather than TSH predominate in the regulation of bone state.

Criteria for thyroid suppression in differentiated thyroid cancer

Differentiated thyroid carcinoma (CDT) is the most common endocrine neoplasia (accounting for 1% of all cancers). 85-90% of thyroid cancers are CDT, which includes two variants, the papillary carcinoma (the most frequent) and the follicular carcinoma. Its incidence has increased in the last 10 years, but its mortality rate remains the same[29]. This increase is due in large part to the increase and improvement of resolution of the diagnostic tests, with greater detection of incidental microcarcinoma.

Treatment indicated in the CDT includes total thyroidectomy completed with ablative dose of radioactive iodine. Subsequently, based on the risk of relapse, a dose of oral replacement (very low risk) or suppressive levothyroxine is given. The suppressive dose aims to induce hyperthyroxinemia with pituitary suppression of TSH that could be a potential stimulus for tumor remnants. The initial suppressive dose of levothyroxine is calculated at 1.8-2.2 µg/kg/day, which is modified according to successive controls. Based on the suppression obtained during hormone therapy, the American Thyroid Association (ATA) has established the following risk groups for treatment with levothyroxine: 1) Low-risk group >0.5 mIU/L; 2) Intermediate risk group: 0.1-0.5 mIU/L and; 3) High risk group: <0.1 mIU/L. Patients with exogenous treatment (by CDT) as well as those with endogenous hyperthyroidism are subjected to prolonged periods of the effect of thyroid hormones on the bone. Many of the aspects related to the bone loss that this therapy can cause, either directly or by suppression of the pituitary-thyroid axis, are now known.

Suppression of TSH in thyroid carcinoma. Bone loss and relation to the risk of relapse

Treatment with levothyroxine in CDT is based on doses that suppress serum TSH levels below the normal range, resulting in a condition similar to that of subclinical hyperthyroidism. We have already pointed out how TSH behaves as a stimulus for the proliferation of thyroid cells, in addition to the uptake of radioiodine and the production of thyroglobulin, so suppression seeks to remove this effect and prevent a recurrence. TSH receptors have been described in the membranes of CDT tumor cells whose concentrations are affected by the reduction of TSH by levothyroxine therapy[30]. There are also observational epidemiological studies in which a positive correlation has been

found between elevated serum TSH levels and risk of malignancy in nodules or more advanced stages of CDT (Table 1). Finally, McGriff et al., in a meta-analysis involving 4,174 patients with CDT, demonstrated a decreased risk of tumor progression in patients receiving levothyroxine suppressive therapy (RR=0.73; 95% CI: 0.6-0.88, p<0.05)[31].

Although there is no general consensus about optimal TSH levels to decrease relapses and minimize the adverse effects of subclinical hyperthyroidism, the American Thyroid Association (ATA) recently defined the impact of TSH suppression in patients with CDT Characterized by low, intermediate and high risk of relapses taking into account several clinical factors[32].

It should be noted that previously Biondi and Cooper, in a review, concluded that aggressive suppression of TSH is important in patients with CDT and high risk, and is much less critical in the other groups[33]. Based on these criteria, Wang et al. Recently studied 306 non-suppressed patients and 465 suppressed patients with CDT classified as low or intermediate risk, and who presented similar recurrence rates after 6 years of follow-up[34]. However, patients with TSH suppression <0.4 mIU/L had a higher incidence of osteoporosis and atrial fibrillation compared with non-suppressed patients (HR 2.1; p=0.05), meaning that prolonged treatment with levothyroxine with Suppressive effect increases the risk of postoperative osteoporosis in patients with low and moderate risk of CDT, according to the ATA classification.

It can be concluded that the optimal dose of maintenance of TSH in patients with CDT of low or intermediate risk of relapse has not yet been well established. Studies suggest that a level of 0.9-1.0 mIU/L could be the optimal suppression value for low- and intermediate-risk CDTs, in order to further reduce the development of osteoporosis and long cardiologic complications Term, without increasing the risk of relapse. It is possible, therefore, that TSH suppression is an independent predictor of bone damage that, moreover, does not seem to diminish relapses in these low- and intermediate-risk patients.

Impact of TSH suppression: adverse effects and quality of life

The prescription of thyroid hormones is ample, reaching almost 5.1% of the adult population. It is generally a well tolerated medication and few immediate side effects. In the last years, publications are being made regarding whether levothyroxine therapy increases the incidence of fracture in the long term. Current evidence is not definitive, although Turner et al. Showed an increase in fractures in elderly patients (>70 years) treated for long periods with thyroxine[35]. The mechanisms by which thyroxine would induce these fractures are unknown, but it has been suggested that bone mineral density would be decreased through induction of subclinical hyperthyroidism, or that normal-high levels cause it . The greater frequency of falls due to arrhythmias favored by this increase of thyroid hormones would be another cause.

The main adverse effects of TSH suppression affect the cardiovascular system, bone metabolism and quality of life (Table 2). In clinical hyperthyroidism, the incidence of atrial fibrillation, myocardial infarction, and mortality increased markedly in the elderly[36]. It is known that atrial fibrillation can triple in the course of 10 years of treatment (TSH <0.1 mIU/L) in those over 65, euthyroid subjects (TSH at the limit of normal). In subclinical CDT hyperthyroidism, in patients treated with levothyroxine, the risk of atrial fibrillation may reach 10.3% (17.5% in the >60 years), according to a study conducted in a population-based population register Million in Denmark[37]. Finally, overall mortality has also been increased (OR: 1.20, 95% CI: 1.06-1.36) in situations of hyperthyroidism in patients with TSH <0.03 mIU/L, compared with those of those With values ranging from 0.04 to 0.4 mIU/L[38].

The increase of thyroid hormones can cause emotional alterations (nervousness, anxiety), mood disorder (depression, sleep disorders, asthenia) and various cognitive alterations, which can influence the quality of life of the patient. Samuel et al. Describe greater items of fatigue and depression in patients treated with levothyroxine suppressive doses[39]. Jarcas et al. Reported cognitive alterations in 31 patients with CDT and suppressive therapy with thyroid hormones[40]. In front of these, Moon et al. Have pointed out that the cognitive functions studied in a group of 50 patients with CDT over 65 years were positively correlated with the higher serum T_4 elevation of these patients in relation to the controls[41].

An observational study by Flynn et al. has studied the effects on the cardiovascular system and fractures in a population of 17,684 subjects on prolonged T_4[38] treatment. They found that patients with elevated (>4 mIU/L) or suppressed (<0.03 mIU/L) TSH had an increased risk of cardiovascular disease, with HR=1.95 (95% CI: 1.73-2.21), for arrhythmias of 1.80 (95% CI: 1.33-2.44) and for fractures of 1.83 (95% CI: 1.41-2.37); had low but not suppressed TSH (0.04-0.4 mIU/L) did not present increased risk in any of these objectives. These authors conclude that it might be safe for patients who ingest T_4 to maintain low but not suppressed TSH.

Thyroid hormones, thyroid suppression and differentiated thyroid cancer

Clinical hyperthyroidism is a recognized risk factor for bone loss, promoting bone remodeling, trabecular perforation, and increased risk for fractures. At the level of subclinical hyperthyroidism (TSH suppressed with normal range HT) the effects of HT on bone are more controversial. Experimental studies and clinical data have demonstrated that thyroid cell proliferation is dependent TSH[42]. The start of treatment with suppression of TSH causes a situation of subclinical hyperthyroidism. Baliran et al.[43] have shown that excess of HT and low TSH levels stimulate bone resorption. This should be taken into account, given the general good prog-

Table 1. TSH targets for prolonged treatment with thyroid hormone in differentiated thyroid carcinoma. According to Haugen BR et al.[32]

TSH targets for prolonged treatment with thyroid hormone				
Risk of suppression of TSH	**Excellent**	**Undetermined**	**Incomplete biochemistry****	**Structural incomplete**
Not known		Mild suppression. Target TSH 0.1-0.5 mIU/L		Moderate or complete suppression. TSH <0.1 mIU/L
Menopause	No deletion. Target TSH 0.5*-2.0 mIU/L			
Tachycardia				
Osteopenia				
Age >60				
Osteoporosis				
Atrial fibrillation				

*0.5 mIU/L represents the lowest reference limit of the TSH determination method which may vary between 0.3-0.5 mIU/L depending on the method.
**The TSH target for patients with incomplete biochemical response may vary depending on the initial ATA risk, Tg levels, Tg trend over time, and risk of suppression.

nosis of these patients, which could lead to the appearance of fractures in prolonged periods of suppressive therapy. In general, the studies describe more aggressive treatments for suppression of TSH in patients at high risk of disease or tumor recurrence, while a less aggressive suppression seems advisable in patients with low risk. In addition, it should be noted that, in recent years, the increase in the prevalence of papillary microcarcinomas with good survival requires modification of these suppression criteria. The maintenance of TSH numbers in the normal range may be advisable for long-term treatment in patients with advanced CDT and relapse-free.

Suppressive treatment with HT in cancer differentiated from thyroid and bone. Longitudinal studies vs transverse

To date, a large number of cross-sectional studies have been published on the effect of suppressive therapy with HT on CDT in both premenopausal and postmenopausal women. In premenopausal studies, there are three studies that find a decrease in BMD in some of the studied areas[44-46]. In front of them, there are three times more studies that do not find any deleterious effect of TSH suppression on the bone in these patients[47].

In postmenopausal patients with CDT, there is a greater disparity of results: some report a decrease in lumbar and neck BMD, and in some, there is also bone loss in radio[46,48,49], in contrast to a large majority who register changes in BMD with Suppressive treatment[50-53]. It is possible that the heterogeneity of the thyroid cancer patients selected for the studies, the different levels of TSH suppression and the different techniques used for hormonal determinations and bone mineral density may influence the significant differences of these results.

For the above reasons, we believe that the study of bone mass follow-up in these patients with CDT is of more value, disregarding cross-sectional studies that reflect a specific situation. Compared with longitudinal studies, cross-sectional analyzes are more susceptible to sample error and other bias[54]. The objective was to review the publications with prospective criteria, the possible bone losses in the different areas studied with bone densitometry, with time of treatment and detailed follow-up, as well as the criteria and times of TSH suppression of these patients. Following these objectives we found in PubMed 11 publications, with longitudinal follow-up, including one from our group[55], which we will analyze next (Table 2).

The first longitudinal study was Pioli et al.[56], who studied 14 premenopausal patients (age 43±6.8 years) with TCD, with densitometries every six months and during follow-up with levothyroxine reaching 3 years. Although ten of these patients underwent almost total thyroidectomy and 5 to subtotal, the HT and suppression patterns were similar, reaching suppression at 4 months, which was maintained during the study. The authors reported bone loss at the spine level of 2.6±1.9% per year, vs the 0.2±1% found in the control group of 15 normal. Paradoxically, if this loss were continued for ten years, it would be 26% in excess of the controls, a fact that has not been repeated in any other study. The radial bone density was normal. It is possible that in these results the large inter-individual variety of the bone para-

Table 2. Relationship of longitudinal studies on the effect of TSH suppression with levothyroxine on bone mineral density (BMD) in pre and postmenopausal women with thyroid cancer

Authors/year	Pre-menopausal with cancer thyroid	Post-menopausal with cancer thyroid	% patients with suppression of TSH	Duration average tracing with DXA	Duration average treatment with HT	Effect on BMD
Pioli G and cols. 1992[56]	14	-	100%	1-3 years	1-3 years	Decrease in BMD-L
Muller CG and cols. 1995[57]	15	10	40%	1,5 years	10 years	No decreases in BMD-L BMD-CF
Fujiyama K and cols. 1995[59]	-	24	50%	1 year	11-15 years	No decreases in BMD-L, BMD-RD
Kung AWC and cols. 1996[60]	-	15	100%	2 years	11,3±6 years	Decreases BMD-L, BMD-CT, BMD-CF, BMD triangle Ward
Guo CY and cols. 1997[61]	-	23	100%	2 years	NE	No decrease in BMD-L, BMD-CF and BMD-CT
Jódar E and cols. 1998[55]	14	13	50%	2,3 years	5,7 years	No decrease in BMD-L, BMD-CF and minimal reduction BMD-RD
Sijanovic S and cols. 2001[62]	19	-	100%	4 years	9 years	No decrease in BMD-L, BMD-CF and minimal reduction BMD-RD
Sugitani I and cols. 2001[63]	-	120	100%	5 years	NE	Decreased BMD-L alone in patients >50 years
Karner and cols. 2005[64]	19	-	100%	1 year	9,4±6 years	No decrease in BMD-L, BMD-CF or in BMD-RD Schneider R and cols. 2012[65]
Kim MK and cols. 2015[66]	49	44	NE	1 year	2 months-1 year	Decrease BMD-L, BMD-CF and BMD-CT in postmenopausal
Kim CW and cols. 2015[67]	24	100	100%	1-1,5 years	NE	No decreases in BMD-L and CF

BMD L: lumbar bone mineral density; BMD CT: bone mineral density total hip; BMD T: trochanteric lumbar bone mineral density; BMD RD: bone mineral density ultradistal radio; BMD CT: total body; NE: not specified; -: they do not have patients in that group.

meters referred to is affected, as well as the use of two different techniques, such as SPA (single photon absorptiometry) and DXA.

The second longitudinal study is Mulller et al.[57]. They studied 15 premenopausal women and 10 postmenopausal women in T_4 suppressive treatment for a variable period of 1.5 years. Of this group, 24 patients with CDT were re-evaluated with DXA with a follow-up interval of 1.5±0.5 years. They selected 15 matched controls in sex, menopausal status, age and BMI. They concluded that suppression of TSH was accompanied by non-significant reductions (2-5%) of lumbar BMD and femoral neck BMD, without any incident fractures. The decrease in BMD found is lower than the classic one described by Mazess, in which the increased risk of vertebral fracture increases 1.5-2 times for each standard deviation (DS) that decreases BMD[58], which senses no effect to this level.

In the Fujiyama et al. series[59], 24 postmenopausal patients were described, divided into two groups, with and without TSH suppressor doses, with 12 patients with CDT each. Both groups had a similar bone loss rate: -0.849±0.605 in the suppressed ones, and -0.669±0.659 in the non-suppressed ones. On the other hand, Z-score values for lumbar and total body BMD were similar to those reported for healthy controls.

In 1996, Kung et al.[60] detailed a study in CDT-operated postmenopausal women who distributed in three subgroups with 15 patients in each: the first, in treatment with calcitonin; The second,

with calcium alone; And the third group, with placebo without any treatment, which is the group we included in this review. Patients in this third group were followed for two years after the administration of levothyroxine and effective suppression of TSH (<0.03 mIU/L) postoperatively for approximately 9 years. They found a significantly superior bone loss at the lumbar, total hip, trochanter and Ward triangles (5.0%, 6%, 4.7%, 8.8%, respectively, p<0.05). However, when no fractures were found, they thought that the clinical importance of this bone decrease should be questioned.

Guo et al.[61] performed a prospective study in 23 postmenopausal women with intervened CDT and subsequent TSH suppression, followed by 2 years with bone densitometry and bone markers. Serum TSH levels were measured every 6-12 months to control TSH suppression. TSH levels were correlated with bone markers (osteocalcin, bone alkaline phosphatase and NTX). This group of postmenopausal women was compared with two other control groups (with and without suppressed TSH levels) who had primary hypothyroidism or Hashimoto's thyroiditis (n=41). They found that control patients had an increase in lumbar and femoral neck BMD and a decrease in bone markers, whereas patients with CDT had decreased bone markers without modifying BMD. Their results suggest that in postmenopausal women in T_4 treatment, bone remodeling is related to the degree of TSH suppression, and that the decrease in T_4 dose in those with suppressed TSH may induce a decrease in bone remodeling.

In our experience[55], we studied 14 premenopausal and 13 postmenopausal women with CDT and TSH suppression followed in our service since their total thyroidectomy with dual photon densitometry repeated for two years. Fifty percent of our patients had TSH below 0.1 mIU/L. The dose of LT4 showed a positive predictive value in each studied bone site which had been scarcely described. None of the bone and mineral parameters studied were correlated with bone mass, except for alkaline phosphatase at Ward's triangle level and ultradistal radius. This is consistent with normal BMD and bone remodeling values found in these patients with prolonged suppressive treatments. The suppressed patients showed a small reduction in BMD in 1/3 distal radius (Z-score -=0.77±0.98, CI 95: -1.11, -0.44), without differences between the pre and postmenopausal.

The study with longer duration of follow-up is that of Sijanovic et al.[62]. These authors studied 19 premenopausal women with intervened CSD (mean age 39±8 years) who underwent T_4 suppressive treatment for an average of 9.4 years. The prospective study with bone densitometry was performed in 4 years. They noted that after one year there was no significant bone loss in any region of the skeleton, and yet, after performing 3 measurements, at 4 years they recorded significant loss of BMD in the distal radius and not in other areas. They commented, surprisingly, that in their analysis there is a decrease although not significant of bone mass in other areas (data not given), so suppressive TSH therapy with thyroxine in a period of approximately 10 years may induce a risk of osteopenia In premenopausal women who reach menopause.

In a selective, rather large group of postmenopausal patients with papillary CDT, Sugitani et al.[63] analyzed the effect of post-operative suppressive TSH therapy on disease-free survival and its effects on BMD. Two groups were analyzed: 140 patients with suppression (mean TSH: 0.07±0.10 mIU/L, and 127 without suppression (mean TSH: 3.14±1.60 mIU/L) .In the non-suppressed group, 120 patients Postmenopausal women were followed for 5 years, showing a decrease in lumbar BMD subgroup of postmenopausal women over 50 years of age. TSH suppression had no significant effects on the prevention of relapses in papillary CDT, although most of its In the end, it is recommended that suppression of TSH, especially in patients with low risk and in elderly patients, is not indicated, taking into account that it has not been shown to decrease recurrences even in patients with high risk.

The longitudinal study by Karner et al.[64] was carried out in premenopausal women with CDT for one year. The duration of TSH suppression at the start of the study was 9.4±6.4 years, and therefore broad. BMD measurements were performed twice over a period of one year. Using single photon absorptiometry (SPA) for extremities and DXA, they found no decrease in BMD at the distal radius, or in lumbar and/or hip BMD. It is a longitudinal study of short duration, small number of subjects (19 premenopausal). Its main recommendation is to practice the bone densitometry study before initiating the suppressor therapy of TSH to identify the patients with high risk of osteoporosis.

More recently a study was published by Schneider R et al.[65] to evaluate the potential effects of LT4 suppressive treatment in 46 premenopausal women undergoing CDT on BMD and bone and muscle strength. It is a prospective, cohort-controlled, 1-year follow-up, in which bone mass is measured by dual lumbar and hip photometry, and bone and muscle strength using the polar stress index with dynamometry. They are simultaneously studying 23 premenopausal women undergoing LT4 replacement therapy. In both premenopausal populations, with suppressive treatment or with substitutive treatment, they do not find a decrease in axial BMD; The annual loss (g/cm^2) in patients with CDT was not significantly different from those receiving LT4 replacement therapy (BMD -0.005 vs +0.004; BMD femoral neck: -0.005 vs +0.00; total hip BMD: +0.001 vs +0.003, respectively). The authors concluded that there is little evidence of adverse effects of levothyroxine on bone, and that premenopausal women with CDT may be at risk for lower BMD at the ultradistal radius. In spite of their null data in this sense, they attribute loss of unbalanced cortical BMD by trabecular augmentation, probably indicating a high endocortical trabecularization.

Kim et al.[66], in a one-year prospective study, found a decrease in bone mass that predominantly affects postmenopausal women compared to premenopausal women in their study. The annual postmenopausal loss was -2.1% in the lumbar spine, -2.2% in the femoral neck, -2.1% in the total hip, significantly higher than the premenopausal women ($p<0.05$ for all). Although the authors report that bone loss was primarily during the early post-thyroidectomy period, a longer study might confirm this.

Finally, Kim et al.[67] conducted a prospective 12-18 month study in 24 premenopausal women with CDT (6 hypoparathyroid glands) and 100 postmenopausal women (50 hypoparathyroid glands), concluding that they found no deleterious effect of suppressive therapy with T_4, Even a protective effect in patients with post-operative hypoparathyroidism.

Risk factors in patients with CDT and TSH suppressor therapy

López Alvarez et al.[68] studied the risk factors involved in possible bone loss in 43 premenopausal and 53 postmenopausal women with TDC treated with suppressive thyroid hormones and followed up for an average of 75 months. Age, as a risk factor, and weight as a protective factor were the variables that most influenced BMD. No significant differences were found when comparing patients with normal concentrations of free thyroxine versus those who had them slightly elevated. In postmenopausal women, there was greater lumbar BMD in the group with adequate calcium intake (957 mg/day) compared to those who did not (855 mg/day) ($p<0.05$). At the level of the femoral neck and lumbar region, TSH, along with age and weight, were the variables that influenced the most. Gómez de Melo et al.[69] carried out another similar study in 109 postmenopausal women with CDT and suppressive treatment, in which they identified that, in the multivariate logistic regression analysis, the factors significantly related to lower BMD values were: low BMI and TSH; Do not find relation between the BMD and the average values of free T_4. They suggest that TSH can have negative effects on BMD only when levels are suppressed.

In summary, most of what has been reported in relation to premenopausal women with CDT and suppressive therapy of TSH shows no deleterious effects on BMD in any anatomical site. In postmenopausal women with CDT and TSH suppression the studies are more heterogeneous, but, nevertheless, it must be pointed out that there are three studies, commented, with important population that refer to bone loss

Conclusions

In recent years, there have been important contributions to the better understanding of the regulation of the skeleton by HT and the hypothalamic-pituitary axis. The deiodination of the HT during its metabolism is considered an important determinant of the thyroid state at the circulating level and of the peripheral tissues. In bone, the activity of deiodinase D2 is involved in osteoblasts and in maintaining adequate mineralization and bone strength. Deiodinase D3 would intervene very early, at cartilage level favoring skeletal growth and development.

In subjects with subclinical hyperthyroidism other than CDT, controlled trials with a significant number of patients are considered necessary to evaluate the efficacy of normalizing TSH levels associated with fracture risk. The accumulated experience with the suppressive treatment of TSH in the CDT is configuring a therapeutic strategy with greater evidence for the treatment of patients with low risk and intermediate, in whom this approach would not be necessary. In contrast, patients at high risk could benefit; However, it is the elderly patients with high risk who usually have greater comorbidities and, in whom the indication will often have to be evaluated.

Bibliography

1. Gogakos AI, Duncan Basset JH, Williams GR. Thyroid and bone. Arch Biochem Biophys. 2010;503:129-36.
2. Duncan Basset, Williams GR. Role of thyroid hormones in skeletal development and bone maintenance. Endocr Rev. 2016;37(2):135-87.
3. Waung JA, Duncan Basset, Williams GR. Thyroid hormone metabolism in skeletal development and adult bone maintenance. Trends Endocrinol Metab. 2012;23:155-62.
4. Wojcicka A, Duncan Basset, Williams GR. Mechanisms of action of thyroid hormones in the skeleton. Biochim Biophys Acta. 2013;1830:3979-86.
5. Schwartz CE, Stevenson RE. The MCT8 thyroid hormone transporter and Allan-Herndon-Dudley syndrome. Best Prac Test Clin Endocrinol Metab. 2007;21:307-21,
6. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network.Cell. 2006 Aug 25;126(4):789-99.
7. Williams GR, Bland R, Sheppard MC. Characterization of thyroid hormone (T3) receptors in three osteosarcoma cell lines of distint osteoblast phenotype:interaction among T3, vitamin D3 and retinoid signalling. Endocrinology. 1994;134:2375-85.
8. Saraiva PP, Teixeira SS, Padovani CR, Nogueira CR. Triiodothyronine (T3) does not induce Rankl expresión in rat Ros 17/2.8 cells. Arq Bras Endocrinol Metab. 2008;52:109-13.
9. Varga F, Spitzer S, Klaushofer K. Triiodothyronine (T3) and 1,25 dihydroxyvitamin D3 (1.25D3) inversely regulate OPG gene expression in dependence of the osteoblastic phenotype. Calcif Tissue Int. 2004;74:382-7.
10. Bochukova E, Schoenmaker N, Agostini M, Schoenmakers E, Rajanayagan O, Keogh JM, et al. Mutation in the thyroid hormone receptor alpha gene. N Engl J Med. 2012;366:243-9.
11. Kim TH, Joung JY, Kang M, Choi SK, Kim K, Jang JY, et al. A modest protective effect of thyrotropin against bone loss is associated with plasma triiodothyronine levels. PLoS One. 2015;10(12):e0145292.
12. Leader A, Ayzenfeld RH, Lishner M, Cohen E, Segev D, Hermoni D. Thyrotropin levels within the lower normal range are associated with an increased risk of hip fractures in euthyroid women, but no men, over the age of 65 years. J Clin Endocrinol Metab. 2014;99:2665-73.
13. Murphy E, Gluer CC, Reid DM. Felsenberg D, Roux C, Eastell R, et al. Thyroid function within the upper normal range is associated with reduced bone mineral-

density and an increased risk of non vertebral fractures in healthy euthryoid postmenopausal women. J Clin Endocrinol Metabol. 2010;95:3173-81.
14. Blum MR, Bauer DC, Collet TH, Fink Ha, Cappola AR, da Costa BR, et al. Subclinical thyroid diysfuncition and fracture risk: a meta-analsis. JAMA. 2015;313:2055-65.
15. Cummings S, Nevitt MC, Browner WS, Stone K, Fox KM, Kristine E, et al. Risk factors for hip fracture in white women. N Engl J Med. 1995;332:767-74.
16. Bauer DC, Ettinger B, Nevitt MC, Stone KL. Study of osteoprotic fractures research group. Risk for fractures in women with low serum levels of TSH. Ann Intern Med. 2001;134:561-8.
17. Baqi L, Payer J, Killinger Z, Hruzikova P, Cierny D, Susienkova K, et al. Thyrotropin versus thyroid hormone in regulating bone density and turnover in premenopausal women. Endocr Regul. 2010;44(2):57-63.
18. Valdiveloo T, Donnan PT, Cochrane L, Leese Gp. The thyroid epidemiology, audit and research study (TEARS): morbidity in patients with endogenous subclinical hyperthyroidism. J Clin Endocrinol Metab. 2011;96:1344-51.
19. Turner MR, Camacho X, Fischer HD, Turner MR, Camacho X, Fischer HD, et al. Levothyroxine dose and risk of fractures in older adults:nested case control study. BMJ. 2011:342:561-8.
20. Garin MC, Arnold AM, Lee JS, Robbins J, Cappola AR. Subclinical thyroid dysfunction and hip fracture and bone mineral density in older subjects: the cardiovacscular health study. J Clin Endocrinol Metab. 2014;99:2657-64.
21. Faber J, Galloc AM. Changes in bone mass during prolonged subclinical hyperthyroidism due to L thyroxine treatment: a meta analysis. Eur J Endocrinol. 1994;130:350-6.
22. Uzzan B, Campos J, Cucherat M, Nony P, Boissel JP, Perret GY. Effect on bone mass of long term treatment with thyroid hormones: a meta analysis. J Clin Endocrinol Metab. 1996;81:4278-89.
23. Wirth CD, Blum MR, da Costa BR, Baumgartner C, Collet TH, Medici M, et al. Subclinical thyroid dysfunction and the risk for fractures: a systematic review and meta analysis. Ann Intern Med. 2014;161:189-99.
24. Kim DJ, Khang YH, Koh JM, Shong YK, Kim GS. Low normal TSH levels are associated with low bone mineral density in healthy postmenopausal women. Clin Endocrinol. 2006;64;86-90.
25. Morris M. The association between serum thyroid-stimulating hormone in its reference range and bone status in postmenopausal American women. Bone. 2000:40:1128-34.
26. Tuchendler D, Bolanowski M. Assessment of bone metabolism in premenopausal femles with hyperthyroidism and hypothyroidism. Endokrynol Pol. 2013;64:40-4.
27. Basset JH, O´Shea PJ, Sriskantharaj S, Rabier B, Boyde A, Howell PG, et al. Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Mol Endocrinol, 2007;21:1095-107.
28. Hwangbo Y, Kim JH, Kim SW, Park YJ, Park DJ, Kim SY, et al. High normal free thyroxine levels are associated with low normal trabecular bone scores in euthryoid postmenopausal women. Osteoporos Int. 2016;27:457-62.
29. Morris LG, Sikora AG, Tosteson TD, Davies L. The increasing incidence of thyroid cancer: the influence of access to care.Thyroid. 2013;23(7):885-9.
30. Vassart G, Dumont JE. The thyrotropin receptor and the regulation of thyrocyte function and growth. Endocr Rev. 1992;13:596-611.
31. McGriff NJ, Csako G, Gourgiotis L, Lori CG, Pucino F, Sarlis NJ. Effects of thyroid hormone suppression therapy on adverse clinical outcomes in thyroid cancer. Ann Med. 2002;34(7-8):554-64.
32. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016;26(1):1-133.
33. Biondi B, Cooper DS. Benefits of thyrotropin suppression versus the risks of advere effects in differentiated thyroid cancer. Thyroid. 2010;20:135-45.
34. Wang Y, Smith AW, Palmer FL, Tuttle RM, Mahros A, Nixon IJ, et al. Thyrotropin suppression increases the risk of osteoporosiswsithout decreasing recurrence in ATA low and intermediate risk patients with differentiated thyroid carcinoma. Thyroid. 2015;25:300-7.
35. Turner MR, Camacho X, Fischer HD, Austin PC, Anderson GM, Rochon P, et al. Levothyroxine dose and risk of fractures in older adults: nested case-control study. BMJ. 2011;342:d2238.
36. Do Cao C, Wemeau JL. Risk benefit ratio for TSH suppressive Levothyroxine therapy in differentiated thyroid cancer. Ann Endocrinol. 2015;76:1S47-52.
37. Aboowara A, Quraishi A, Sapp IL, Alqambar MH, Sarie A, O Connell CM, et al. Prevalence of atrial fibrillation in patients taking TSH suppression therapy for management of thyroid cancer. Clin Invest Med. 2012;35:E152-6.
38. Flyn RW, Bonellie SR, Jung RT, McDonald TM, Morris AD, Leese GP. Serum thyroid stimulating hormone concentration and morbidity from cardiovascular disease and fractures in patients on longterm thyroxine therapy. J Clin Endocrinol Metab. 2010;95:186-93.
39. Samuels MH.Thyroid disease and cognition. Endocrinol Metab Clin North Am. 2014;43(2):529-43.
40. Jaracz J, Kucharska A, Rajewska-Rager A, Lacka K. Cognitive functions and mood during chronic thyrotropin-suppressive therapy with L-thyroxine in patients with differentiated thyroid carcinoma. J Endocrinol Invest. 2012;35(8):760-5.
41. Moon JH, Ahn S, Seo J, Han JW, Kim KM, Choi SH, et al. The effect of long-term thyroid-stimulating hormone suppressive therapy on the cognitive function of elderly patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2014;99(10):3782-9.
42. Papaleontiou M, Hawley ST, Haymart MR. Effect of thyrotropin suppression therapy on bone in thyroid cancer patients. Oncologist. 2016;21(2):165-71.
43. Baliram R, Sun L, Cao J, Li J, Latif R, Huber AK, et al. Hyperthyroid associated osteoporosis is exacerbated by the loss of TSH signaling. J Clin Invest. 2012;122:3737-41.
44. Ross DS, Neer RM, Ridgway EC, Daniels GH. Subclinical hyperthyroidism and reduced bone density as a possible result of prolonged suppression of the ituitary thyroid axis with L thyroxine. Am J Med. 1987;82:1167-70.
45. Paul Tl, Kerrigan J, Kelly AM, Braverman LE, Baran DT. Long term L thyroxine therapy is associated with decreased hip bone density in premenopausal women. JAMA. 1988;259:3137-41.
46. Diamond T, Nery L, Hales A. A therapeutic dilemma: suppressive doses of thyroxine significantly reuce bone mineral measurements in both premenoausal and postmenopausal women wih thyroid carcinoma. J Clin Endocrinol Metab. 1991;72:1184-8.
47. Reverte JL, Holgado S, Alonso N, Salinas I, Granada ML, Sanmarti A. Lack of deleterious effect on bone mineral density of long term thyroxine suppressive therapy for differentiated thyroid carcinoma. Endocr Relat Cancer. 2005;12:973-81.
48. Kung AWC, Lorentz T, Tam SCF. Thyroxine suppressive therapy decreases bone mineral density in postmenopausal women. Clin Endocrinol. 1993;39:535-40.
49. Stepan JJ, Limanova Z. Biochemical assessment of bone losss in patients on long term thyroid hormone treatment,. Bone Miner. 1992;17:377-88.
50. Hawkins F, Rigopoulou D, Papapietro K, Lopez Alvarez MB. Spinal bone mass alter long-term treatment with L-Thyroxine in postmenopausal women with thyroid cancer and chronic lymphocytic tiroiditis. Calcif Tissue Int. 1994;54:16-9.
51. Franklyn JA, Betteridge J, Daykin J, Holder R, Oates GD, Parle JV, et al. Long-term thyroxine treatment and bone mineral density. Lancet. 1992;340:9-13.
52. De Melo TG, da Assumpcao LV, Santos Ade O, Zantut-Wittmann DE. Low BMI and low TSH value as risk fac-

tors related to lower bone mineral density in pstmenoausal women under levothyroxine herapy for differeniated thyroid carcinoma. Thyroid Res. 2015;8:7.
53. Tournis S, Antoniou JD, Liakou CG, Christodoulou J, Papakitsou E, Galanos A, et al. Volumetric bone mineral density and bone geometry assessed by peripheral quantitative computed tomography in women with differentiated thyroid cancer under TSH suppression. Clin Endocrinol. 2015;82:197-204.
54. Newman T, Browner W, Cumming S, Hulley S. Designing a new study: cross-sectional and case control studies. In: Hulley S, Cumming S, (eds.) Designing Clinical Research. Baltimore: Williams and Wilkings; 1988. p. 77-78.
55. Jodar E, López B, García L, Rigopoulou D, Martinez G, Hawkins F. Bone Changes in pre and postmenopausal women with thyroid cancer and levothyroxine therapy: Evolution of axial and appendicular bone mass. Osteoporos Int. 1998:8;311-6.
56. Pioli G, Pedrazzoni M, Palummeri E, Stanesi M, Del Frate R, Vescovi PP, et al. Longitudinal study of bone loss after thyroidectomy and suppressive thyroxine therapy in premenopausal women. Acta Endocrinol. 1992;126:238-42.
57. Muller CG, Bayley TA, Harrison JE, Tsang R. Possible limited bone loss with suppressive thyroxine therapy is unlikely to have clinical relevance. Thyroid. 1995;5(2);817-21.
58. Mazes RB. Peak bone mass, reference values, and T-scores. J Clin Densitom. 2001;4(1):73-7.
59. Fujiyama K, Kiriyama T, Ito M, Kimura H, Ashizawa K, Tsuruta M, et al. Suppressive doses of thyroxine do not accelerate age-related bone loss in late postmenopausal women. Thyroid. 1995 5(1):13-7.
60. Kung AWC, Yeung SSC. Prevention of bone loss induced by thyroxine suppressive therapy in postmenopausal women: The effect of calcium and calcitonin. J Clin Endocrinol Metab. 1996;81:1232-6.
61. Guo CY, Weetman AP, Eastell R. Longitudinal changes of bone mineral density and bone turnover in postmenoausal women on thyroxine. Clin Endocrinol. (Oxf) 1997;46:301-7.
62. Sijanovic S, Karner I. Bone loss in premenopausal women on long-term suppressive therapy with thyroid hormone. Medscape Womens Health. 2001;6(5):3.
63. Sugitani I, Fujimoto Y. Effect of postoperative thyrotropin suppressive therapy on bone mineral density in patients with papillary thyroid carcinoma: A prospective controlled study. Surgery. 2011;150:1250-7.
64. Karner I, Hrgović Z, Sijanović S, Buković D, Klobucar A, Usadel KH, et al. Bone mineral density changes and bone turnover in thyroid carcinoma patients treated with supraphysiologic doses of thyroxine. Eur J Med Res. 2005;10:480-8.
65. Schneider R, Schneider M, Reiners C, Schneider P. Effects of levothyroxine on bone mineral density, muscle force, and bone turnover markers: a cohort study. J Clin Endocrinol Metab. 2012;97(11):3926-34.
66. Kim MK, Yun KJ, Kim MH, Lim DJ, Kwon HS, Song KH, et al. The effects of thyrotropin-suppressing therapy on bone metabolism in patients with well-differentiated thyroid carcinoma. Bone. 2015;71:101-5.
67. Kim CW, Hong S, Oh SH, Lee JJ, Han JY, Hong S, et al. Change of bone mineral density and biochemical markers of bone turnover in patients on suppressive levothyroxine therapy for differentiated thyroid carcinoma. J Bone Metab. 2015;22:135-41.
68. Lopez Alvarez MB, Hawkins F, Rigopoulou D, Martinez G, Estenoz J, Orutño B, et al. Factores de riesgo y densidad mineral osea en mujeres en tratamiento prolongado con levotiroxina. Med Clin. 1999;112:85-9.
69. Gomes de Melo T, Montalli da Assumpcao LV, Oliveria Santo A, Engelbrecht D. Low BMI and low TSH value as risk factors related to lower bone mineral density in postmenopausal women under levothyroxine therapy for differentiated thyroid carcinoma. Thyroid Res. 2015;8:1-7.


se iomm
Sociedad Española de Investigación Ósea
y del Metabolismo Mineral


fe iomm
Fundación Española de Investigación Ósea
y del Metabolismo Mineral