April-June **2023** ■ Volume **15** ■ No.-**2** Pages: **43-91**

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Revista de Osteoporosis y Metabolismo Mineral

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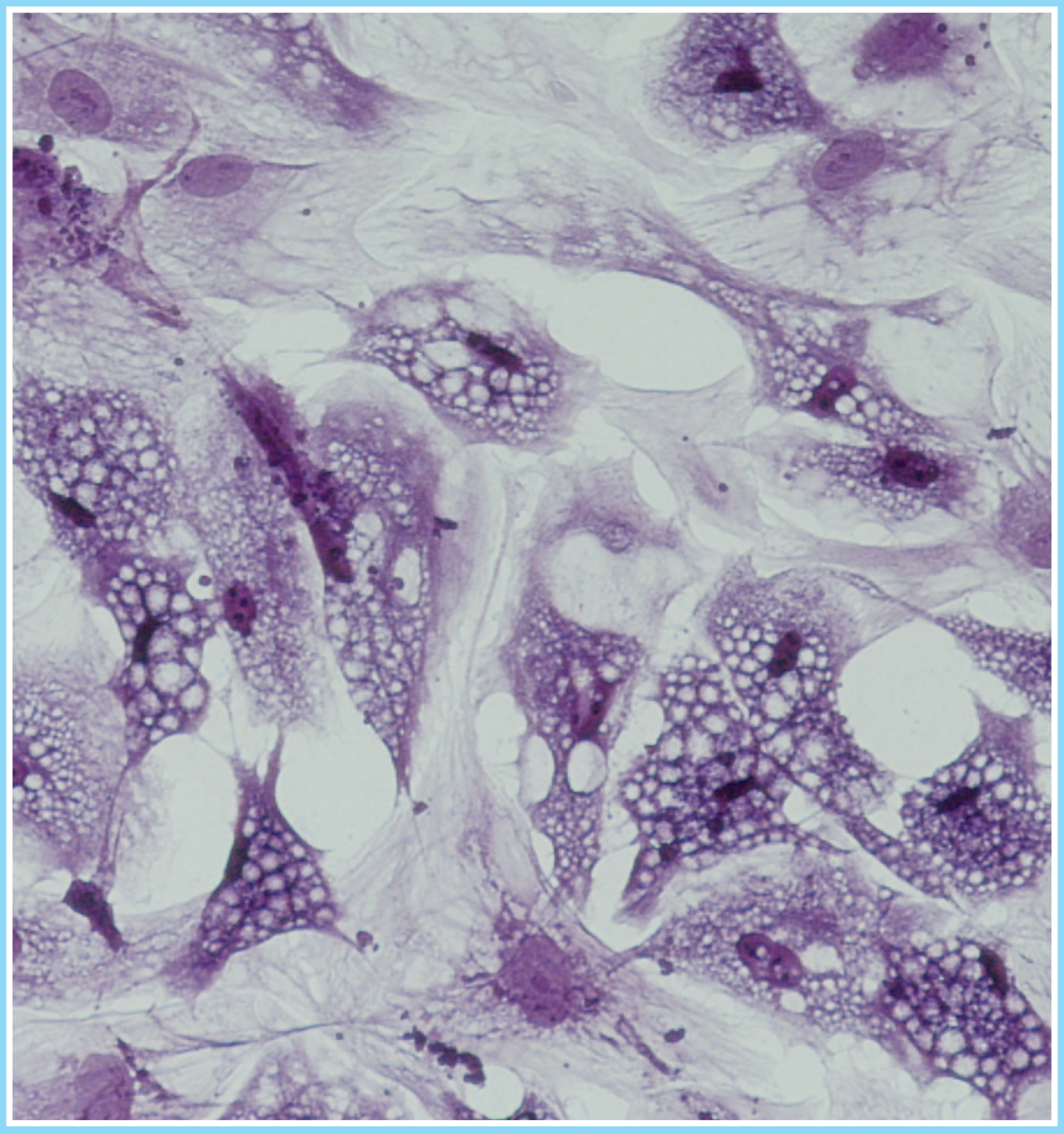

ARÁN Ediciones, S.L. ISSN (print version): 1889-836X. ISSN: (online version): 2173-2345

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ISSN (print version): 1889-836X. ISSN: (online version): 2173-2345
Legal Deposit: M-8158-2023

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Summary

Vol. **15** ■ April-June ■ **No.** 2

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Special Article

Clinical Setting and Decision-Making

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Original

High and very high risk of osteoporotic fractures in Chilean women

Luis Imaicela Naula, Enrique López Gavilánez, Manuel Navarro Chávez, Mario Hernández Bonilla, Noemí Bautista Litardo, Mario Navarro Grijalva

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Abstract

Objective: to evaluate the application of intervention thresholds based on FRAX in Chilean women. Recategorize the risk of osteoporotic fracture to optimize the selection of eligible women for intervention.

Methods: we selected 1782 women aged 50 and older from the 2016-2017 National Health Survey (third version). We estimated the probability of major osteoporotic fractures and hip fractures using the Chilean FRAX model. We estimated the percentage of women eligible for treatment and assessment of bone mineral density by applying specific intervention thresholds by age from 50 to 90 years and a hybrid threshold that combines age-dependent thresholds up to 75 years and, thereafter, a fixed threshold with a single fracture probability up to 90 years.

Results: twenty-two women (1.23 %) had a previous fracture and were eligible for treatment for this reason. Using age-specific thresholds, another 33 women were eligible for treatment because the probability of major osteoporotic fracture was above the upper assessment threshold. In 1107 (62.12 %) women, bone mineral density measurement is recommended to recalculate FRAX with the inclusion of femoral neck bone mineral density. With the hybrid threshold, an additional 44 (3.69 %) women were eligible for treatment, and bone density measurement was advised in 1169 women (65.50 %). If treatment was assigned based on FRAX without bone mineral density alone, the number of women eligible for treatment was 70 (5.15 %) with an age-specific intervention threshold and 120 (6.72 %) with the hybrid threshold.

Conclusions: the hybrid threshold identifies more women eligible for treatment compared to age-specific thresholds. The average fracture probability was higher with the hybrid threshold. Based on this, our position is to recommend the hybrid threshold.

Keywords:
FRAX®. Intervention threshold. Hybrid threshold. Risk of fracture. osteoporosis. Chile.

Received: 10/03/2023 • Accepted: 18/05/2023

Conflicts of interest: the authors LIN, ELG, MNC, MHB, NBL, and MNG declared no conflicts of interest whatsoever. The principles set forth in the Declaration of Helsinki on clinical trials were observed at all time.

Imaicela Naula L, López Gavilánez E, Navarro Chávez M, Hernández Bonilla M, Bautista Litardo N, Navarro Grijalva M. High and very high risk of osteoporotic fractures in Chilean women. Rev Osteoporos Metab Miner 2023;15(2):43-53

DOI: 10.20960/RevOsteoporosMetabMiner.00001

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INTRODUCTION

The worldwide prevalence of osteoporosis has significantly increased and will continue to grow in the future. The main causes of this increase are the global aging population and lifestyle changes. Consequently, there will also be an increase in both the prevalence and incidence rate of fragility-related fractures associated with osteoporosis (1).

According to the latest audit conducted in 19 Latin American countries published by the IOF (2), in Chile, the incidence rate of hip fractures in people aged 50 and older is 144 cases per 100 000 inhabitants, a rate that did not vary from 2015 through 2019 (2). Recently, Quevedo et al. reported a 40 % increase in hospital discharges due to hip fractures in patients aged ≥ 45 years from 2006 through 2017 (3).

The primary endpoint of osteoporosis treatment is to prevent fractures, so it is essential to recognize and treat individuals at high risk of fractures. Several simple and cost-effective alternatives have been developed to identify and select individuals at risk who are eligible for treatment and bone mineral density (BMD) measurement (4).

The FRAX tool to predict risk of fracture was developed back in 2008 by Kanis et al. and is currently the most widely used tool across the world. It is available cost-free on the internet and allows us to assess the risk of fracture based on clinical risk factors and the elective inclusion of BMD. Its use has been added to most national and international clinical practice guidelines on the management of osteoporosis (5) including those in Latin America (6-11). Country-specific FRAX models (FRAX v4.1) are currently available in 14 countries in Asia, 36 countries in Europe, 11 in the Middle East and Africa, 2 in North America, and 2 in Oceania (https://www.sheffield.ac.uk/FRAX/) back in Jannuary 21, 2023) (12). In Latin America, the FRAX tool is available in 7 countries (13-16) including Chile, where it was built based on population data provided by Riedemann and Neira (2001-2006) (17).

The establishment of country-specific intervention thresholds based on age (following the methodology of the United Kingdom National Osteoporosis Guideline Group - NOGG) allows us to select patients eligible for treatment and refer them for BMD measurement to recalculate the risk of fracture. However, we should mention that the addition of BMD into the FRAX form is not essential to calculate the risk of fracture, which is particularly significant in countries with limited access to this technique (17). One disadvantage of age-dependent FRAX thresholds obtained using the NOGG strategy is that inequalities in treatment access arise among older individuals (18). To overcome this setback, the latest NOGG guidelines add a combination of age-dependent thresholds up to 70 years and thereafter a fixed threshold with a single fracture probability across all age groups (hybrid threshold) (18). This strategy has also been adopted by other authors who argue that the use of hybrid thresholds may be appropriate in countries with low incidence rates of hip fracture like some countries in the Middle East, Southern Europe, and Latin America (19-21).

Back in 2019, the NOGG guidelines refined the categorization of risk of fracture into high and very high to optimize treatment selection (anabolic or antiresorptive) in high risk patients (5). Using this risk recategorization, the percentage of women characterized as very high risk increased with age.

In Latin America, with the exception of Ecuador, the effectiveness of FRAX thresholds in the identification of the percentage of individuals who were eligible for intervention in respective populations has not yet been determined. This study aims to analyze the effectiveness of the Chilean FRAX model (without BMD) in identifying women who would be eligible for treatment and BMD evaluation according to age-specific intervention thresholds and a combination of fixed thresholds (hybrid threshold). An additional objective is to review and update the categorization of risk of fracture as "high" and "very high" to better guide therapeutic interventions for the prevention of fragility fractures in Chilean women.

METHODS

POPULATION

In the present study, data from participants from the 2016-2017 National Health Survey (ENS), third version, were used. This survey is a national cross-sectional study that collected information from, at least 6027 individuals aged 15 years and older, residing in metropolitan and rural areas of the 15 regions of Chile from August 2016 through January 2017. The population sample was probabilistic and geographically stratified. The complete sample design and methodological details have been described elsewhere (23). The ENS primarily employed internationally validated instruments and was designed to estimate the prevalence of priority health problems and associated risk factors. The survey forms, database, manuals, and codebooks are publicly available and can be downloaded from the website (24). For this study, all women aged 50 years and older from the ENS 2016-2017 survey were selected (n = 1782) including 1760 women without previous fractures and 22 with previous fractures. The study was approved by the ethics committee of the Hospital Docente Policía Nacional Guayaquil No.2, Ecuador.

Age and sex were self-reported. Height was measured in centimeters, weight in kilograms, and body mass index (BMI) was estimated as well (kg/m^2). The questions

and responses described in the supplementary table 1 were used to obtain the risk factors associated with osteoporotic fractures. Some responses needed to be transformed and recoded following the FRAX® tool recommendations to convert them into dichotomous variables. The questions and responses associated with arthritis were not used as the survey indicates they have not been validated nor were those associated with glucocorticoids since the dosage and duration of use cannot be established. Smoking habits were categorized as current, former, or never. Self-reported average alcohol consumption was recoded to adjust it as equivalent to ≥ 3 units per day. Forearm and hip fractures over the past year were self-reported. The diagnosis of secondary osteoporosis or rheumatoid arthritis (RA) was not confirmed, and data were recorded as "NO" following the FRAX questionnaire recommendations (Supplementary Table I).

POSSIBILITIES OF FRACTURE

The 10-year probability of major osteoporotic fracture and hip fracture was estimated using the FRAX® tool version 4.2 specific to the Chilean population, available online (https://www.sheffield.ac.uk/FRAX/tool.aspx?country=50). The estimates did not include BMD measurement.

INTERVENTION THRESHOLDS

Two intervention thresholds were explored: an age-specific threshold and a hybrid threshold as shown on figure 1.

To establish the intervention thresholds and BMD assessment, the methodology adopted by the NOGG in FRAX-based guidelines for the United Kingdom (25) and previously described for the Chilean population was used (13).

The number of women aged 50 years or older exceeding the intervention threshold (and thus eligible for treatment) was estimated as a total and in 5-year age intervals using FRAX probabilities (BMD was not included in the calculation). Since a previous fracture is considered to carry sufficient risk according to the NOGG to ill-advise treatment, the intervention threshold for women without previous fractures was set at the 10-year probability (age-specific) of experiencing a major osteoporotic fracture (MOF) (hip, spine, forearm, or humerus), which is equivalent to women with prior fragility fractures according the Chilean FRAX model (version 4.2). Body mass index was set at 25 kg/m^2.

ASSESSMENT THRESHOLDS TO RECOMMEND BONE MINERAL DENSITY MEASUREMENT

Two assessment thresholds were considered to make recommendations for BMD measurement. Lower assessment threshold (LAT): probability level below which neither treatment nor a BMD test should be considered. Upper assessment threshold (UAT): probability level above which treatment can be recommended regardless of BMD. The LAT was set to exclude the need for BMD measurement in women without clinical risk factors, as indicated in the European clinical practice guidelines. An UAT was selected to minimize the probability that an individual identified as high risk (based on clinical risk factors alone) could, with

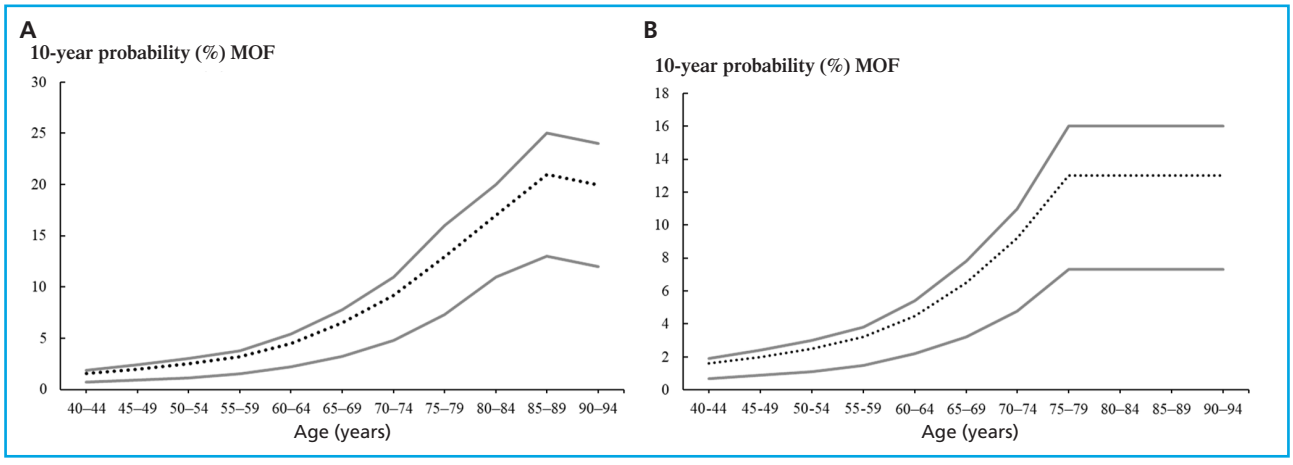

Figure 1. Graphs of intervention and assessment thresholds showing the original (A) and current (B) NOGG thresholds applied to the FRAX model for Chile. The dotted line represents the intervention threshold while the solid gray lines represent the upper and lower assessment thresholds. IT: intervention threshold; LAT: lower assessment threshold; UAT: upper assessment threshold; MOF: major osteoporotic fracture (13).

additional BMD information, be recategorized into a low-risk category. The UAT was set at 1.2 times the intervention threshold (25).

HYBRID THRESHOLD

The hybrid threshold is an alternative threshold that combines age-dependent thresholds up to 75 years and, thereafter, a fixed threshold with a single fracture probability up to 90 years. This threshold was adopted following NOGG recommendations because age-specific thresholds lead to disparities in access to treatment especially at older ages (≥ 70 years) based on the presence or absence of a prior fracture. The hybrid threshold reduces disparities in treatment access and decreases the need to perform bone densitometries (18,26).

ASSESSMENT STRATEGY

The strategy to establish intervention thresholds and BMD assessment followed the FRAX-based methodology approved by NOGG in the United Kingdom (26) and subsequently recommended by the European clinical practice guidelines (5). Women with a prior fragility fracture are considered eligible for treatment without further evaluation. In women without a prior fragility fracture, the strategy was based on assessing the probability of experiencing a MOF within the next 10 years. Women with probabilities below the lower assessment threshold were considered ineligible for treatment. Women with probabilities above the upper assessment threshold were considered eligible for treatment. Women with probabilities between the upper and lower limits of the assessment threshold would be referred for BMD measurement and risk of fracture reassessment.

RISK CATEGORIZATION

Women with probabilities below the LAT can be considered at low risk. Women with probabilities above the UAT can be considered for treatment. Women with probabilities between the UAT and LAT would be referred for BMD measurement and risk of fracture reassessment (intermediate risk) (5).

In addition to the low and high risk categories mentioned in the current IOF-ESCEO guidelines (5), a very high risk category can be identified. Very high risk is defined as a probability of fracture that is 1.2 times higher compared to the intervention threshold (e. g., the UAT) following a FRAX evaluation with or without BMD inclusion. In other words, the same probability threshold can be used when BMD testing is not available (27). The justification for a more refined risk characterization is to guide patients towards the most appropriate treatments (anabolic or antiresorptive) (27). The high risk category would now fall above the intervention threshold but below the UAT while the low-risk category would be below the intervention threshold (27).

STATISTICAL ANALYSIS

The characteristics of the information collected were described using descriptive analysis. Qualitative variables were expressed as absolute frequency and percentage while the quantitative ones were expressed as mean and standard deviation. Participant filtration from the database, variable transformation or recoding, and descriptive statistics were performed using IBM SPSS Statistics software package for Windows, version 26.0 (IBM Corp, Armonk, NY, New York, United States).

In this study, information from Health Surveys on epidemiological surveillance conducted by the Ministry of Public Health was used. The authors with to thank the Chilean Ministry of Health for providing us with the database. All the results obtained from the study or research are the sole responsibility of the authors and do not compromise the aforementioned institution whatsoever [http://epi.minsal.cl/encuesta-nacional-de-salud-2015-2016/].

RESULTS

A total of 1782 women over the age of 50 from the ENS 2016-2017 survey were selected. Twenty-two participants had a previous fracture. The mean age was 65.09 (10.16) years, and they had a body mass index (BMI) of 29.84 (5.58) kg/m^2. A total of 376 (21,10) were current smokers, and 71 (3.98) used ≥ 3 units of alcohol per day. The baseline characteristics are shown on table I.

The 10-year probability (mean) of MOF and hip fracture (estimated without BMD) was 4.18 (3.72) and 1.50 (2.18), respectively (Table II).

THRESHOLDS

The intervention and assessment thresholds specific to the Chilean population and the methodology to obtain them have been described in a former publication (13) and are shown on the supplementary table II.

Table I. Summary description of baseline variables in women ≥ 50 years (n = 1782)

	n	%	Mean	SD
Age (years)			65.09	10.16
Weight (kg)			69.42	13.52
Height (cm)			152.53	6.40
BMI (kg/m²)			29.84	5.58
Previous fracture	22	1.23		
Relative to hip fracture	120	6.73		
Current smoker	376	21.10		
Alcohol ≥ 3 units/day	71	3.98		

Table II. Ten-year probability of major osteoporotic fracture (MOF) and hip fracture (n = 1782) (13)

			MOF		HF	
Age	n	%	Mean	SD	Mean	SD
50-54	306	17.17	1.25	0.38	0.14	0.09
55-59	334	18.74	1.67	0.52	0.26	0.17
60-64	288	16.16	2.41	0.81	0.50	0.46
65-69	277	15.54	3.52	0.95	0.92	0.45
70-74	218	12.23	5.57	2.11	2.19	1.72
75-79	182	10.21	8.41	3.00	3.77	2.48
80-84	113	6.34	11.55	3.06	5.20	2.56
85-89	41	2.30	12.23	2.47	5.63	2.31
90	23	1.29	12.67	3.44	6.79	3.36
Total	1782	100	4.18	3.72	1.50	2.18

HF: hip fracture; MOF: major osteoporotic fracture; SD: standard deviation.

The intervention threshold in Chilean women increased with age from a 10-year probability of 2.5 % at 50 years to 20 % at 90 years.

The age-specific UAT and LAT to recommend BMD measurement are also shown on supplementary table 2. For example, at 65 years, a BMD test would be ill-advised for an individual with a fracture probability rate < 3.2 %. At the same age, a BMD test would, however, be advised for an individual with fracture probabilities between 3.2 % and 7.8 %. Treatment would be advised without the need for a BMD test (for risk of fracture assessment, but possibly for treatment monitoring) in individuals with fracture probabilities > 7.8 %. In women who undergo a BMD test, treatment would be advised for those with fracture probabilities ≥ 6.5 %.

MANAGEMENT PATHWAY

With age-specific thresholds, 22 women (1.23 %) had a previous fracture and would, therefore, be eligible for treatment. A total of 1107 women (62.12 %) had probabilities above the LAT but below the UAT, thus indicative of the need for BMD testing. Treatment without the need for a BMD test could be advised in 33 women (0.61 %). In the case of using a hybrid threshold, 1169 women (65.50 %) had probabilities above the LAT but below the UAT, thus indicative of the need for a BMD test. Treatment without a BMD test could be advised for 44 women (3.69 %) (including the 22 women with previous fractures). If treatment was assigned based solely on FRAX without BMD measurement (including those with a previous fracture), the number of women eligible for treatment was 70 (5.15 %) with an age-specific intervention threshold, and 120 (6.72 %) with the hybrid threshold. Distribution accross the different possible scenarios is shown on figure 2.

The hybrid threshold (compared to the age-specific threshold) increases the number of women selected for treatment by 1.3 times when considering an intermediate risk category and by 1.71 times when treatment is assigned based solely on FRAX without BMD measurement.

Table III shows fracture probabilities in women eligible for treatment based on categorization. Fracture probabilities were higher in those eligible for treatment based on the hybrid thresholds, intermediate with age-specific thresholds, and lower in those with a previous fracture (Table III).

Using the age-specific threshold, the number of patients categorized as high risk doubled down (2.12 times; from 33 to 70). With the hybrid threshold, the number of high risk patients almost tripled (increased by 2.72 times; from 44 to 120) (Fig. 2).

Adopting the methodology used by NOGG (26), the European clinical practice guidelines, and IOF-ESCEO (5,27) for risk categorization refinement, we found that with age-specific thresholds, 50 women were categorized as high risk and 15 as very high risk whereas with the hybrid threshold, 87 women were categorized as high risk and 28 as very high risk (Fig. 3).

Table III. Ten-year fracture probabilities (%) in women eligible for treatment according to the specified criteria

Criterion for treatment		MOF		HF	
	n	Mean	SD	Mean	SD
Previous fracture	22	10.00	8.01	4.36	5.32
Age-specific	65	10.93	6.54	6.01	5.18
Hybrid	115	12.33	5.27	6.70	4.23
All women treated	1782	4.18	3.72	1.50	2.18

HF: hip fracture; MOF: major osteoporotic fracture.

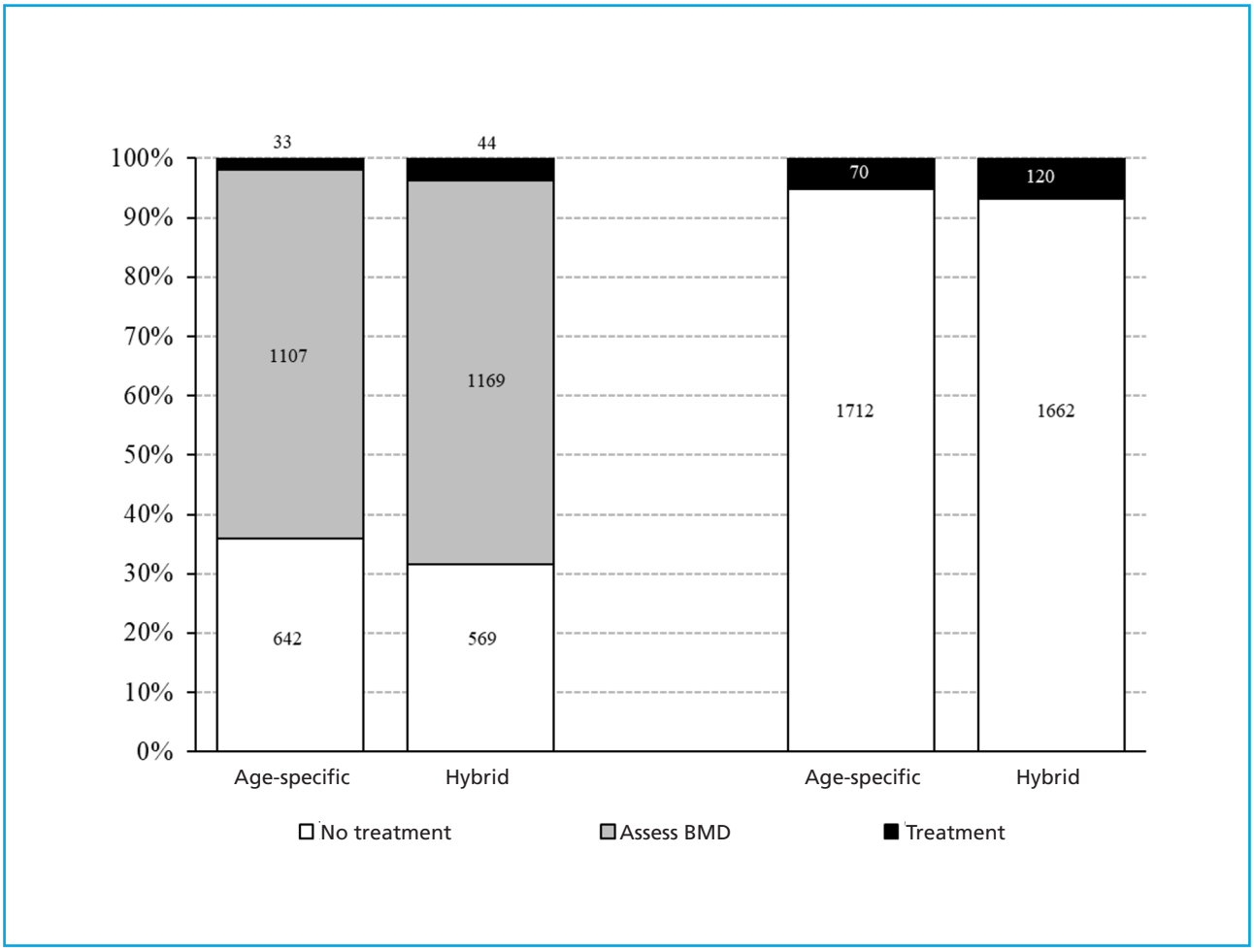

Figure 2. Distribution (n) of women assessed for risk of fracture (MOF) using age-specific or hybrid intervention and assessment thresholds. The bars on the left predict the subsequent use of DMO in those with intermediate risk. The bars on the right guide treatment using FRAX without DMO alone. MOF: major osteoporotic fracture.

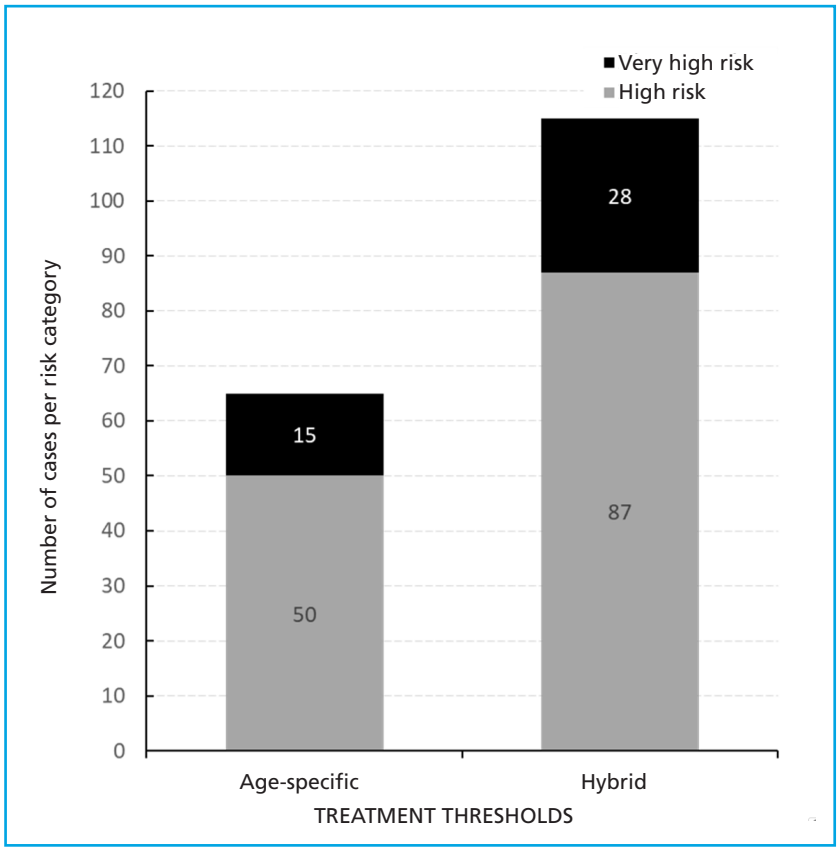

Figure 3. Refinement of the categorization for the risk of mayor osteoporotic fracture in Chilean women, according to the methodology used by NOGG [26], the European clinical practice guidelines, and IOF-ESCEO (5,27).

DISCUSSION

In this study, following the same methodology adopted by NOGG and the European clinical practice guidelines (5,25,27,28), we categorized Chilean women aged 50 and above according to fracture probabilities based on the country-specific FRAX model into low, intermediate, and high risk categories. It is demonstrated that the hybrid threshold identifies more women at high risk compared to the age-dependent threshold. The percentage of female population potentially eligible for treatment was 3.6 % or 6.4 % depending on the intervention threshold used (age-specific or hybrid). As expected, a higher percentage of women were eligible for treatment when hybrid thresholds were applied. Approximately 65 % of women would be advised to take a BMD test. BMD was not measured in this population sample, so we do not know what percentage of women would exceed the intervention threshold and, therefore, be eligible for treatment. However, we should mention that NOGG guidelines state that FRAX can be used without BMD measurement as the performance of FRAX with and without BMD is approximately equivalent (29).

In the United Kingdom National Osteoporosis Guideline Group (NOGG) and more recently in the European clinical practice guidelines (5,28), the intervention threshold is set at the fracture probability equivalent of a woman of the same age with a previous fragility fracture because if women with a previous fragility fracture are considered eligible for treatment, women without a fracture but with equivalent probabilities would also be eligible for treatment. The screening strategy for women is based on opportunistic case-finding as general population screening is ill-advised (except in North America) (30,31). Based on the NOGG guidelines, women with fracture probabilities equivalent or lower compared to those without clinical risk factors should not be evaluated with BMD. In high risk individuals, BMD measurement is ill-advised. When the estimate risk of fracture without BMD is close to the intervention threshold, adding a BMD test increases the possibility of risk re-stratification (from high to low and *vice versa*) (32), the use of BMD becomes more efficient.

Although FRAX is available online in 72 countries and 81 different populations, the availability of country-specific (or ethnicity-specific) intervention thresholds is limited. In a systematic review, out of 120 articles recommending FRAX for treatment decision-making, 38 did not provide clear thresholds to identify those in need for treatment (21). Most countries that have not

established their own intervention thresholds have added fixed thresholds recommended by the National Osteoporosis Foundation (NOF) for the United States population (21). The NOF-recommended intervention thresholds are the result of an economic analysis conducted in 2008 and their application outside the United States is not justified (21). On the other hand, the United Kingdom NOGG clinical practice guidelines use a clinically oriented approach to determine the percentage of individuals who would be eligible for treatment. Also, costs associated with treatment depend on the algorithm used to analyze them (12).

Previously, clinical practice guidelines worldwide were based on the diagnosis of osteoporosis to make treatment decisions for each patient. Currently, there has been a paradigm shift away from diagnosing osteoporosis to assessing the risk of fracture to guide the clinical decision-making process (3,34). However, the international clinical practice guidelines on the management of osteoporosis do not consistently provide treatment recommendations based on the risk of fracture (33).

The European clinical practice guidelines have proposed risk refinement into high and very high categories to optimize the selection of anabolic or antiresorptive treatment following the evidence available on the imminent risk of fracture and previous fractures (5).

In a review of 70 English-language guidelines, 63 discussed the idea of risk of fracture, but only 34 recommended using FRAX alone to classify such risk of fracture (33). A total of 28 provided a risk category or threshold that made up an indication for drug therapy. A total of 12 guidelines reported a moderate, medium, or intermediate risk category, and management recommendations were made based on this categorization.

In Latin America, only 5 out of 7 countries with FRAX have published national osteoporosis guidelines (6-11). All of them include FRAX as a tool to assess to risk of fracture. However, in 3 of these countries, starting drug therapy to treat osteoporosis is advised based on the NOF thresholds. The Chilean guidelines do not provide a clear recommendation on whether to use NOGG- or NOF-based thresholds for treatment decision-making. Only in Colombia and Brazil, is it recommended to use the NOGG thresholds (specific to age and country) to start treatment. None of the guidelines differentiate among different types of anti-osteoporotic treatment (anabolics or bisphosphonates) based on FRAX-derived risk categorization.

Except for Ecuador (22), the application of intervention thresholds in Latin America has not been described. The findings of this study are similar to those published by López Gavilánez et al. (22) on the Ecuadorian population, in the sense that the application of risk categorization using FRAX alone without measuring BMD allowed selecting more candidates for treatment with both age-specific and hybrid thresholds.

The most appropriate intervention thresholds for a country should be decided locally considering economic factors, availability of healthcare resources, and physician based preferences (12). In Chile, the individual cost of treating a hip fracture goes from USD 4000 to USD 9000 in the public and private healthcare systems, respectively. The cost of hospitalization due to hip fractures back in 2020 was 34 million dollars per year (3). While the use of intervention thresholds has been shown to be cost-effective in Europe (5) and the UK (35) the risk of fracture (13) and healthcare costs associated with fracture care (3) in Chile are different. Therefore, the cost-effectiveness of using these intervention thresholds in the Chilean population has not yet been established through their use in the routine clinical practice and should be backed by a comprehensive economic study of the healthcare system.

There are several limitations to consider in this study. Firstly, although the survey was broad and representative of the Chilean population, there were fewer women surveyed in the older age groups (≥ 80 years = 10 %), which could affect the accuracy of our estimates and therefore the number of women eligible for treatment. Secondly, in the NOGG guidelines (26) two different FRAX results are used: the thresholds for major osteoporotic fracture and hip fracture probabilities, and treatment is advised if fracture probability exceeds the intervention threshold in either of the 2 FRAX results (or in both). The hip fracture probability varies across countries and regions worldwide while the probability of major osteoporotic fractures is less well-known. We should mention that, in most studies, the thresholds for major osteoporotic fracture probability are based on more assumptions compared to those for hip fracture (36). This study was limited to the thresholds for major osteoporotic fracture probability. Therefore, using the fracture probabilities of both sites may increase the number of women identified as high risk.

We should mention that if we defined very high risk by multiplying the intervention threshold by 1.6, as later Kanis et al. (37) did in the hybrid FRAX model, the percentage of women classified as very high risk would be much lower compared to that obtained in this study.

Thirdly, it is important to support the application of these risk of fracture assessment thresholds using cost assessment analyses too. In the United Kingdom, this approach has been proven cost-effective. However, cost-effectiveness (35) will be necessarily different in the Chilean population due to different risk of fractures and healthcare costs. This study did not take into consideration the financial impact of applying these thresholds across different healthcare institutions.

Finally, the results of this study can only be applied to the Chilean population and are not applicable to other countries in Latin America.

CONCLUSIONS

This study proved that a risk of fracture assessment strategy following the methodology proposed by NOGG, but based on the Chilean FRAX, allows us to identify many more women at high risk of fracture and, therefore, eligible for treatment according to different age-specific thresholds and an alternative threshold for older women. The study demonstrates that the hybrid threshold identifies more women at high risk compared to the age-dependent threshold. Based on this, our recommendation is to use the hybrid threshold.

The addition of country-specific intervention thresholds and risk re-stratification into high and very high categories into the national osteoporosis guidelines will positively impact treatment decision-making by physicians in countries throughout the region.

Supplementary Table I. Questionnaire including questions and answers from the ENS survey used in this study

Risk factor	Question asked	Codes	Meaasured by	Answered given
Age	At what age did you break your bone?	Age	Self-reported	Expressed in years
Sex	Sex	Sex	Self-reported	Woman
Weight	Weight	m4p1_1 m4p1_2	OMRON HN289 electronic scale	Expressed in kilos
Height	Height	m4p2_1 m4p2_2	L-shaped ruler and chromed measuring tape	Expressed in centimeters
Previous fracture*	At what age did you break your bone?	o3:	Self-reported	If the following 3 conditions are met:
	a. Age of fracture	o3a_1 until o3a_10		Time ≤ 1 year
	b. Cause of fracture	o3b_1 until o3b_10		Incidental fall on the ground (e. g., slipped, tripped fell of the bed)
	c. What bone did you break?	o3c_1 until o3c_10 o3c_1_esp until o3c_10_esp		- The hip, the wrist
Hip fracture in a relative	In your immediate family: Has anyone suffered or died from a hip fracture? (referring to children, parents, or siblings).	af1i	Self-reported	Yes
Active smoker**	Do you currently smoke cigarettes?	ta3	Self-reported	- Yes, 1 or more cigarettes a day - Yes, ocasionally
Alcohol**	Over the past year, how often did you drink alcoholic beverages?	m7p9	Self-reported	- 2 to 3 times a week - 4 or more times a week

*Elaborated by the authors. *Recodified as YES if meets the 3 answers. **Recodified as YES if meets any of the 2 answers.*

Supplementary Table II. Intervention and BMD assessment thresholds (lower and upper) derived from FRAX for Chile (13)

Age	Intervention threshold	Lower assessment threshold	Upper assessment threshold
40-44	1.6	0.7	1.9
45-49	2	0.9	2.4
50-54	2.5	1.1.	3
55-59	3.2	1.5	3.8
60-64	4.5	2.2	5.4
65-69	6.5	3.2	7.8
70-74	9.2	4.8	11
75-79	13	7.3	16
80-84	17	11	20
85-89	21	13	25
90	20	12	24

REFERENCES

1. Reginster JY, Burlet N. Osteoporosis: a still increasing prevalence. Bone 2006;38(2 Suppl 1):S4-S9. DOI: 10.1016/j.bone.2005.11.024
2. LATAM AUDIT 2021. Epidemiología, costo e impacto de la osteoporosis y las fracturas por fragilidad. Available from: https://www.osteoporosis.foundation/sites/iofbonehealth/files/2022-08/LATAM %20Audit %202021 %20- %20FINAL. pdf accessed 21 Jan 2023
3. Quevedo I, Ormeño JC, Weissglas B, Opazo C. Epidemiology and Direct Medical Cost of Osteoporotic Hip Fracture in Chile. J Osteoporos 2020;2020:5360467. DOI: 10.1155/2020/5360467
4. Kanis JA, Johnell O, De Laet C, Jonsson B, Oden A, Ogelsby AK. International variations in hip fracture probabilities: implications for risk assessment. J Bone Miner Res 2002;17(7):1237-44. DOI: 10.1359/jbmr.2002.17.7.1237.
5. Kanis JA, Cooper C, Rizzoli R, Reginster J-Y; Scientific Advisory Board of the European Society for Clinical and Economic Aspects of Osteoporosis (ESCEO) and the Committees of Scientific Advisors and National Societies of the International Osteoporosis Foundation (IOF). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int 2019;30(1):3-44.
6. Brazilian guidelines for the diagnosis and treatment of postmenopausal osteoporosis. Rev Bras Reumatol Engl Ed 2017;57(Suppl 2):452-66. DOI: 10.1016/j.rbre.2017.07.001.
7. Diagnóstico y tratamiento de osteoporosis en mujeres posmenopáusicas. Actualización 2018. Disponible en: https://www.imss.gob.mx/sites/all/statics/guiasclinicas/673GER.pdf
8. Schurman L, Galich AM, González C, González D, Messina OD, Sedlinsky C, et al. Guías Argentinas para el Diagnóstico, la Prevención y el Tratamiento de la Osteoporosis MEDICINA (Buenos Aires) 2017;77:46-60.
9. Arinoviche Schenker R, Arinoviche Numhauser, Arriagada Maldini M, Campusano M C, Fuentealba Perez C, Gajardo Lamas H, et al. Nueva guía SCHOMM 2018. Guías de diagnóstico, prevención y tratamiento de la osteoporosis. Disponible en: http://schomm.cl/Pdfs/SCHOMM_Guias2018_Final.pdf. Sociedad Chilena de Osteología y Metabolismo Mineral. consultado 21september 2021
10. Medina Orjuela A, Rosero Olarte O, Rueda Plata PN, Sánchez Escobar F, Chalem Chouekae M, González Reyes MA, et al. II Consenso Colombiano para el Manejo de la Osteoporosis Posmenopáusica. Rev Colomb Reumatol 2018;25(3):184-210.
11. Prevención, diagnóstico y tratamiento de la osteoporosis [2019]. Guía de Consulta Para el Médico de Primer Nivel de Atención. Secretaria de Salud de México. Disponible en: cenetec-difusion.com/gpc-sns/wp-content/uploads/2019/06/GuiaConsulta_Osteoporosis.pdf
12. Li-Yu J, Lekamwasam S. Intervention thresholds to identify postmenopausal women with high fracture risk: A single center study based on the Philippines FRAX model. Osteoporos Sarcopenia 2021;7(3):98-102. DOI: 10.1016/j.afos.2021.09.003
13. Clark P, Denova-Gutiérrez E, Zerbini C, Sanchez A, Messina O, Jaller JJ, et al. FRAX-based intervention and assessment thresholds in seven Latin American countries. Osteoporos Int 2018;29(3):707-15. DOI: 10.1007/s00198-017-4341-4
14. Zerbini CA, Szejnfeld VL, Abergaria BH, McCloskey EV, Johansson H, Kanis JA. Incidence of hip fracture in Brazil and the development of a FRAX model. Arch Osteoporos 2015;10:224. DOI: 10.1007/s11657-015-0224-5
15. Jaller-Raad JJ, Jaller-Char JJ, Lechuga-Ortiz JA, Navarro-Lechuga E, Johansson H, Kanis JA. Incidence of hip fracture in Barranquilla, Colombia, and the development of a Colombian FRAX model. Calcif Tissue Int 2013;93(1):15-22. DOI: 10.1007/s00223-013-9717-7
16. Lopez Gavilanez E, Johansson H, McCloskey E, Harvey NC, Segale Bajana A, Marriott Blum D, et al. Assessing the risk of osteoporotic fractures: the Ecuadorian FRAX model. Arch Osteoporos 2019;14(1):93. DOI: 10.1007/s11657-019-0644-8
17. Kanis JA, Odén A, McCloskey EV, Johansson H, Wahl DA, Cooper C. A systematic review of hip fracture incidence and probability of fracture worldwide. Osteoporos Int 2012;23(9):2239-56. DOI: 10.1007/s00198-012-1964-3.
18. McCloskey E, Kanis JA, Johansson H, Harvey N, Oden A, Cooper A, et al. FRAX-based assessment and intervention thresholds–an exploration of thresholds in women aged 50 years and older in the UK. Osteoporos Int 2015;26:2091-9.
19. Chakhtoura M, Leslie WD, McClung M, Cheung AM, Fuleihan GE. The FRAX-based Lebanese osteoporosis treatment guidelines: rationale for a hybrid model. Osteoporos Int 2017;28(1):127-37. DOI: 10.1007/s00198-016-3766-5
20. Goldshtein I, Ish-Shalom S, Leshno M. Impact of FRAX-based osteoporosis intervention using real world data. Bone 2017;103:318-24. DOI: 10.1016/j.bone.2017.07.027
21. Kanis JA, Harvey NC, Cooper C, Johansson H, Oden A, McCloskey EV. 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;11(1):25. DOI: 10.1007/s11657-016-0278-z
22. López Gavilánez E, Johansson H, Harvey N, Lorentzon M, McCloskey E, Valdivieso Jara J, et al. The application of FRAX in Ecuador. Rev Colomb Reumatol 2021 DOI: 10.1016/j.rcreu.2021.07.004
23. Departamento de Epidemiología (1). Ministerio de Salud. Gobierno de Chile. Encuesta Nacional de Salud (ENS) 2016-2017. 2018. Disponible en: http://epi.minsal.cl/encuesta-ens-descargable/
24. Departamento de Epidemiología (2). Ministerio de Salud. Gobierno de Chile. Encuesta Nacional de Salud 2016-2017. 2018. Disponible en: http://epi.minsal.cl/encuesta-nacional-de-salud-2015-2016/
25. Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A. Case finding for the management of osteoporosis with FRAX–assessment and intervention thresholds for the UK. Osteoporos Int 2008;19:1395-408. DOI: 10.1007/s00198-008-0712-1
26. Compston J, Cooper A, Cooper C, Gittoes N, Gregson C, Harvey N, et al. UK clinical guideline for the prevention and treatment of osteoporosis. Arch Osteoporos 2017;12(1):43. DOI: 10.1007/s11657-017-0324-5
27. Kanis JA, Harvey NC, McCloskey E, Bruyère O, Veronese N, Lorentzon M, et al. Algorithm for the management of patients at low, high and very high risk of osteoporotic fractures. Osteoporos Int 2020;31(1):1-12. DOI: 10.1007/s00198-019-05176-3
28. Compston J, Cooper A, Cooper C, Francis R, Kanis JA, Marsh D, et al. Guidelines for the diagnosis and management of osteoporosis in postmenopausal women and men from the age of 50 years in the UK. Maturitas 2009;62(2):105-8. DOI: 10.1016/j.maturitas.2008.11.022
29. 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. DOI: 10.1007/s00198-007-0343-y
30. Papaioannou A, Morin S, Cheung AM, Atkinson S, Brown JP, Feldman S, et al; Scientific Advisory Council of Osteoporosis Canada. 2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary. CMAJ 2010;182(17):1864-73. DOI: 10.1503/cmaj.100771
31. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int 2014;25:2359-81. DOI: 10.1007/s00198-014-2794-2
32. Johansson H, Oden A, Johnell O, Jonsson B, de Laet C, Oglesby A, et al. Optimization of BMD measurements to identify high risk groups for treatment - A test analysis. J Bone Miner Res 2004;19(6):906-13. DOI: 10.1359/jbmr.2004.19.6.906
33. Sale JEM, Gray M, Mancuso D, Inrig T, Boire G, Beaulieu MC, et al. Treatment recommendations based on fracture risk status are not consistently provided in osteoporosis guidelines. Rheumatol Int 2018;38(12):2193-208. DOI: 10.1007/s00296-018-4181-3
34. Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A. How to decide who to treat. Best Pract Res Clin Rheumatol 2009;23(6):711-26. DOI: 10.1016/j.berh.2009.09.002
35. Kanis JA, Adams J, Borgström F, Cooper C, Jönsson B, Preedy D, et al. The cost-effectiveness of alendronate in the management of osteoporosis. Bone 2008;42(1):4-15. DOI: 10.1016/j.bone.2007.10.019
36. Leslie WD, Kanis JA. Calibration of FRAX: A Journey, not a Destination. Calcif Tissue Int 2021;109(6):597-9. DOI: 10.1007/s00223-021-00891-8
37. Kanis JA, Johansson H, Harvey NC, Lorentzon M, Liu E, Vandenput L, McCloskey EV. An assessment of intervention thresholds for very high fracture risk applied to the NOGG guidelines: A report for the National Osteoporosis Guideline Group (NOGG). Osteoporos Int 2021;32(10):1951-60. DOI: 10.1007/s00198-021-05942-2

Revista de Osteoporosis y Metabolismo Mineral

Original

Effect of extracellular vesicles derived from hypoxia-preconditioned human mesenchymal stem cells on osteoblastogenesis and adipogenesis *in vitro*

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Abstract

Objectives: mesenchymal stem cells (MSC) are characterized by their anti-inflammatory, immunosuppressive activity, and their ability to differentiate. This makes them an interesting therapeutic tool in cell therapy and regenerative medicine. In part, the therapeutic effect of MSC is mediated by the secretion of extracellular vesicles (EV). The preconditioning of MSC in hypoxia can enhance the regenerative capacity of the secreted EV. In this context, the aim of the study was to evaluate whether EV derived from human MSC cultured in normoxic and hypoxic conditions affect the osteoblastogenesis and adipogenesis of MSC.

Material and methods: EV were isolated from MSC maintained for 48 hours in normoxic or hypoxic conditions (3 % O_2) using ultrafiltration and size exclusion chromatography. The EV were characterized by Western blot, electron microscopy, and nanoparticle tracking analysis. In MSC cultures, the effect of the EV on viability was evaluated using an MTT assay, migration was assessed with the Oris assay while differentiation into osteoblasts and adipocytes was also studied.

Results: the EV increased viability and migration, but no differences were seen between those derived from normoxic and hypoxic culture conditions. The EV, mainly those derived from hypoxia, increased both mineralization, and the expression of osteoblastic genes. However, they did not affect adipogenesis significantly.

Conclusions: the EV derived from MSC in hypoxia do not affect adipogenesis but have a greater ability to induce osteoblastogenesis. Therefore, they could potentially be used in bone regeneration therapies and treatments for bone conditions like osteoporosis.

Keywords: Extracellular vesicles. Mesenchymal stem cells. Hypoxia. Cell differentiation. Osteoblasts. Adipocytes.

Received: 17/08/2022 • Accepted: 18/04/2023

Authors' contributions: María Ángeles Gálvez-Moreno, and Antonio Casado-Díaz contributed equally to the study.

Conflicts of interest: the authors declare no conflict of interest.

Acknowledgements: Beca FEIOMM de Investigación Traslacional 2016, Proyecto PI18/01659 del Instituto de Salud Carlos III (ISCIII).

Jiménez-Navarro C, Torrecillas-Baena B, Camacho-Cardenosa M, Quesada-Gómez JM, Gálvez-Moreno MA, Casado-Díaz A. Effect of extracellular vesicles derived from hypoxia-preconditioned human mesenchymal stem cells on osteoblastogenesis and adipogenesis *in vitro.* Rev Osteoporos Metab Miner 2023;15(2):54-65

DOI: 10.20960/RevOsteoporosMetabMiner.00012

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INTRODUCTION

Mesenchymal stem cells or MSC (mesenchymal stem cells or mesenchymal stromal cells) are multipotent cells (1,2). The ability of MSC to differentiate into various cell lineages, as well as their anti-inflammatory and immunosuppressive activities has turned them a tool with great potential in cellular therapy and regenerative medicine. MSC participate in the body's homeostasis through tissue regeneration and repair. Osteoblasts are among the cell types MSC can differentiate into. Osteoblastic differentiation is controlled by several signaling pathways including the canonical Wnt/β-catenin pathway, and the increased expression and activation of the transcription factor RUNX2 (3,4). MSC can also differentiate into adipocytes through the induction of the transcription factor PPARγ (peroxisome proliferator-activated receptor γ). This factor regulates the expression of adipogenic genes and induces the development of the adipocytic phenotype (4).

During the aging process or in certain diseases such as diabetes, MSC tend to differentiate into adipocytes at the expense of osteoblastogenesis (5,6). This increases the adiposity of bone marrow, thus decreasing the capacity for bone formation. This favors the loss of bone mass and the onset of osteoporosis (7,8). Therefore, therapeutic strategies that promote osteoblastic differentiation and inhibit adipogenesis can contribute to bone regeneration (9).

The therapeutic applications of MSC face several obstacles. These include the low viability of transplanted cells, their inherent heterogeneity, unidentified factors associated with the age of the donor, and the tumorigenic potential of these cells (10). Recent studies indicate that a large part of the therapeutic properties of MSC are associated with their paracrine effects exerted through their secretome (11) that includes a soluble fraction rich in growth factors and cytokines plus a vesicular fraction that contains various types of molecules with high regenerative capabilities (12). Different studies have shown that there is a synergistic effect when both fractions are used together for regenerative or immunomodulatory purposes (13,14). However, former studies have demonstrated that the vesicular fraction is mainly responsible for the induction of certain potentially regenerative physiological processes. For example, extracellular vesicles (EV) from bone marrow MSC increased the migration and proliferation of dermal fibroblasts in vitro and angiogenesis in human umbilical vein endothelial cells (HUVEC) while EV-depleted conditioned media did not have that effect (15). Due to the regenerative properties of these vesicles and their stability in the medium, the use of MSC-derived EV, instead of the cells themselves, has been proposed as a suitable therapeutic alternative (1,16).

Due to the lack of consensus on the classification of EV, the International Society for Extracellular Vesicles (ISEV) declared that the preferred generic term that should be used is extracellular vesicle (17). The content of EV depends on the type of the original cell and its physiological state. The main components of EV are proteins, lipids, and nucleic acids (RNA and DNA). Proteins, from the family of tetraspanins, stand out among them. CD63, CD81, and CD9 are among the tetraspanins we can find, which are considered exosomal markers (10,18). MicroRNAs (miRNAs) are notable nucleic acids present in EV with the ability to alter gene expression in recipient cells (19). EV participate in intercellular communication, cellular maintenance, immune response, and tumor progression (20). In the case of EV derived from MSC, they play important roles in biological processes like angiogenesis, antigen presentation, apoptosis, coagulation, cellular homeostasis, inflammation, differentiation, proliferation, and intercellular signaling (16).

The use of MSC-derived EV allows us to design cell-free therapies. This can help avoid the difficulties and potential adverse effects associated with the application of cells in cellular therapy (10). Therefore, MSC-derived EV have been evaluated for the treatment of various respiratory, musculoskeletal, cardiovascular, neurological, hepatic, gastrointestinal, dermatological, and renal diseases (21).

The secretion and content of EV vary substantially depending on the physiological state of the MSC from which they derive, which is, in turn, impacted by the environmental conditions of their niche (22). In this context, it has been proven that preconditioning MSC under different culture conditions can stimulate the secretion of EV, thus enhancing their therapeutic efficacy. These conditions include cytokines, hypoxia, trophic and physical factors, as well as chemical and pharmacological agents (23).

The reduced oxygen availability following hypoxia induces a cellular adaptive response that includes alterations in the content of secreted EV (24). At cellular level, hypoxia induces the activation of hypoxia-inducible factor 1-alpha (HIF1α). Under normoxic conditions, this transcription factor is hydroxylated and degraded by the cytoplasmic proteasome. However, in the presence of tissue damage, ischemic processes, or exposure to hypoxia, the decreased availability of oxygen inhibits the hydroxylation of HIF1α, thus leading to its accumulation and translocation into the nucleus where it induces the expression of genes involved in the adaptation of low oxygen levels. These genes include those associated with angiogenesis, wound healing, anaerobic glucose metabolism, erythropoiesis, proliferation, differentiation, and apoptosis, among others. It has been demonstrated that hundreds of genes can be transcriptionally regulated by HIF1α (25). Regarding the effect of hypoxia on the content of EV, numerous studies have shown that MSC-derived EV preconditioned under hypoxia have greater therapeutic capabilities in regenerative medicine (24).

Although it is known that the paracrine effects of MSC mediated by secretion affect various cellular physiological aspects, there is still limited information on how these vesicles can influence the adipogenic and osteogenic differentiation of precursor cells. Therefore, the objective of this study was to investigate how EV derived from cultures of human bone marrow MSC grown under hypoxic or normoxic conditions affect the osteoblastic and adipogenic differentiation of MSC. The aim is to contribute to the necessary knowledge for the possible development of new therapeutic approaches for the management of conditions associated with bone and/or adipose tissue.

MATERIALS AND METHODS

CULTURE AND EXPANSION OF MESENCHYMAL STEM CELLS

Human bone marrow derived MSC were obtained from cryopreserved and previously characterized cultures from our group's cell line collection. MSC from a healthy 31-year-old male donor were used for this study. A vial of cryopreserved MSC (8×10^5 cells) was seeded into a 75 cm^2 culture flask in a α-MEM culture medium (Cambrex Bio Science-Lonza; Basel, Switzerland) supplemented with 10 % FBS (Gibco-Thermo Fisher Scientific), 1 % ultraglutamine (Cambrex Bio Science-Lonza), 0.1 mg/mL streptomycin, 100 U of penicillin, and 1 ng/mL FGF-2 (Fibroblast Growth Factor-2) from Sigma-Aldrich (Saint Louis, MO, United States). The culture medium was changed every 3-4 days. Upon reaching 80 %-90 % confluency, the cells were detached using trypsin-EDTA (Gibco-Thermo Fisher Scientific) and re-seeded in 1:3 dilution.

ISOLATION OF MSC-DERIVED EXTRACELLULAR VESICLES

In MSC cultures at passages 4 to 6, when they reached approximately 70 % confluency, the culture medium was replaced with a fresh medium supplemented with 5 % exosome-depleted FBS (Gibco-Thermo Fisher Scientific). Cells were maintained in this medium for 48 hours under 2 oxygen concentration conditions: hypoxia (5 % CO_2, 3 % O_2, and 37 °C) and normoxia (5 % CO_2, 16 % O_2, and 37 °C). Afterwards, the culture medium from three 75 cm^2 flasks (approximately 50 mL) for each condition was collected and centrifuged at 4 °C for 10 min at 300 g, 20 min at 1200 g, and 30 min at 10 000 g. After the final centrifugation, the culture medium was concentrated using the Amicon®Ultra-15 Centrifugal Filter Device 100 kDa (Millipore; Merck KGaA, Darmstadt, Germany) to approximately 2 mL. EV were purified from the concentrated medium using size-exclusion chromatography with PURE-EV Columns (HansaBioMed; Tallinn, Estonia) following the manufacturer's instructions for use. The EV-containing fractions were finally concentrated to a volume of 300-400 µL using ultrafiltration with an Amicon®Ultra-15 Centrifugal Filter Device 10 kDa (Millipore).

MORPHOLOGICAL CHARACTERIZATION AND QUANTIFICATION OF EXTRACELLULAR VESICLES

The morphology of EV was analyzed using transmission electron microscopy (TEM). In short, 20 µL of the sample were applied to carbon-coated copper grids. After drying, the grids were stained with 2 % (w/v) uranyl acetate (UrAc) for 1 min. Images were captured using a JEOL JEM 1400 High-resolution transmission electron microscope (SCAI, University of Córdoba, Córdoba, Spain) at an acceleration voltage of 80-200 keV.

The nanoparticle concentration was determined using a Nanosight NS300 at the University Institute of Nanochemistry, Universidad de Córdoba, Córdoba, Spain.

Western blotting

For total protein extraction from different cell cultures, cells were lysed with the Cell Extraction Buffer (Thermo Fisher Scientific) that was supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF) and a 50 µL/mL protease inhibitor cocktail (PIC) (both from Sigma-Aldrich). The lysate was incubated on ice for 30 min with vortexing every 10 min. Finally, the lysate was centrifuged for 10 min at 13 000g at 4 °C, the precipitated cellular debris was discarded, and the supernatant was stored at -20 °C until further use. Protein concentration was quantified using the Bio-Rad DC Protein Assay kit (Bio-Rad) following the manufacturer's protocol. For the extraction and quantification of proteins from EV, the same protocol was used after lysing the vesicles with the Cell Extraction Buffer.

The protein concentration obtained from EV ranged from 0.1 µg/µL to 0.3 µg/µL. For Western blotting, a total of 2 µg to 10 µg of protein from each sample were loaded onto an 8 %-16 % acrylamide gel (nUView Tris-Glycine Precast Gels, NuSeP) in denaturing conditions using a Mini-Protean electrophoresis system (Bio-Rad). After electrophoresis, proteins were transferred to Polyvinylidene difluoride (PVDF) membranes (Bio-Rad) using a Trans-Blot Turbo Transfer System (Bio-Rad). The membranes were blocked with a 5 % non-fat dry milk solution in T-TBS buffer (20 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.05 % Tween) for 1 hour at room temperature. Afterwards, the membranes were

incubated overnight at 4 °C with primary antibodies, anti-CD9 (1:700), anti-CD63 (1:700) (both from Invitrogen, ThermoFisher Scientific), or anti-calnexin (1:1000) from Sigma-Aldrich, in 1 % milk in T-TBS. After washing the membranes 3 times with T-TBS, they were incubated with the secondary antibody, anti-Mouse IgG H & L-HRP (1:5000) (Invitrogen, ThermoFisher Scientific) for CD9 and CD63, and anti-Rabbit IgG H & L-HRP (1:3000) (Abcam) for calnexin, in 1 % milk in T-TBS for 1 hour. Finally, the excess secondary antibody was washed with T-TBS, and the membrane was developed using Clarity Western ECL Substrate (Bio-Rad). The bands were visualized using a Bio-Rad ChemiDoc™ XRS+ Gel Documentation System through the ImageLab software from the same company. The band intensity was later quantified using ImageJ 1.53t software.

QUANTIFICATION OF GENE EXPRESSION THROUGH POLYMERASE CHAIN REACTION (PCR)

RNA from cultures induced to differentiate into osteoblasts or adipocytes was isolated using the NZY total RNA isolation kit (NZYTech Lda; Lisbon, Portugal) following the manufacturer's instructions for use. RNA was quantified using a NanoDrop ND-1000 spectrophotometer from Thermo Fisher Scientific, and 900 ng were reverse transcribed into cDNA using the iScript cDNA Synthesis Kit (Bio-Rad) again according to the manufacturer's instructions for use.

Real-time quantitative PCR (qRT-PCR) was performed using a Roche Applied Science LightCycler 96 Instrument. Each PCR reaction was performed in a volume of 10 µL containing 1 µL of cDNA, 1 µM primers (Table I), and 1X SensiFAST Sybr No-Rox Mix (BIOLINE). The PCR amplification program included an initial cycle at 95 °C for 2 min (DNA denaturation and activation of DNA polymerase) and 40 to 45 cycles of 95 °C for 5 seconds (DNA denaturation) at 65 °C for 30 seconds (primer annealing and product extension). The results were analyzed using the LightCycler 1.1 software from the same manufacturer. The POLR2A gene (polymerase [RNA; DNA-directed] II polypeptide A) was used as a constitutive gene.

CELL VIABILITY ASSAY

Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich). MSC were seeded in 96-well plates at a density of 4000 cells per well in culture medium. Cells were treated in a culture medium supplemented with EV-free FBS and different concentrations of MSC-derived EV kept under normoxic culture conditions (MSC-EvN) or hypoxia (MSC-EvH) (3×10^7, 9×10^7, and 15×10^7 particles/mL). After 48 hours, the culture medium was removed, and 100 µL of DMEM (Dulbecco's Modified Eagle Medium; Capricorn Scientific GmbH) without FBS or phenol red, containing 1 mg/mL MTT, were added. Cells were incubated at 37 °C for 2 hours,

Table I. Primer sequences and amplicon sizes

Gene	Direct and reverse primer sequence (5 '→ 3')	Size of byproduct (bp)
Runt-related transcription factor 2 *(RUNX2)*	TGGTTAATCTCCGCAGGTCAC ACTGTGCTGAAGAGGCTGTTTG	143
Osterix *(SP7)*	AGCCAGAAGCTGTGAAACCTC AGCTGCAAGCTCTCCATAACC	163
Collagen, type I, alpha 1 *(COL1A1)*	CGCTGGCCCCAAAGGATCTCCTG GGGGTCCGGGAACACCTCGCTC	263
Integrin-binding sialoprotein *(BSP)*	AGGGCAGTAGTGACTCATCCG CGTCCTCTCCATAGCCCAGTGTTG	171
Peroxisome proliferator-activated receptor gamma 2 *(PPARG2)*	GCGATTCCTTCACTGATACACTG GAGTGGGAGTGGTCTTCCATTAC	136
Lipoprotein lipase *(LPL)*	AAGAAGCAGCAAAATGTACCTGAAG CCTGATTGGTATGGGTTTCACTC	113
Fatty-acid-binding protein 4 *(FABP4)*	TCAGTGTGAATGGGGATGTGAT TCTGCACATGTACCAGGACACC	162
Fatty acid synthase *(FASN)*	AAGCTGAAGGACCTGTCTAGG CGGAGTGAATCTGGGTTGATG	146
Polymerase (RNA; DNA directed) II polypeptide A *(POLR2A)*	TTTTGGTGACGACTTGAACTGC CCATCTTGTCCACCACCTCTTC	125

and the formazan crystals formed during incubation were dissolved in 100 % isopropanol. The absorbance of the resulting solution was measured at 570 nm, with a reference at 650 nm using a PowerWave XS microplate spectrophotometer (BioTek Instruments).

CELL MIGRATION ASSAY

Cell migration of MSC was evaluated using the Oris™ Cell Migration Assay (Platypus Technologies). MSC were seeded in 96-well plates (15 000 cells/well) and incubated at 37°C with cell seeding stoppers in each well until reaching 90 % confluency. Afterwards, the stoppers were removed, leaving a 2 mm halo in the center of each well. After washing with PBS, α-MEM + 2 % EV-free FBS was added containing MSC-EvN or MSC-EvH at a concentration of 3×10^7, 9×10^7, or 15×10^7 particles/mL. At 0 h, 12 h, and 18 h, images were captured using an Incucyte® Systems for Live-Cell Imaging phase-contrast microscope. Migration was measured by calculating the percentage of wound closure area compared to the initial open area (t = 0) using the following formula: migration area (%) = (A0-At)/A0 × 100, where A0 represents the initial open area, and At the residual area at the measurement time. ImageJ software was used to quantify areas in the images.

ADIPOCYTE AND OSTEOBLAST DIFFERENTIATION

MSC were seeded in P12 or P24 culture plates (Nalgene-Nunc-Thermo Fisher Scientific) at a density of 3000 cells/cm^2. Once they reached 60 %-80 % confluency, they were differentiated into adipocytes or osteoblasts in the presence or absence of MSC-EvN or MSC-EvH. To induce adipocyte differentiation, the culture medium without FGF was supplemented with 5×10^{-7}M dexamethasone, 50 µM indomethacin, and 0.5 mM isobutylmethylxanthine. For osteoblast differentiation, the medium was supplemented with 10^{-8} M dexamethasone, 10 mM β-glycerophosphate, and 0.2 mM ascorbic acid. All inducers were obtained from Sigma-Aldrich.

After 13 days of differentiation, samples were taken from the cultures for RNA extraction and analysis of gene expression of adipocyte or osteoblast markers.

MINERALIZATION STAINING OF THE EXTRACELLULAR MATRIX

Mineralization of the matrix in osteoblast-induced MSC was evaluated using alizarin red S staining at 21 days. Cultures were fixed for 10 min with 3.7 % formaldehyde and stained with a 40 mM alizarin red S solution at pH 4.1. All reagents were from Sigma-Aldrich. The wells were then washed with 60 % isopropanol, dried, and images were captured. To quantify mineralization, the staining was eluted with 10 % acetic acid and neutralized with 10 % ammonium hydroxide. The resulting solution's absorbance was measured at 405 nm using a PowerWave XS microplate spectrophotometer from BioTek Instruments.

OIL RED STAINING OF LIPID DROPLETS

The formation of lipid droplets in adipocyte-induced cultures was evaluated using oil-red O staining at 13 days of differentiation. Cultures were fixed with 3.7 % formaldehyde for 20 min and stained with a solution of 60 % 0.3 % oil-red (w/v in isopropanol) and 40 % distilled water. After 15 to 20 min of incubation, cells were washed with distilled water, stained with hematoxylin, and images were taken using an optical microscope for each well. Oil-red O staining was quantified using image analysis software ImageJ. The area of the oil-red O stained image was normalized to the corresponding cell number.

STATISTICAL ANALYSIS

Comparison between different treatments was performed using ANOVA to detect significant changes followed by Tukey's test to identify significant differences between pairs of treatments. Significant changes (*) were considered with p values < 0.05. At least 3 data points were obtained per studied parameter. Data are expressed as mean ± standard error of the mean (mean ± SEM).

RESULTS

CHARACTERIZATION OF EXTRACELLULAR VESICLES

In size exclusion chromatography of concentrated media from MSC grown under normoxic or hypoxic culture conditions, 10 fractions were obtained and their protein concentration was estimated by measuring absorbance at 280 nm. As shown on figure 1A, the amount of eluted protein increased from fraction

7 onwards, which is indicative that protein-free EV are present in previous fractions.

After mixing and subsequent concentration of fractions 1 to 6 by ultrafiltration, the nanovesicles obtained were quantified and analyzed by Nanoparticle Tracking Analysis. The average size of the EV obtained from this analysis was approximately 150 nm (Fig. 1B). Transmission electron microscopy (TEM) images showed the spherical morphology of the isolated EVs (Fig. 1C). Furthermore, the presence of surface markers CD63 and CD9 were detected in the nanovesicles while the cellular protein calnexin was not detected (Fig. 1D).

EFFECT OF MSC-EvN AND MSC-EvH APPLICATION ON MSC VIABILITY AND MIGRATION

MSC were grown in the presence or absence of 30, 60, or 150 x 10^6 particles/mL of MSC-EvN or MSC-EvH for 3 days, time after which cell viability was quantified. As shown in figure 2A, MSC viability tended to increase with the concentration of EV. This increase was significant with the highest concentration used for both types of EV being slightly higher in cells treated with EV derived from hypoxia conditions (Fig. 2A).

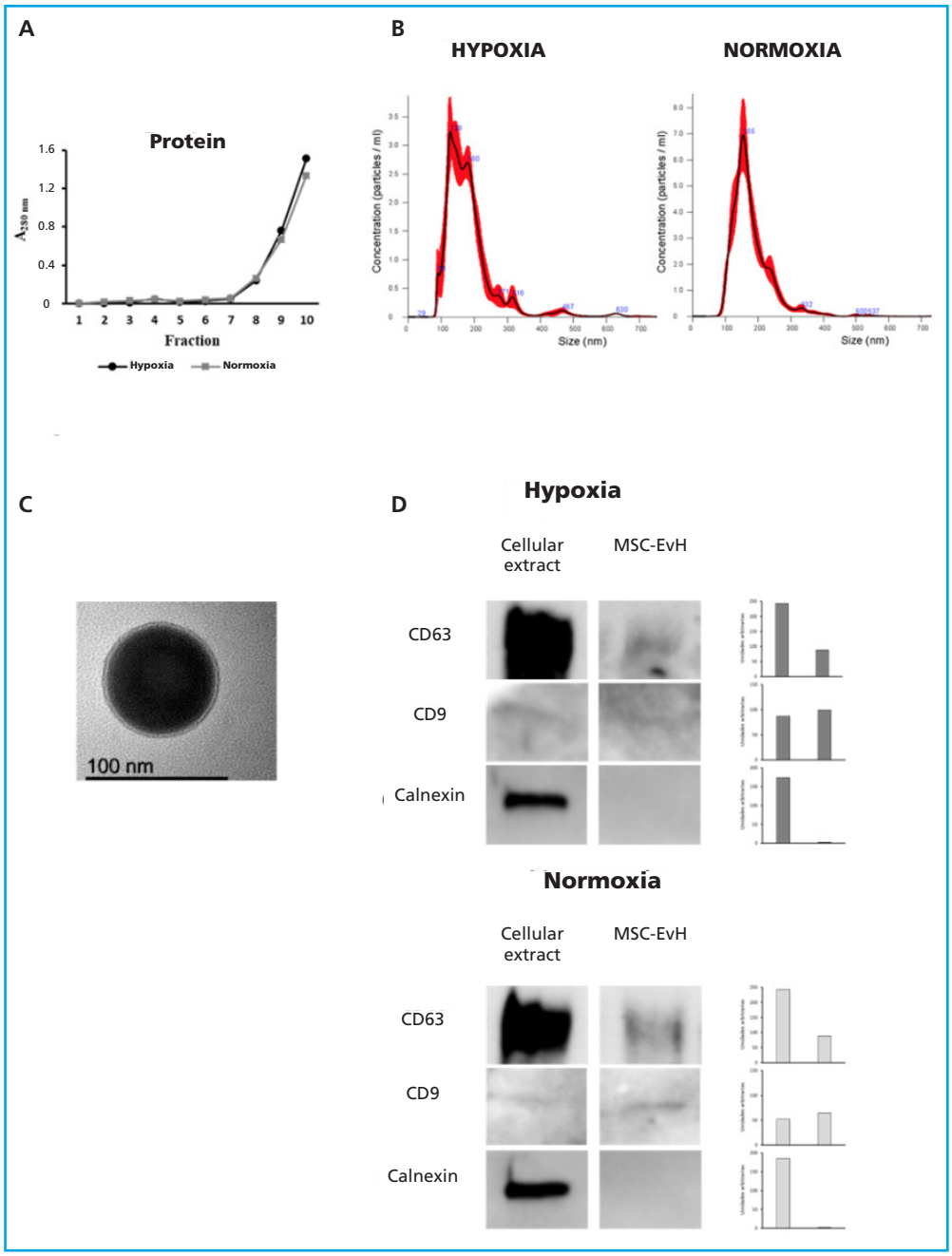

Figure 1. Characterization of extracellular vesicles derived from MSC in normoxic and hypoxic conditions. A. Absorbance at 280 nm of the fractions obtained through size exclusion chromatography. B. Particle size distribution of MSC-EvN and MSC-EvH obtained through Nanosight. C. TEM image of an EV showing its morphology and size. D. Western blot analysis of the protein expression pf positive EV markers (CD63 and CD9) and negative markers (calnexin) in cell and EV extracts (MSC-EvH and MSC-EvN). The graphical representation of their expression quantification is shown to the left of each marker.

Cell migration of MSC in the presence of different EV concentrations also tended to be higher. However, these changes were not significant in the case of treatments with MSC-EvH. On the other hand, MSC-EvN treatment increased cell migration significantly, but only when the highest concentration was used (Fig. 2B).

EFFECT OF EXTRACELLULAR VESICLES ON MSC DIFFERENTIATION INTO OSTEOBLASTS

Considering the results obtained regarding MSC viability and migration, a concentration of 15 × 10^7 particles/mL of MSC-EvN and MSC-EvH was selected to study and assess their effect on cell differentiation.

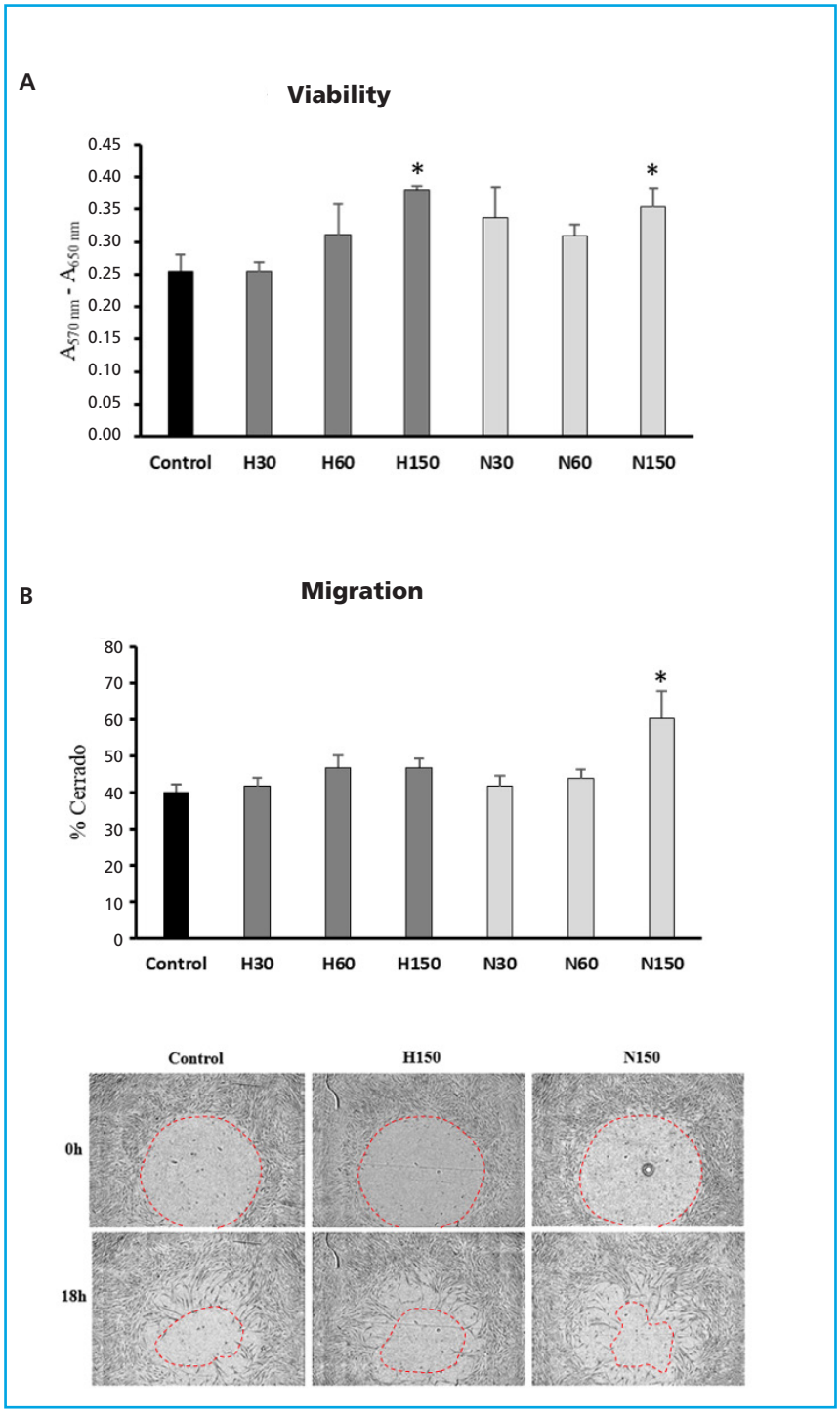

Figure 2. Viability and migration of MSC in the presence or absence of MSC-EvH or MSC-EvN. A. Effect of treatment with MSC-EvH (H) or MSC-EvN (N) at concentrations of 3 × 10^7 particles/mL (30), 6 × 10^7 particles/mL (60), and 15 × 10^7 particles/mL (150) on MSC culture viability. B. Same as (A), but for cell migration. The images represent representative cultures treated 0 and 18 h after the start of migration. *$p < 0.05$ vs control (untreated cells).

Mineralization of MSC differentiated into osteoblasts increased significantly with both types of vesicles. This increase was higher in cultures treated with EV derived from cultures under hypoxia conditions (Fig. 3A). Regarding the expression of osteoblastic marker genes, no significant changes were found in the genes encoding the transcription factor RUNX2 and the extracellular matrix protein collagen type 1 alpha (COL1A1). However, a significant increase was observed in the expression of the SP7 transcription factor gene, also known as osterix, with MSC-EvH treatment. Additionally, treatments with MSC-EvH and MSC-EvN significantly induced the expression of the integrin-binding sialoprotein gene (IBSP). In this case, the change was greater in EV derived from MSC in hypoxia compared to those obtained from normoxic culture conditions (Fig. 3B). These results suggest that EV derived from MSC in hypoxia have a greater capacity to promote osteoblastogenesis compared to those obtained from cultures obtained in normoxic conditions.

EFFECT OF EXTRACELLULAR VESICLES ON MSC DIFFERENTIATION INTO ADIPOCYTES

In the phenotypic analysis of MSC differentiated into adipocytes treated with MSC-EvH or MSC-EvN, no significant changes were seen in the formation of fat vesicles

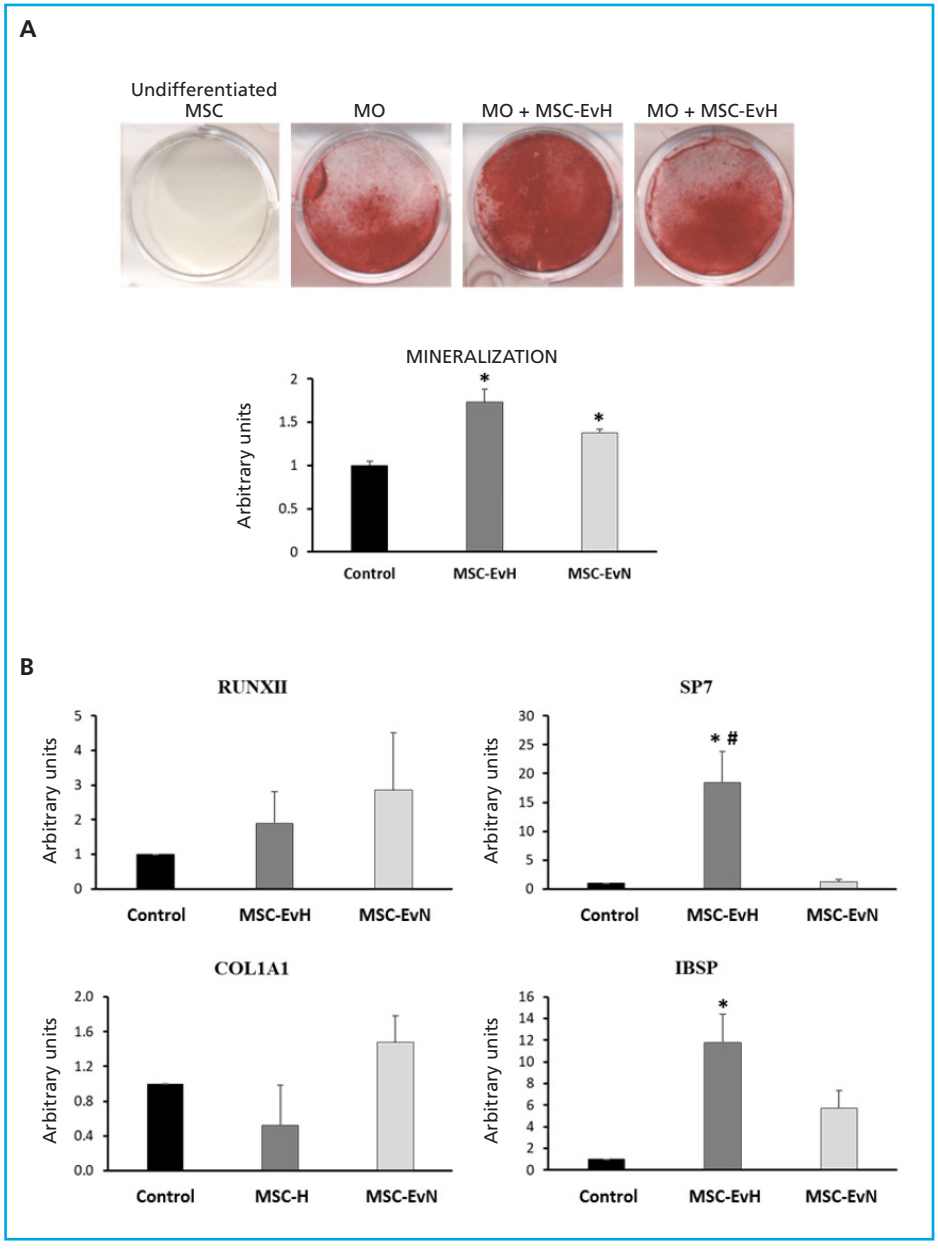

Figure 3. Effect of extracellular vesicles derived from MSC on osteogenic differentiation. A. Representative images and quantification of alizarin red S staining of MSC cultures at 21 days of differentiation in osteoblastic medium (OM) in the presence or absence of MSC-EvH or MSC-EvN. B. Expression of osteogenic genes RUNX2, SP7, COL1A1, and IBSP at 13 days of differentiation in cultures treated with MSC-EvH and MSC-EvN. Data are expressed as mean ± SEM. *$p < 0.05$ vs control (untreated cultures); #$p < 0.05$ vs MSC-EvN.

compared to untreated cultures (Fig. 4A). Regarding the expression of adipogenic genes PPARG2, LPL, and FASN, no differences were seen between the different treatments and the control. However, the expression of FABP4 in cultures treated with MSC-EvN increased significantly compared to the control and cultures treated with MSC-EvH. The expression of the latter showed no changes compared to untreated cultures (Fig. 4B).

DISCUSSION

Our study demonstrates that size exclusion chromatography for EV isolation produces highly pure vesicles with low contamination of soluble proteins (26). The results of treatments with both types of EV, MSC-EvH and MSC-EvN, indicate that they increase the viability

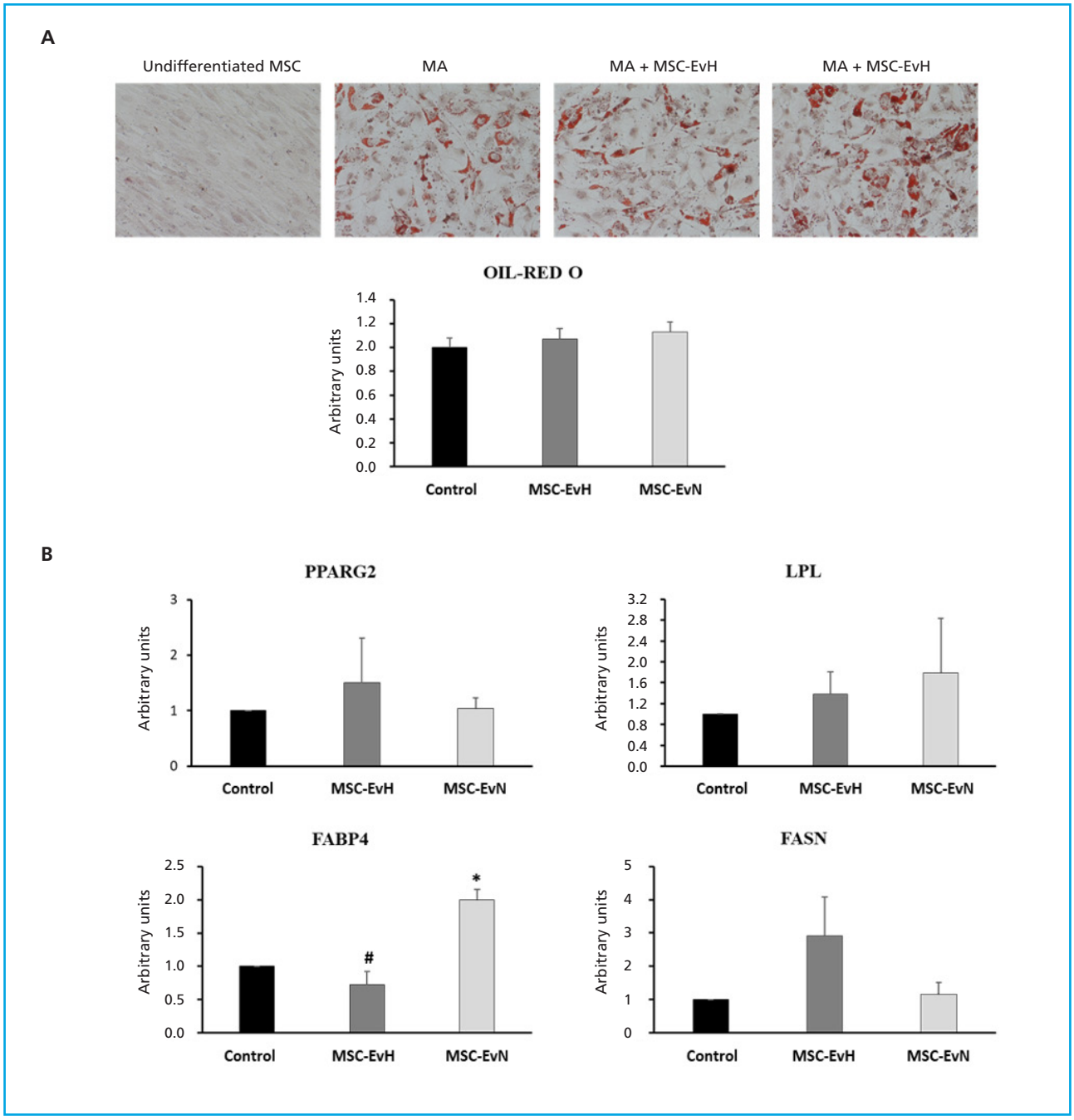

Figure 4. Effect of extracellular vesicles derived from MSC on adipogenic differentiation. A. Images and quantification of oil red O staining in MSC cultures after 13 days in adipogenic medium (AM) in the presence or absence of MSC-EvH or MSC-EvN. (Images at 200x magnification). B. Expression of adipogenic genes PPARG2, LPL, FABP4, and FASN at 13 days of differentiation in cultures treated with MSC-EvH and MSC-EvN. Data are expressed as mean ± SEM. *$p < 0.05$ vs control (untreated cultures); #$p < 0.05$ vs MSC-EvN.

of MSC cultures in vitro when applied at a concentration of 15 × 10^7 particles/mL. The positive effect of EV derived from MSC on the viability of different cell types has been described in various studies (15,27). Some authors have shown that MSC-derived EV do not affect the viability of bone marrow-derived MSC (28). However, these results were obtained from EV obtained by ultracentrifugation and after maintaining the cells for 12 hours in fresh culture media (28), not 48 hours as it was our case. Therefore, the different methodological conditions can affect the content of EV and explain the differences seen among different studies. Our data do not show differences between the effects of MSC-EvH and MSC-EvN on the viability of MSC cultures. However, some authors have described that EV derived from MSC cultured in hypoxic conditions have a greater capacity to increase cell viability compared to those obtained in normoxic conditions (29,30). However, we should mention that these studies have been mainly conducted on endothelial cells, not MSC. We should, therefore, remember that hypoxia causes the production of factors that stimulate and induce endothelial cells to form new vessels to compensate for the decreased oxygen levels like the vascular endothelial growth factor (VEGF) (31). These factors may be abundant in EV derived from MSC maintained in hypoxic conditions. However, our data suggest that they may not have a significant effect on the viability MSC.

Treatment with MSC-EvH and MSC-EvN tended to increase MSC migration. In other cell types such as endothelial cells, fibroblasts, and keratinocytes, it has been shown that MSC-derived EV enhance their proliferation and migration capabilities (32,33). The increased migration induction is associated with greater regenerative capabilities of EV (34). Our results show that MSC migration was not significantly influenced when treated with MSC-EvH. This suggests that the hypoxic culture conditions used did not produce EV enriched in factors that would stimulate the migration of these cells.

The use of EV derived from bone cells like bone marrow MSC is emerging as a possible therapeutic strategy to treat bone conditions including osteoporosis (35,36). Our results show that in vitro osteogenic differentiation of MSC is enhanced when cultures are treated with EV derived from MSC, primarily with MSC-EvH. Cultures treated with these EV exhibited greater mineralization and expression of osteoblastic genes such as *SP7* and *IBSP*. The former encodes a transcription factor essential for osteogenic differentiation (37) while the latter encodes integrin-binding sialoprotein, an extracellular matrix protein involved in mineralization (38). These results support what has been previously described by other studies demonstrating the osteogenic capabilities of EV obtained from MSC (39-41). *In vivo* experiments in a bone fracture model have shown that EV derived from MSC cultured under hypoxic conditions promote bone fracture healing to a greater extent compared to EV obtained from MSC under normoxic conditions. This is partly due to their promotion of angiogenesis through miR-126, which regulates the SPRED1/Ras/Erk angiogenic signaling pathway (40). In our case, we have not evaluated the potential effect of MSC-EvH on endothelial cells, but we have demonstrated that they induce osteoblastogenesis in precursor cells. Therefore, treatment with MSC-EvH could promote bone regeneration through its induction of angiogenesis in endothelial cells and osteoblastic differentiation of MSC. Other studies also support the high potential of MSC-derived EV regarding bone regeneration due to their ability to promote angiogenesis and osteoblastogenesis (39). The positive effect of MSC-derived EV on osteoblastogenesis *in vitro* and *in vivo* has been observed to involve miRNAs such as miR-196a, miR-335, and miR-27a (41-43).

MSC are also precursors of adipocytes. Overall, factors that promote adipogenic differentiation negatively affect osteogenesis and vice versa (9). However, our results indicate that neither MSC-EvH nor MSC-EvN affected adipogenesis significantly. Only the mRNA levels of FABP4 increased with MSC-EvN treatment. The FABP4 gene encodes a fatty acid-binding protein involved in various extracellular functions, so the application of MSC-EvN may affect aspects related to fatty acid metabolism during adipogenesis (44), which in our case did not affect the accumulation of lipid droplets.

In conclusion, our data demonstrate that treatment with MSC-derived EV enhances the viability, migration, and osteogenic differentiation of human bone marrow MSC. Osteoblastic differentiation is primarily induced when EV are derived from MSC exposed to hypoxia. This suggests that preconditioning cells under low oxygen levels could induce the secretion of EV enriched in osteogenic factors. The identification of these factors in the future may provide insights into the mechanism of action of these EV regarding osteoblastogenesis opening up other possibilities to design more efficient therapeutic strategies to treat different bone conditions. The results of this study support the potential use of cell-free therapy based on the application of EV to treat systemic bone diseases like osteoporosis and promote bone formation in difficult-to-heal fractures.

REFERENCES

1. Kim S, Lee SK, Kim H, et al. Exosomes secreted from induced pluripotent stem cell-derived mesenchymal stem cells accelerate skin cell proliferation. Int J Mol Sci 2018;19:3119. DOI: 10.3390/ijms19103119
2. Rani S, Ryan AE, Griffin MD, et al. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. Mol Ther 2015;23:812-23. DOI: 10.1038/mt.2015.44
3. Gomathi K, Akshaya N, Srinaath N, et al. Regulation of Runx2 by post-translational modifications in osteoblast differentiation. Life Sci 2020;245:117389. DOI: 10.1016/j.lfs.2020.117389

4. Takada I, Kouzmenko AP, Kato S. Wnt and PPARγ signaling in osteoblastogenesis and adipogenesis. Nat Rev Rheumatol 2009;5:442-7. DOI: 10.1038/nrrheum.2009.137
5. Moerman EJ, Teng K, Lipschitz DA, et al. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: The role of PPAR-γ2 transcription factor and TGF-β/BMP signaling pathways. Aging Cell 2004;3:379-89. DOI: 10.1111/j.1474-9728.2004.00127.x
6. Singh L, Brennan T, Russell E, et al. Aging alters bone-fat reciprocity by shifting in vivo mesenchymal precursor cell fate towards an adipogenic lineage. Bone 2016;85:29-36. DOI: 10.1016/j.bone.2016.01.014
7. Qadir A, Liang S, Wu Z, et al. Senile osteoporosis: The involvement of differentiation and senescence of bone marrow stromal cells. Int J Mol Sci 2020;21:349. DOI: 10.3390/ijms21010349
8. Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? Nat Clin Pract Rheumatol 2006;2:35-43. DOI: 10.1038/ncprheum0070
9. Chen Q, Shou P, Zheng C, et al. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ 2016;23:1128-39. DOI: 10.1038/cdd.2015.168
10. Joo HS, Suh JH, Lee HJ, et al. Current knowledge and future perspectives on mesenchymal stem cell-derived exosomes as a new therapeutic agent. Int J Mol Sci 2020;21:727. DOI: 10.3390/ijms21030727
11. Kusuma GD, Carthew J, Lim R, et al. Effect of the Microenvironment on Mesenchymal Stem Cell Paracrine Signaling: Opportunities to Engineer the Therapeutic Effect. Stem Cells Dev 2017;26:617-31. DOI: 10.1089/scd.2016.0349
12. Vizoso FJ, Eiro N, Cid S, et al. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. Int J Mol Sci 2017;18(9):1852. DOI: 10.3390/ijms18091852
13. Vilaça-Faria H, Marote A, Lages I, et al. Fractionating stem cells secretome for Parkinson's disease modeling: Is it the whole better than the sum of its parts? Biochimie 2021;189:87-98. DOI: 10.1016/j.biochi.2021.06.008
14. González-Cubero E, González-Fernández ML, Olivera ER, et al. Extracellular vesicle and soluble fractions of adipose tissue-derived mesenchymal stem cells secretome induce inflammatory cytokines modulation in an in vitro model of discogenic pain. Spine J 2022;22:1222-34. DOI: 10.1016/j.spinee.2022.01.012
15. Shabbir A, Cox A, Rodriguez-Menocal L, et al. Mesenchymal Stem Cell Exosomes Induce Proliferation and Migration of Normal and Chronic Wound Fibroblasts, and Enhance Angiogenesis in Vitro. Stem Cells Dev 2015;24:1635-47. DOI: 10.1089/scd.2014.0316
16. Casado-Díaz A, Quesada-Gómez JM, Dorado G. Extracellular Vesicles Derived From Mesenchymal Stem Cells (MSC) in Regenerative Medicine: Applications in Skin Wound Healing. Front Bioeng Biotechnol 2020;8:1-19. DOI: 10.3389/fbioe.2020.00146
17. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 2018;7:1535750. DOI: 10.1080/20013078.2018.1535750
18. Jeppesen DK, Fenix AM, Franklin JL, et al. Reassessment of Exosome Composition. Cell 2019;177:428-45.e18. DOI: 10.1016/j.cell.2019.02.029
19. Deng H, Sun C, Sun Y, et al. Lipid, Protein, and MicroRNA Composition Within Mesenchymal Stem Cell-Derived Exosomes. Cell Reprogram 2018;20:178-86. DOI: 10.1089/cell.2017.0047
20. Doyle L, Wang M. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells 2019;8:727. DOI: 10.3390/cells8070727
21. Willis GR, Kourembanas S, Mitsialis SA. Toward Exosome-Based Therapeutics: Isolation, Heterogeneity, and Fit-for-Purpose Potency. Front Cardiovasc Med 2017;4:63. DOI: 10.3389/fcvm.2017.00063
22. Costa LA, Eiro N, Fraile M, et al. Functional heterogeneity of mesenchymal stem cells from natural niches to culture conditions: implications for further clinical uses. Cellular and Molecular Life Sciences 2021;78:447-67. DOI: 10.1007/s00018-020-03600-0
23. Ding J, Wang X, Chen B, et al. Exosomes Derived from Human Bone Marrow Mesenchymal Stem Cells Stimulated by Deferoxamine Accelerate Cutaneous Wound Healing by Promoting Angiogenesis. Biomed Res Int 2019;2019:9742765. DOI: 10.1155/2019/9742765
24. Pulido-Escribano V, Torrecillas-Baena B, Camacho-Cardenosa M, et al. Role of hypoxia preconditioning in therapeutic potential of mesenchymal stem-cell-derived extracellular vesicles. World J Stem Cells 2022;14:453-72. DOI: 10.4252/wjsc.v14.i7.453
25. Mole D, Blancher C, Copley R, et al. Genome-wide association of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha DNA binding with expression profiling of hypoxia-inducible transcripts. J Biol Chem 2009;284:16767-75. DOI: 10.1074/jbc.M901790200
26. Gámez-Valero A, Monguió-Tortajada M, Carreras-Planella L, et al. Size-Exclusion Chromatography-based isolation minimally alters Extracellular Vesicles' characteristics compared to precipitating agents. Sci Rep 2016;6:1-9. DOI: 10.1038/srep33641
27. Wang Y, Yao J, Cai L, et al. Bone-targeted extracellular vesicles from mesenchymal stem cells for osteoporosis therapy. Int J Nanomedicine 2020;15:7967-77. DOI: 10.2147/IJN.S263756
28. Wei F, Li Z, Crawford R, et al. Immunoregulatory role of exosomes derived from differentiating mesenchymal stromal cells on inflammation and osteogenesis. J Tissue Eng Regen Med 2019;13:1978-91. DOI: 10.1002/term.2947
29. Almeria C, Weiss R, Roy M, et al. Hypoxia Conditioned Mesenchymal Stem Cell-Derived Extracellular Vesicles Induce Increased Vascular Tube Formation in vitro. Front Bioeng Biotechnol 2019;7:1-12. DOI: 10.3389/fbioe.2019.00292
30. Gao W, He R, Ren J, et al. Exosomal HMGB1 derived from hypoxia-conditioned bone marrow mesenchymal stem cells increases angiogenesis via the JNK/HIF-1α pathway. FEBS Open Bio 2021;11:1364-73. DOI: 10.1002/2211-5463.13142
31. Han Y, Ren J, Bai Y, et al. Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGF-R. Int J Biochem Cell Biol 2019;109:59-68. DOI: 10.1016/j.biocel.2019.01.017
32. Ren S, Chen J, Duscher D, et al. Microvesicles from human adipose stem cells promote wound healing by optimizing cellular functions via AKT and ERK signaling pathways 06 Biological Sciences 0601 Biochemistry and Cell Biology. Stem Cell Res Ther 2019;10:1-14.
33. Wang X, Omar O, Vazirisani F, et al. Mesenchymal stem cell-derived exosomes have altered microRNA profiles and induce osteo-

genic differentiation depending on the stage of differentiation. PLoS One 2018;13:4-6. DOI: 10.1371/journal.pone.0193059
34. Cooper DR, Wang C, Patel R, et al. Human Adipose-Derived Stem Cell Conditioned Media and Exosomes Containing MALAT1 Promote Human Dermal Fibroblast Migration and Ischemic Wound Healing. Adv Wound Care 2018;7:299-308. DOI: 10.1089/wound.2017.0775
35. Li Q-C, Li C, Zhang W, et al. Potential Effects of Exosomes and their MicroRNA Carrier on Osteoporosis. Curr Pharm Des 2022;28:899-909. DOI: 10.2174/1381612828666220128104206
36. Vig S, Fernandes MH. Bone Cell Exosomes and Emerging Strategies in Bone Engineering. Biomedicines 2022;10:767. DOI: 10.3390/biomedicines10040767
37. Zhang C. Transcriptional regulation of bone formation by the osteoblast-specific transcription factor Osx. J Orthop Surg Res 2010;5:1-8. DOI: 10.1186/1749-799X-5-37
38. Ogata Y. Bone sialoprotein and its transcriptional regulatory mechanism. J Periodontal Res 2008;43:127-35. DOI: 10.1111/j.1600-0765.2007.01014.x
39. Takeuchi R, Katagiri W, Endo S, et al. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. PLoS One 2019;14:1-19. DOI: 10.1371/journal.pone.0225472
40. Liu W, Li L, Rong Y, et al. Hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by the transfer of miR-126. Acta Biomater 2020;103:196-212. DOI: 10.1016/j.actbio.2019.12.020
41. Qin Y, Wang L, Gao Z, et al. Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation in vitro and promote bone regeneration in vivo. Sci Rep 2016;6:1-11. DOI: 10.1038/srep21961
42. Wang Y, Zhou X, Wang D. Mesenchymal Stem Cell-Derived Extracellular Vesicles Inhibit Osteoporosis via MicroRNA-27a-Induced Inhibition of DKK2-Mediated Wnt/β-Catenin Pathway. Inflammation 2022;45:780-99. DOI: 10.1007/s10753-021-01583-z
43. Hu H, Wang D, Li L, et al. Role of microRNA-335 carried by bone marrow mesenchymal stem cells-derived extracellular vesicles in bone fracture recovery. Cell Death Dis 2021;12:156. DOI: 10.1038/s41419-021-03430-3
44. Hotamisligil GS, Bernlohr DA. Metabolic functions of FABPs - Mechanisms and therapeutic implications. Nat Rev Endocrinol 2015;11:592-605. DOI: 10.1038/nrendo.2015.122

ROMM
Revista de Osteoporosis y Metabolismo Mineral

Original

Polygenic risk of bone fractures in Spanish women with osteoporosis

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Abstract

Background: osteoporosis is a highly polygenic trait characterized by low bone mineral density (BMD) and/or fragility fractures. Over the past decade, polygenic risk scores (PRS) are an emerging tool to try to predict the risk of complex disorders with a genetic component.

Objective: to analyze the capacity of different PRSs to predict osteoporosis in the Spanish population.

Material and methods: our dataset consisted of two differentiated groups. The first group included osteoporosis cases diagnosed and treated at the Marques de Valdecilla University Hospital (n = 304; 293 women) while the second group consisted of people from the overall Spanish population (n = 3199; 1458 women). Four previously generated PRSs were compared with generalized linear models.

Results: the osteoporosis group showed a significantly higher genetic risk compared to the control group in 3 PRSs (PRS-1 p = 1e-7; PRS-2 p = 1.87e-15; PRS-3 p = 0.1477; PRS-4 p = 8.98e-9). In addition, in these PRSs, the individuals in the upper quartile of risk had a significantly higher risk of osteoporosis, compared to those individuals in the other quartiles (PRS-1 OR, 1.83; PRS-2 OR, 2.11; PRS-3 OR, 0.96; PRS-4 OR, 1.72).

Conclusions: in summary, the application of PRSs shows significant differences between the overall Spanish population and patients with osteoporosis, which is suggestive of its utility within strategies for the identification of subjects at risk based on clinical-genetic criteria.

Keywords:
Osteoporosis. Polygenic risk scores. Genomics. Bone mineral density.

Received: 19/10/2022 • Accepted: 19/04/2023

Acknowledgements: partially supported by a grant from Instituto de Salud Carlos III (PI21/00532). Alvaro del Real received support by the postdoctoral grant Margarita Salas in the University of Cantabria, Spain.

Conflicts of Interest: the authors declare no conflict of interest.

Del Real Á, Cruz R, Olmos JM, Hernández JL, Valero C, Riancho JA. Polygenic risk of bone fractures in Spanish women with osteoporosis. Rev Osteoporos Metab Miner 2023;15(2):66-71

DOI: 10.20960/RevOsteoporosMetabMiner.00014

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INTRODUCTION

Osteoporosis is the most prevalent bone disease characterized by low bone mineral density (BMD) leading to an increased risk of fracture. Osteoporosis-related fractures represent an immense economic burden on the healthcare systems. Common diseases such as osteoporosis are usually polygenic, involving many genetic variants rather than rare monogenic mutations (1). Several studies have shown that BMD is a highly polygenic trait, which is directly associated with bone fracture (2). Over the past 15 years, genome-wide association studies (GWAS) have identified many genomic loci as related to the risk of various complex diseases (3). The knowledge of the genetic variants involved in a specific trait allows the early identification of subjects at risk and the initiation of preventive measures. Thus, in the GWAS era, several genetic variants have been related to BMD and fracture risk. The seminal study of Estrada et al. identified 56 loci associated with BMD and 13 SNPs with bone fractures (4). Another more recent study from the UK Biobank database analyzed the association of genetic variants and heel quantitative ultrasound (eBMD) in a total of 426 824 individuals to finally identify 518 significant loci (5).

The results of those studies are being used to develop risk scores based on the analysis of multiple gene variants (polygenic risk scores, PRSs). Hence, a PRS can be defined as an individual's mark made of the allelic signature at a number of polymorphic loci (often, tens or hundreds, or even thousands) related to the genetic susceptibility to develop a disorder (2). A representative analysis with PRSs involves an association between a PRS and a trait from the main data. This association can be evaluated with standard analytical procedures such as the *p* value to test a null hypothesis; effect size estimate (OR of high vs low risk individuals), and/or with measures of discrimination like the area under the curve (AUC). Several statistical tests can be applied to check the significance of the association including linear or logistic regression with or without adjusting for covariates like sex and age (6).

Several PRSs have been generated in relation to BMD and/or risk of fracture. First, the 56 loci identified (*n* = 63 probes) from the seminal study described above (4) were used as a PRS related to femoral neck BMD. Richards' lab developed a prediction of fracture risk PRS (*n* = 21717 probes) by using ultrasonography data of the calcaneus as an intermediate phenotype (7). Their polygenic risk score was more strongly associated with the risk of fracture than many other clinical risk factors, including age, sex, BMI and FRAX clinical factors (8). Additionally, Tanigawa et al. generated two distinct PRS models, incorporating the results at 316 and 1270 loci, respectively (9).

The objective of this study was to analyze the capacity of the previously mentioned PRSs to discriminate between patients with osteoporosis and controls in the Spanish population.

METHODS

SAMPLE RECRUITMENT

Our dataset included two groups. The group of cases corresponds to patients with osteoporosis recruited at the Marques de Valdecilla University Hospital (*n*= 304; 293 women; mean age, 65 years; range 47 to 87 years). Subjects with secondary osteoporosis were excluded. BMD was measured by dual X-ray densitometry (DXA) at the spine (mean BMD 0.744 [Interquartile range, IR, 0.692 to 0.792]) and the hip (mean BMD 0.737 [IR 0.679 to 0.803]) using a Hologic QDR 4500 densitometer (Waltham, MA, United States).

The control included samples from the overall Spanish population that were provided by the "Banco Nacional de ADN Carlos III (BNADN; www.bancoadn.org) (*n* = 3199; 1458 women; mean age, 48 years; range 18 to 104 years).

These data were not included in former GWAS. The study protocol was approved by the institutional review board (Comité de Ética en Investigación Clínica de Cantabria). All patients gave their informed written consent.

DNA ISOLATION AND GENOTYPING

DNA was isolated from aliquots of peripheral blood using commercially available column-based kits, following the manufacturer's instructions for use. Quantification of DNA was performed using Qubit dsDNA BR Assay Kit (ThermoFisher, Waltham, MA, United States). DNA samples from both groups were genotyped at the Spanish National Genotyping Center ("Centro Nacional de Genotipado-Fundación Pública Galega de Medicina Xenómica"), using the Axiom™ Spain Biobank array following the manufacturer's instructions for use (Axiom™ 2.0 Assay 96-Array Format Manual Workflow; ThermoFisher Scientific). Briefly, total genomic DNA (200 ng) was amplified and randomly fragmented into 25 to 125 base pair fragments, which were then purified and resuspended in a hybridization cocktail. The hybridization-ready targets were then transferred to the GeneTitan Multichannel Instrument for automated, hands-free processing (including hybridization to Axiom array plates, staining, washing and imaging). CEL files were automatically processed for allele calling using the Axiom GT1 algorithm available through the Axiom Analysis Suite v4.0.3.3 and following the Axiom™ Genotyping Solution Data Analysis User Guide (ThermoFisher Scientific, Waltham, MA, United States).

QUALITY CONTROL AND PRS ANALYSIS

The Axiom Analysis Suite was applied to conduct the quality control of genotyped data. Thresholds applied were DQC ≥ 0.85, and call rate ≥ 97 %. The percent of

passing samples was ≥ 95, and the average call rate for passing samples was ≥ 98.5. To assess the existence of stratification and to identify kinship relationships, PCA and IBD analyses were implemented with PLINK software. After that, genotyped data was imputed with TOPMED imputation software. Minor allele frequency (MAF) or Hardy-Weinberg equilibrium (HWE) thresholds were not implemented because of the possibility of losing selected probes from the PRSs databases.

Four different PRSs datasets were computed based upon previous publications. Details for each PRS are shown on table I.

Statistical tests were performed with the R software (version 4.2.1). Moreover, ROC curves were generated with the "pROC" package (10).

RESULTS

Three of the 4 PRSs showed significantly higher scores in the osteoporosis group compared to the control group (PRS-1 p = 1e-7; PRS-2 p = 1.87e-15; PRS-3 p = 0.1477; PRS-4 p = 8.98e-9). Moreover, in those three PRSs with significant differences the individuals from the risk quartile (which corresponds to the first quartile for PRS-2 and upper quartile for PRS-1 and PRS-4) had a significantly higher risk of osteoporosis compared to those individuals from the other quartiles (PRS-1 OR, 1.83 [CI, 1.41-2.36]; PRS-2 OR, 2.11 [CI, 1.64-2.71]; PRS-3 OR, 0.96 [CI, 0.72-1.27]; PRS-4 OR, 1.72 [CI, 1.32-2.21]) (Fig. 1).

Furthermore, the frequencies and the probability of disease are shown on figure 2. PRS-1 and PRS-4 have

Table I. Details of the published PRSs used in this article

	Associated phenotype	Probes	Merged probes	Ancestry	Reference
PRS_1	Femur neck BMD	63	63	Mainly European and partly East Asian	Estrada K, et al. Nat Genet 2012;44:491-501
PRS_2	Heel quantitative speed of sound (SOS)	21716	15 721	Predominantly white British for training, testing and validation sets	Forgetta V, et al. PLOS Medicine 2020;17(7):e1003152
PRS_3	Osteoporosis	316	273	White British for training, testing and validation sets.	Tanigawa Y, et al. PLoS Genet 2022;18(3):e1010105
PRS_4	Osteoporosis without fracture	1270	1136		

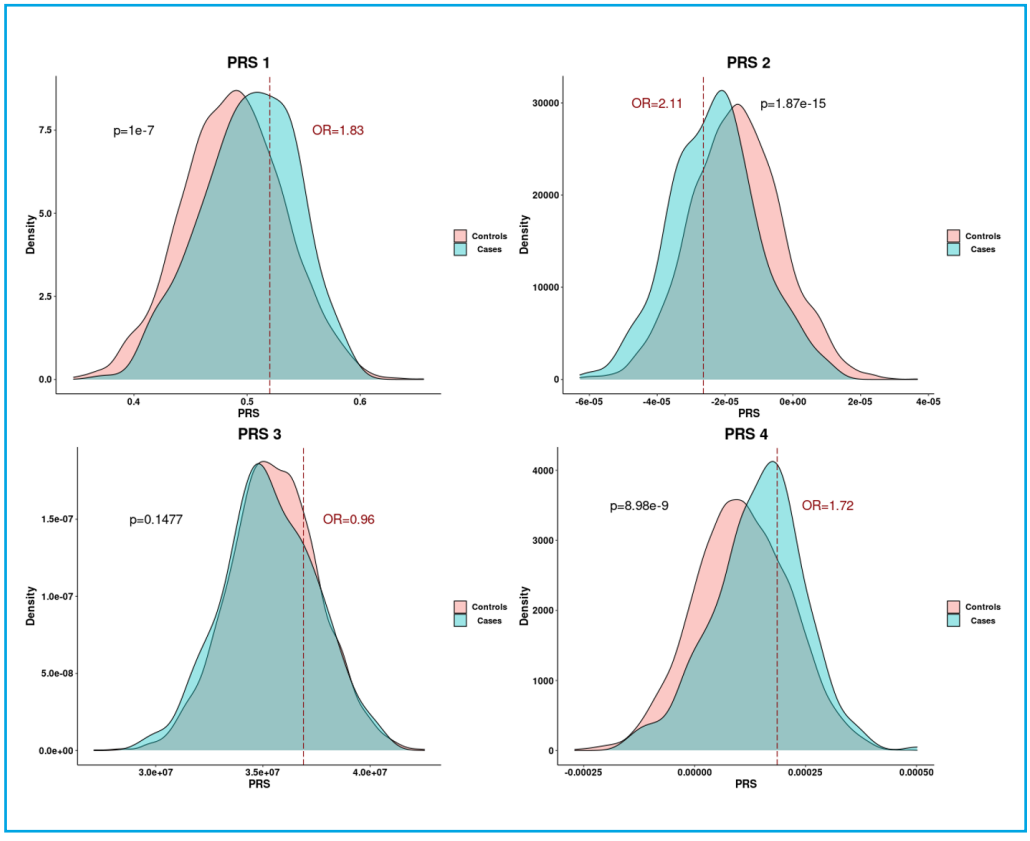

Figure 1. Density plots with osteoporotic cases in blue and controls in orange for each PRS tested in the manuscript. The p value is estimated using Student t tests to look for the differences in the mean of each group. Vertical red dot line limits the upper quartile (first quartile for PRS-2), which should have a higher risk of cases. The quartiles are calculated with all samples together, not by group. Moreover, Odds ratio (OR) are estimated from the quartile selected vs the other quartiles.

an ascending curve because the higher the score the higher the risk of osteoporosis. PRS-2 values are inversely correlated to osteoporosis because it is associated with heel quantitative speed of sound (SOS) data, which are translated into a higher risk when the scores are more negative. PRS-3 has an almost horizontal regression line due to the non-significant association.

The studied PRSs showed a moderate discriminative capacity, as evidenced by the areas under ROC curves (PRS-1 AUC, 0.645; PRS-2 AUC, 0.61; PRS-3 AUC, 0.526; PRS-4 AUC, 0.625) (Fig. 3).

DISCUSSION

In the present study, we tested four previously published PRSs derived from high-powered GWAS of various osteoporosis-related traits. By comparing the results in a group of patients with osteoporosis and in the control Spanish population, we demonstrate that three of the four PRSs tested are significantly associated with osteoporosis. So, our findings confirm that genetic profiling may help in the identification of osteoporosis, although their discriminative capacity is only moderate and their clinical relevance is still to be demonstrated.

The seminal study conducted by Estrada et al. back in 2012 was the largest GWAS on osteoporosis to that date and identified a total of 63 genetic variants associated with femur neck BMD. They showed that their genetic score predicted the risk of osteoporosis (1.56 odds for osteoporosis of women in the highest bin). The prediction ability was small, with an area under the curve (AUC) of 0.59 with the genetic score alone for osteoporosis (4). In the present study the odds ratio was 1.83 for osteoporosis in the last quartile with an AUC of 0.645 with the genetic score alone. Thus, these results appeared to be somewhat better compared to those from the original study. Hence, this predictive model might be of interest for the Spanish population.

More recently, Forgetta et al. created a PRS model trained with LASSO including 21 717 from a total of 345 111 SNPs significantly associated with ultrasound speed of sound (SOS), which decreased the number of people requiring CRF-FRAX and BMD-FRAX assessments (7). The prediction model, known as gSOS, can improve fracture risk prediction. Thus, a lower gSOS that is related to a lower SOS, was associated to a higher rate of major osteoporotic and hip fractures in European populations (8). The authors showed that the population in the quartile with the lowest gSOS had an odds ratio of 1.68 for major osteoporotic fracture risk and 1.57 for hip fracture. They also demonstrated that gSOS predicts major osteoporotic fracture and hip fracture with an AUC of 0.734 and 0.798,

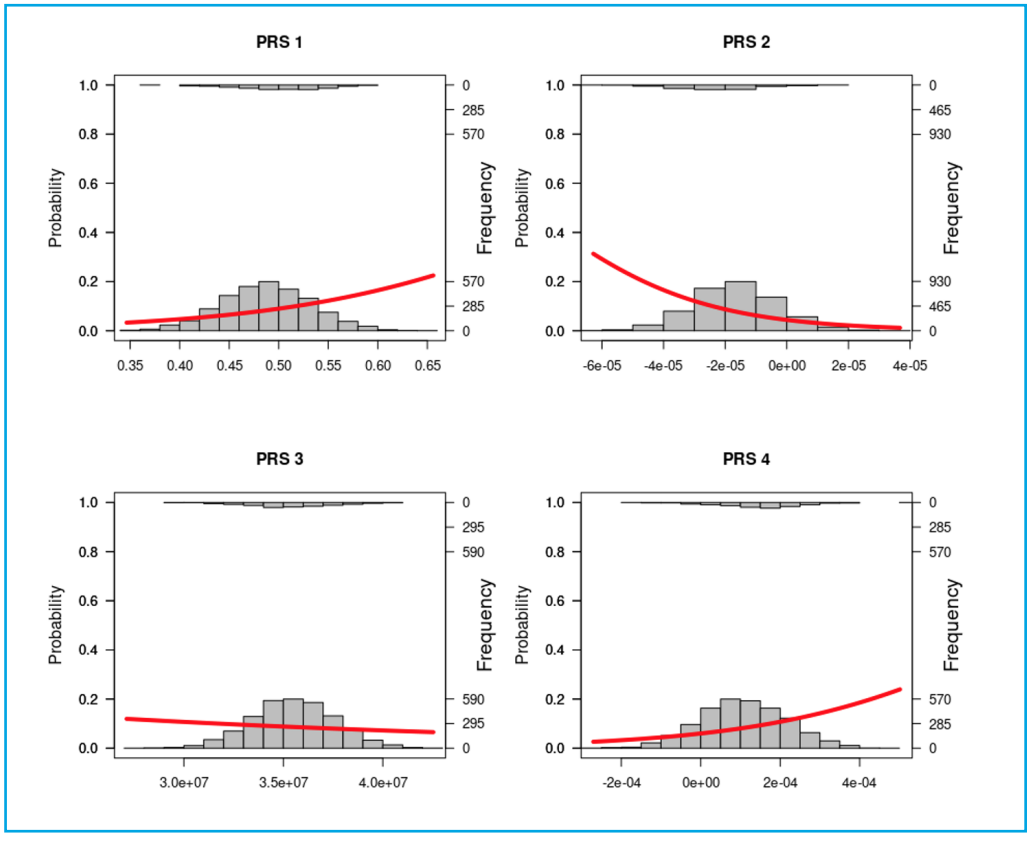

Figure 2. Frequency and linear logistic plots with the controls as zero (the bottom) and the cases as one (the top) for each PRS tested in the manuscript. Left axis is the probability of being control or cases according to the linear logistic regression. Whereas, the right axis shows the frequency of each group in a score range. The X axis scores corresponds to the PRS obtained.

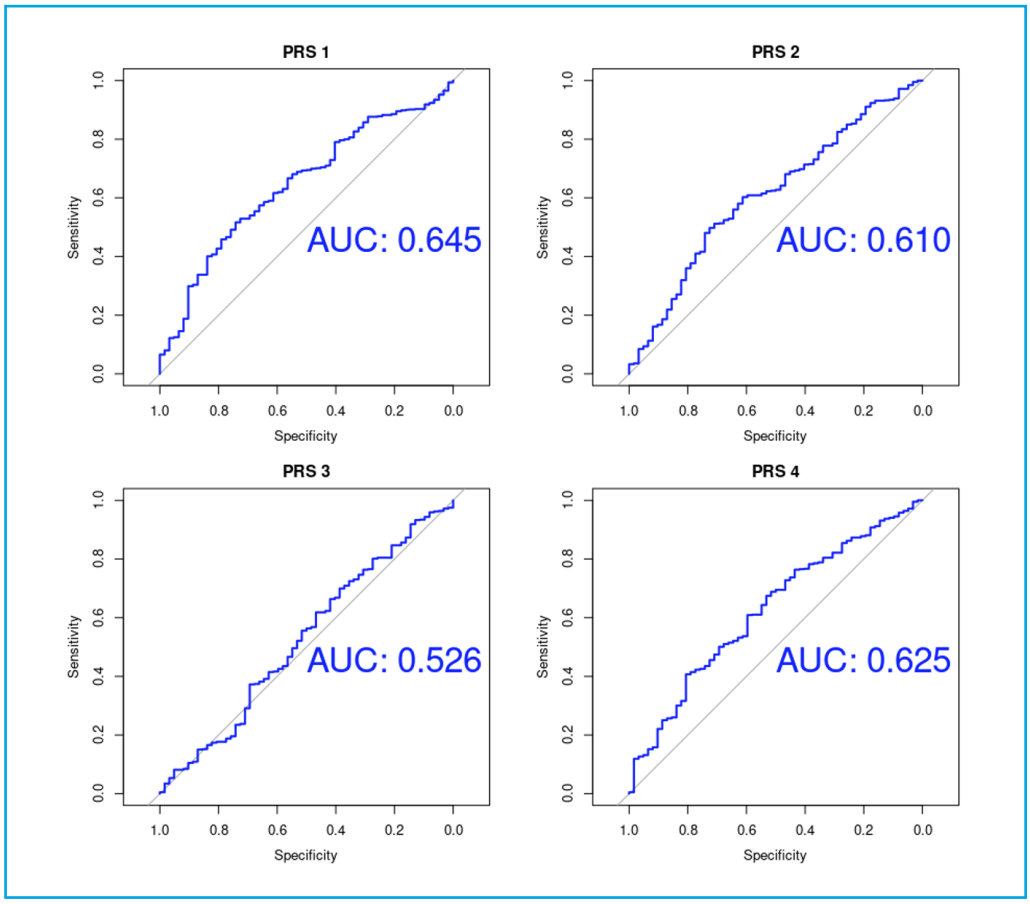

Figure 3. ROC curves for each PRS tested in the manuscript. Area under the curve (AUC) is shown in the center of each plot.

respectively. In our data, people with PRS score in the first quartile had a 2.11-fold risk of osteoporosis and an AUC of 0.61. The AUC is not as high as the one from the reference article. However, we could not use the fractures as a dependent variable because we did not have those data. Hence, our comparisons were not as clean as theirs in terms of the dependent variable.

The UK Biobank project is a noteworthy program that permits to study GWAS data with different phenotypes in a large number of individuals from the UK overall population (11). Thanks to these genetic and phenotypic data, various PRSs have been created. A study conducted by Rivas et al. proposed up to 813 PRSs models to predict over 1500 traits with genetic and phenotypic data from the UK Biobank including 2 PRSs models related to osteoporosis identified as PGS001273 and PGS001274 in the Polygenic Score Catalog (www.pgscatalog.org). Both have been trained with data from more than 260 000 individuals with European ancestry. The first one has been related to 'osteoporosis' (*n* = 316 SNPs) whereas the second one is associated with 'osteoporosis without pathological fracture' (*n* = 1270). Overall, they have found that the size of the PRS model is related to an increased predictive power. Thus, with the score model, they obtained an AUC of 0.629 and 0.718 in PGS001273 and PGS001274, respectively (9). In our own study, PGS001273 did not show a significant association with osteoporosis. However, the largest PRS (PGS001274) was associated with osteoporosis (OR of the first quartile vs other quartiles 1.72), and a predictive power with an AUC of 0.625.

The heterogeneity between PRSs predictions is based on the heterogeneity between GWAS results, which are possibly different due to the use of distinct variables, outcome measurement, and the ancestry of samples (12). That is the reason why combining the results of the PRS tested does not improve the levels of prediction. For the same reasons, prediction models validated in each of these studies are somewhat better compared to ones obtained from our own data except for the study conducted by Estrada. This might also be associated with the fact that BMD was used as an outcome measure in Estrada's report and in the present study whereas calcaneal ultrasound was used in the UK Biobank-derived studies. Nevertheless, the predictive ability of the PRSs is low and, nowadays, they must be used as a complement for diagnosis with other clinical parameters such as FRAX scores.

This study has several limitations. We included a well-characterized group of individuals with primary osteoporosis of Spanish ancestry. However, our con-

trol group obtained from the Spanish DNA biobank is well characterized according to the ancestry, sex and age. However, there are not data regarding clinical bone factors. As a matter of fact, some of them might present osteoporosis, which would decrease the study power. Also, the limited sample size, particularly of the patients' group, limit the statistical power of the study. This, and the lack of data about the controls, precluded the adjustment of the genetic associations by some relevant clinical factors.

In conclusion, several PRSs show significant differences between the overall Spanish population and patients with osteoporosis. This result supports the concept that PRSs may help identify individuals at risk of osteoporosis. Their exact role alone and in combination with other clinical factors remains to be elucidated.

REFERENCES

1. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet 2018;50(9):1219-24. DOI: 10.1038/s41588-018-0183-z
2. Nguyen TV, Eisman JA. Post-GWAS Polygenic Risk Score: Utility and Challenges. JBMR Plus 2020;4(11):e10411. DOI: 10.1002/jbm4.10411
3. Uffelmann E, Huang QQ, Munung NS, de Vries J, Okada Y, Martin AR, et al. Genome-wide association studies. Nat Rev Methods Primer 2021;1(1):59. DOI: 10.1038/s43586-021-00056-9
4. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet 2012;44(5):491-501. DOI: 10.1038/ng.2249
5. Morris JA, Kemp JP, Youlten SE, Laurent L, Logan JG, Chai RC, et al. An atlas of genetic influences on osteoporosis in humans and mice. Nat Genet. 2019;51(2):258-66. DOI: 10.1038/s41588-018-0302-x
6. Choi SW, Mak TS, O'Reilly PF. Tutorial: a guide to performing polygenic risk score analyses. Nat Protoc. 2020;15(9):2759-72. DOI: 10.1038/s41596-020-0353-1
7. Forgetta V, Keller-Baruch J, Forest M, Durand A, Bhatnagar S, Kemp JP, et al. Development of a polygenic risk score to improve screening for fracture risk: A genetic risk prediction study. PLoS Med 2020;17(7):e1003152. DOI: 10.1371/journal.pmed.1003152
8. Lu T, Forgetta V, Keller-Baruch J, Nethander M, Bennett D, Forest M, et al. Improved prediction of fracture risk leveraging a genome-wide polygenic risk score. Genome Med 2021;13(1):16. DOI: 10.1186/s13073-021-00838-6
9. Tanigawa Y, Qian J, Venkataraman G, Justesen JM, Li R, Tibshirani R, et al. Significant sparse polygenic risk scores across 813 traits in UK Biobank. PLoS Genet 2022;18(3):e1010105. DOI: 10.1371/journal.pgen.1010105
10. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12(1):77. DOI: 10.1186/1471-2105-12-77
11. Collins R. What makes UK Biobank special? Lancet Lond Engl 2012;379(9822):1173-4. DOI: 10.1016/S0140-6736(12)60404-8
12. Yang TL, Shen H, Liu A, Dong SS, Zhang L, Deng FY, et al. A road map for understanding molecular and genetic determinants of osteoporosis. Nat Rev Endocrinol 2020;16(2):91-103. DOI: 10.1038/s41574-019-0282-7

ARÁN

ROMM
Revista de Osteoporosis
y Metabolismo Mineral

Original

Follow-up and compliance to anti-osteoporotic treatment from nursing in a fracture liaison service

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Abstract

Introduction: compliance to anti-osteoporotic treatment is essential for the effectiveness of medications in clinical practice and is a priority objective for fracture liaison services (FLS).

Objectives: to describe the follow-up and compliance to treatment of patients assisted by our FLS and identify the reasons for follow-up discontinuation.

Material and methods: this is a descriptive, retrospective, and cross-sectional study of patients aged > 50 years with osteoporotic fractures treated in an FLS from 2016 through 2020. A descriptive statistical analysis of the variables collected was conducted using the SPSS software.

Results: the sample included 1280 patients; 86.2 % were women and 13.8 % were men, 26.7 % of whom had received prior anti-osteoporotic treatment. After inclusion in the FLS, there was an increase of 59.6 % in patients who were started on anti-osteoporotic treatment and a 42.6 % increase in supplementation. A total of 4 different follow-up visits were conducted (at 5.4 months, 14.5 months, 24.3 months, and 33.8 months) with good compliance to treatment at around 72.1 %, 80.6 %, 83.1 %, and 83.7 %, respectively, and compliance to supplements at around 90.1 %, 90 %, 88.2 %, and 87.1 %, respectively. The reasons for follow-up discontinuation were completion of the follow-up program (21.48 %), death (11.02 %), transfer of follow-up to primary care (9.53 %), patient's decision (6.48 %), medical decision (3.83 %), treatment not indicated (3.13 %), and inability to continue follow-up (2.73 %).

Conclusions: the inclusion of these patients in an FLS shows a high percentage of good compliance and improves the percentage of patients with osteoporotic fractures who are started on treatment. The most common reason for follow-up discontinuation was continuation of care by primary care physicians.

Keywords:
Fracture liaison service. Fragility fracture. Medication compliance. Osteoporosis treatment. Case manager.

Received: 10/10/2022 • Accepted: 22/05/2023

Conflict of interest: the authors Leticia Cebollada Gadea, Raquel Laguna Rodrigo, Manuel Jordán Jarque, and Rafael Izquierdo Aviñó declared no conflicts of interest associated with the publication of this manuscript. They also declared to have observed the principles established by the Declaration of Helsinki on clinical trials.

Cebollada Gadea L, Laguna Rodrigo R, Jordán Jarque M, Izquierdo Aviñó R. Follow-up and compliance to anti-osteoporotic treatment from nursing in a fracture liaison service. Rev Osteoporos Metab Miner 2023;15(2):72-80

DOI: 10.20960/RevOsteoporosMetabMiner.00017

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INTRODUCTION

The World Health Organization (WHO) defines osteoporosis (OP) as a chronic disease characterized by low bone mass and deterioration of the microarchitecture of the bone tissue, leading to increased bone weakness and an increased risk of fractures (1,2).

The prevalence of osteoporosis is increasing due to the progressive aging of the population, and it is estimated to cause 9 million fractures worldwide annually, making up a serious public health problem with significant medical, social, and economic impact (1-5). It is known as a silent disease because it often progresses asymptomatically, and its first clinical or sentinel sign is often a fragility fracture (1-4,6,7).

Fragility fracture (FF) is defined as a fracture that occurs without trauma or with low-energy trauma, such as a fall from a height corresponding to standing height or less (1,8). It is estimated that approximately one in three women and one in five men over the age of 50 will experience, at least, one fragility fracture in their lifetime (1,6,8).

The most predictive factor for a FF is the presence of a previous fracture as it increases the risk of subsequent fractures or re-fractures within the next two years (9,10). This increased risk is known as imminent risk of fracture and can trigger what experts call a cascade of fractures (6,8-11).

Despite the wide range of anti-osteoporotic treatments (AOT), available there is a treatment gap, defined as the percentage of eligible individuals who do not receive osteoporosis medication (12,13). According to various studies published, it is estimated that between 63 % and 80 % of the individuals with fragility fractures do not receive any form of treatment, indicative that osteoporosis is possibly both underdiagnosed and undertreated (6,8,13-16).

In addition to the treatment gap, a serious problem is therapeutic compliance, which is clearly observed with the use of oral bisphosphonates, the most commonly prescribed pharmacological drugs these days (6,7,10,17-19).

Treatment compliance refers to adherence and is of great importance for the effectiveness of drugs in clinical practice (2,18,20,21). Factors affecting compliance are diverse, complex, and multidimensional being some associated with treatment itself and its administration schedule, and others with the patients' cognitive status and knowledge (2,14,18,20,21).

Therefore, in response to the urgent need for improving this situation, the International Osteoporosis Foundation (IOF) recommended the implementation, whenever possible, of Fracture Liaison Services (FLS) as a global strategy for secondary fracture prevention. However, according to the IOF, FLS are available in < 10 % of hospitals in Spain (1,11,22).

The FLS model has become increasingly common, and there are various types of FLSs based on the care model used: types A, B, C, and D. Type A represents a coordinated approach to secondary fracture prevention with a central coordinator who identifies, investigates, and initiates treatment, and a follow-up program for patients included in the FLS (6,13,23,24). This approach begins with the identification of patients > 50 years of age with a recent FF followed by the evaluation of clinical risk factors for future fractures, possible causes of secondary osteoporosis, initiation of treatment, and appropriate long-term follow-up to improve compliance to anti-osteoporotic therapy (3,11,12,25).

Several studies have demonstrated the effectiveness and efficacy of FLS in various aspects of FF management including patient identification and increased treatment initiation rates (38 % vs 17.2 %), and improved compliance after sustaining a fracture (57 % vs 34.1 %), thus reducing the risk of new fractures (3,6,8,11,24,26).

The objective of this study is to describe the follow-up and compliance to anti-osteoporotic treatment and supplements in our FLS, and identify the reasons for follow-up discontinuation. Additionally, the study aims to determine the percentage of patients who initiate treatment.

MATERIAL AND METHODS

This study was approved by the Aragón Ethical Committee for Scientific Research (CEICA) of the Government of Aragon, Spain that issued a favorable opinion back in September 28, 2016, and all patients included received an information sheet and signed a written informed consent form.

A descriptive, retrospective, and cross-sectional study was conducted on patients treated at the Fracture Liaison Service (FLS) of Hospital Provincial Nuestra Señora de Gracia in Zaragoza, Aragón (Spain) since the establishment of this unit since November 1, 2016 up until December 31, 2020.

Our FLS is a type A model, operational since November 2016, that consists of a specialized medical coordinator in Traumatology, a specialist in Geriatrics and Gerontology, a case manager nurse, and a nursing care and administrative tasks technician.

The inclusion criteria in our FLS are: patients ≥ 50 years of age from Health Sector I, from Zaragoza and with any of the following diagnostic categories according to ICD-9-CM (International Classification of Diseases):

vertebral fracture (805 and 806), pelvic fracture (808), proximal humerus fracture (812), distal radius and ulna fracture (813), and femoral neck fracture (820, 821). All patients are identified through the emergency department care registry and invited to a weekly consultation where they are offered voluntary inclusion in the unit.

Our follow-up protocol includes phone or in-person follow-ups for, at least, 2 years after the initiation of treatment (at 6 months, 1 year, and 2 years after treatment initiation). Various factors such as the patients' disease progression, therapeutic changes, and detection of fractures at follow-up impact the duration of personalized follow-up. For this study, all the follow-ups conducted on the selected sample were reviewed on 4 different occasions during the 3 years following their inclusion, and sometimes for longer periods, thus verifying therapeutic compliance to AOT and/or supplements administered.

This follow-up was mainly conducted by the case manager nurse, either in person or over the phone. To evaluate compliance to AOT and supplements during different follow-ups, a questionnaire was used that classified compliance as good if medication was used > 80 % of the time, fair if used between 50 % and 80 %, and poor if used < 50 % of the time. Additionally, the tolerance or intolerance to both AOT and supplements was recorded.

An important aspect of the follow-up was patient education and awareness provided by the case manager nurse through detailed written information in the clinical report at the initiation of treatment and during in-person follow-ups, as well as verbally during phone follow-ups. The importance of treatment regarding the risk of sustaining a new fracture and its implications for the patients' quality of life and independence was stressed out as well.

From an initial sample of 1398 patients included during the study period, the final sample of the study included a total of 1280 patients with detailed explanations of the exclusions made in figure 1.

A database was created in the SPSS program for demographic, clinical, initiation, follow-up, and compliance variables associated with anti-osteoporotic treatment and/or supplements, as well as the causes for follow-up discontinuation and risk of fracture. These variables were used for a descriptive analysis.

The variables analyzed in our study include demographic variables (gender, age, and mortality), general clinical variables (height, weight, body mass index), relevant past medical history (treatment with glucocorticoids over the past 6 months and organic diseases that may decrease bone mineral density or other risk factors associated with falling). For fracture risk assessment with the FRAX® tool, the guide-

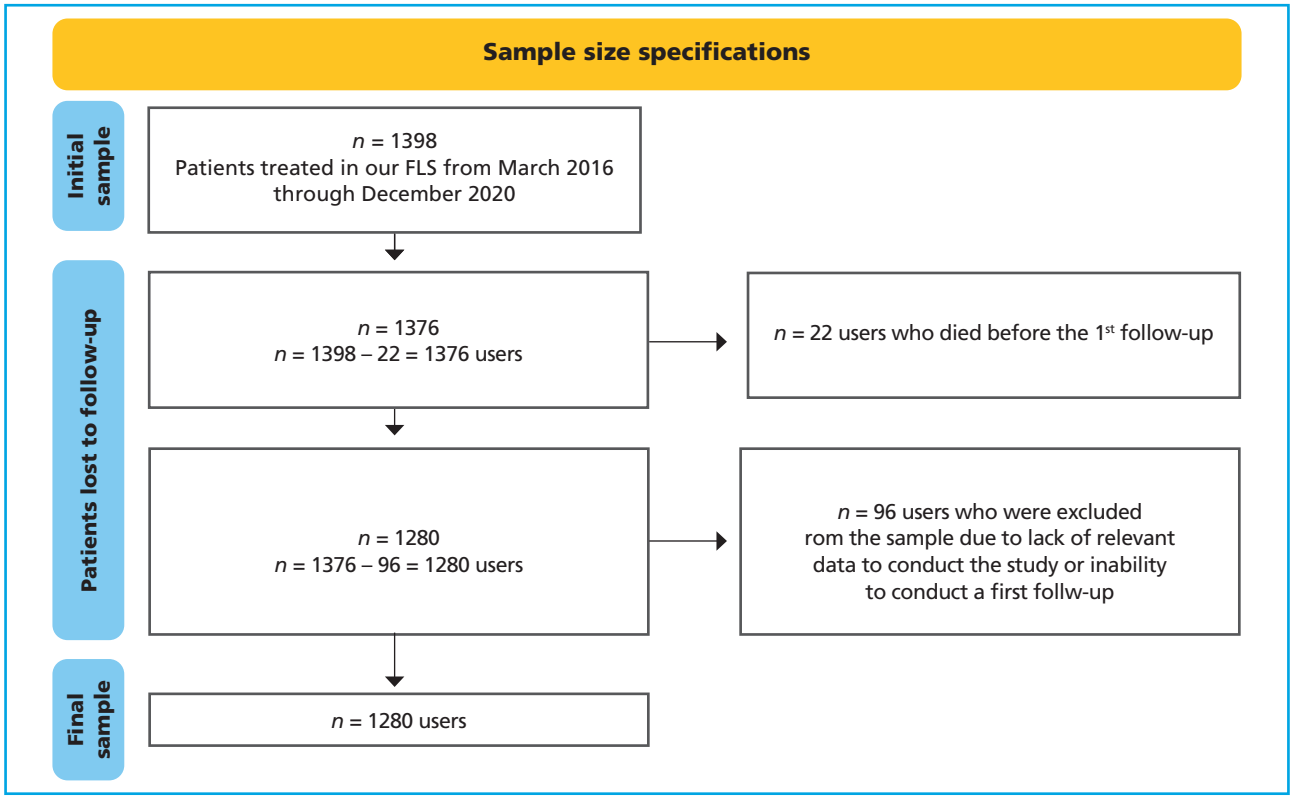

Figure 1. Specifications of sample size variations.

lines published by Azagra et al. (27) were followed, which adjusted the FRAX® for a Barcelona population, describing thresholds that stratify the risk of major fracture as < 5 % for low risk, ≥ 5 % and < 7.5 % for intermediate risk, and ≥ 7.5 % for high risk of fracture (23,27). The number of falls within the year prior to the index fracture, the location of index fractures, and the presence of previous fractures were documented.

Regarding the variable of supplements prescribed in the FLS, the milligrams of calcium used in the diet were estimated using a calcium intake calculator.

Compliance and tolerance variables to AOT and supplements were collected in the 4 follow-ups conducted. Finally, the variable of causes of follow-up discontinuation was analyzed including compliance of the FLS program, follow-up by primary care physician (referral of follow-up to the patient's primary care physician for various reasons like patient preference, initiation of supplements only, etc.), inability to conduct the follow-ups (inability to contact the patient during the follow-ups scheduled), medical decision (when discontinuation was indicated by a clinician assisting the patient, whether the FLS coordinator or a different healthcare professional assisting the patient for other reasons), mortality, treatment discontinuation by patient decision, and treatment not indicated.

Quantitative variables were analyzed as mean ± standard deviation, while the qualitative ones were analyzed as absolute frequency and percentage. A univariate descriptive statistical analysis was performed using the "SPSS Statistics" version 22 for Mac, with statistical significance set at p values < 0.05.

RESULTS

Out of the 1280 patients selected for our study, 1103 (86.2 %, 95 %CI, 84.16–88.02) were women and 177 (13.8 %, 95 %CI, 11.98-15.84) were men, with a mean age of 82.1 ± 9.9 years (Kolmogorov-Smirnov test for normality; p < 0.05.). The mean age in men was 84.3 ± 8.3 years, and 81.7 ± 10.2 years in women.

Table I shows a descriptive analysis of the clinical variables studied.

All patients in our sample had at least one index fracture, which was a prerequisite to be included in the FLS. Additionally, 6.3 % had two index fractures, and 1.9 % three index fractures during recruitment. Furthermore, 39.1 % had previously sustained some type of prior fracture before the index fracture. Among patients with a past medical history of fracture prior to the index fracture, 30.5 % had sustained a major fracture (5.3 %, hip; 5.2 %, humerus; 7.7 %, wrist; 12.1 % vertebra) while the remaining 8.5 % had sustained fractures in different locations. Regarding previous falls including the fall that led to their referral to our FLS, the mean number of falls in the year prior to inclusion was 1.7.

Regarding treatment, only 26.7 % of the patients had received AOT and 34.8 % supplements prior to inclusion. After evaluation in the FLS, 83.3 % received AOT, and 77.4 % supplements. The most prescribed drug after inclusion in the FLS was alendronate (44.9 %) followed by denosumab (40.6 %), risedronate (6.8 %), teriparatide (7.6 %), and IV zoledronic acid (0.1 %). Combined calcium and vitamin D supplements were prescribed to 63.1 % of the patients, and vitamin D alone was prescribed to 36.9 % of the patients.

In our study, the mean time elapsed from treatment initiation to the first follow-up was 5.4 ± 4.8 months. The mean time from treatment initiation to the second follow-up was 14.5 ± 7.4 months, to the third follow-up was 24.3 ± 8.9 months, and to the fourth follow-up was 33.8 ± 14.0 months.

Regarding follow-up continuity, out of the 1280 FLS users who received the first follow-up, 933 (72.9 %) had a second follow-up, 551 (43 %) had a third follow-up, and 209 (16.3 %) a fourth follow-up.

Table II shows the type of follow-up conducted at each control, and compliance to TAO and supplements, and tolerance to both in each individual follow-up. A good compliance to TAO was seen during the 1st, 2nd, 3rd, and 4th follow-ups with rates of 72.1 %, 80.6 %, 83.1 %, and 83.7 %, respectively. Regarding supplements, proper compliance was seen in the during the 1st, 2nd, 3rd, and 4th follow-ups with rates of 90.1 %, 90 %, 88.2 %, and 87.1 %, respectively. Regarding tolerance, the figures obtained for TAO exceeded 88 % in all follow-ups except the 1st one (77.4 %); regarding supplements, tolerance was > 94 % in all the follow-ups conducted.

At the time of data collection, 41.8 % of the patients were still included in the follow-up program while 58.2 % had completed the follow-up. Regarding the reasons for ending the follow-up, the most common reason was compliance with the FLS follow-up program (21.48 %) followed by death (11.02 %), follow-up by the patient's primary care physician (9.53 %), patient's decision (6.48 %), medical decision (3.83 %), treatment not indicated (3.13 %), and the impossibility to contact the patient for follow-up reason (2.73 %). The percentage distribution of the reasons for ending the follow-up is shown on figure 2.

Table I. Clinical variables. sites of index fractures #1 and #2, and presence of prior fractures before inclusion in the FLS

	n % 1280	%	95 %CI
Height (n = 1181)	Mean: 1.6 m ± 0.1		
Weight (n = 1180)	Mean: 66.7 kg ± 12.4		
BMI (n = 1180)	Mean: 27.2 ± 4.8		
BMI according to WHO (n = 1259)			
Low weight	21	1.7%	1.0-2.5
Normal weight	423	33.6 %	31.0-36.3
Overweight	492	39.1 %	36.4-41.8
Grade I obesity	241	19.1 %	17.0-21.4
Grade II obesity	67	5.3 %	4.2-6.7
Grade III obesity	15	1.2 %	0.7-2.0
Past medical history (relevant)			
Yes	912	71.3 %	68.7-73.7
No	368	28.7 %	26.3-31.3
Death (during the study period)			
Yes	141	11 %	9.4-12.9
No	1139	89 %	87.1-90.8
Risk if FRAX fracture (n = 1058)			
Low	54	5.1 %	3.9-6.6
Intermediate	140	13.2 %	11.3-15.4
High	864	81.7 %	79.2-84.0
Falls prior to index fracture	*Mean: 1.7 falls within the year prior to the index fracture*		
Yes	1230	96.1 %	9-98.0
No	50	3.9 %	2.9-5.1
Site of index fracture #1			
Hip	570	44.5 %	41.8-47.3
Humerus	175	13.7 %	11.8-15.7
Wrist/Radius	246	19.2 %	17.1-21.5
Vertebra	229	17.9 %	15.8-20.1
Other	60	4.7 %	3.6-6.0
Site of index fracture #2			
Hip	1	0.1 %	0.0-0.4
Humerus	11	0.9 %	0.4-1.5
Wrist/Radius	10	0.8 %	0.4-1.4
Vertebra	2	0.2 %	0.0-0.6
Other No	28	2.2 %	1.5-3.2
	1228	95.9 %	94.7-97.0
Existence of previous fracture			
Yes	500	39.1 %	37.9-41.3
No	780	60.9 %	58.2-63.6

BMI: body mass index; Fx: fracture; FLS: fracture liaison service; WHO: World Health Organization.

Table II. Compliance and tolerance of AOT and supplements at the follow-ups

	Follow-up #1 (n = 1280)	Follow-up #2 (n = 933)	Follow-up #3 (n = 551)	Follow-up #4 (n = 209)
Type of follow-up				
On-site	867 (67.7 %)	324 (34.7 %)	104 (18.9 %)	28 (13.4 %)
Phone call	413 (32.3 %)	609 (65.3 %)	447 (81.1 %)	181 (86.6 %)
Compliance to AOT				
Good	923 (72.1 %)	752 (80.6 %)	458 (83.1 %)	174 (83.7 %)
Regular	18 (1.4 %)	20 (2.1 %)	8 (1.5 %)	4 (1.9 %)
Poor	82 (6.4 %)	73 (7.8 %)	55 (10 %)	21 (10.1 %)
Not applicable	257 (20.1 %)	88 (9.4 %)	30 (5.4 %)	9 (4.3 %)
Tolerance to AOT				
Yes	991 (77.4 %)	824 (88.3 %)	509 (92.4 %)	195 (93.3 %)
No	32 (2.5 %)	21 (2.3 %)	12 (2.2 %)	5 (2.4 %)
Not applicable	257 (20.1 %)	88 (9.4 %)	30 (5.4 %)	9 (4.3 %)
Compliance to supplements				
Good	1153 (90.1 %)	840 (90 %)	486 (88.2 %)	182 (87.1 %)
Regular	19 (1.5 %)	19 (2 %)	5 (0.9 %)	4 (1.9 %)
Poor	69 (5.4 %)	53 (5.7 %)	48 (8.7 %)	13 (6.2 %)
Not applicable	39 (3 %)	21 (2.3 %)	12 (2.2 %)	10 (4.8 %)
Tolerance to supplements				
Yes	1219 (95.2 %)	896 (96 %)	529 (96 %)	198 (94.7 %)
No	22 (1.7 %)	16 (1.7 %)	10 (1.8 %)	1 (0.5 %)
Not applicable	39 (3 %)	21 (2.3 %)	12 (2.2 %)	10 (4.8 %)

AOT: anti-osteoporotic treatment.

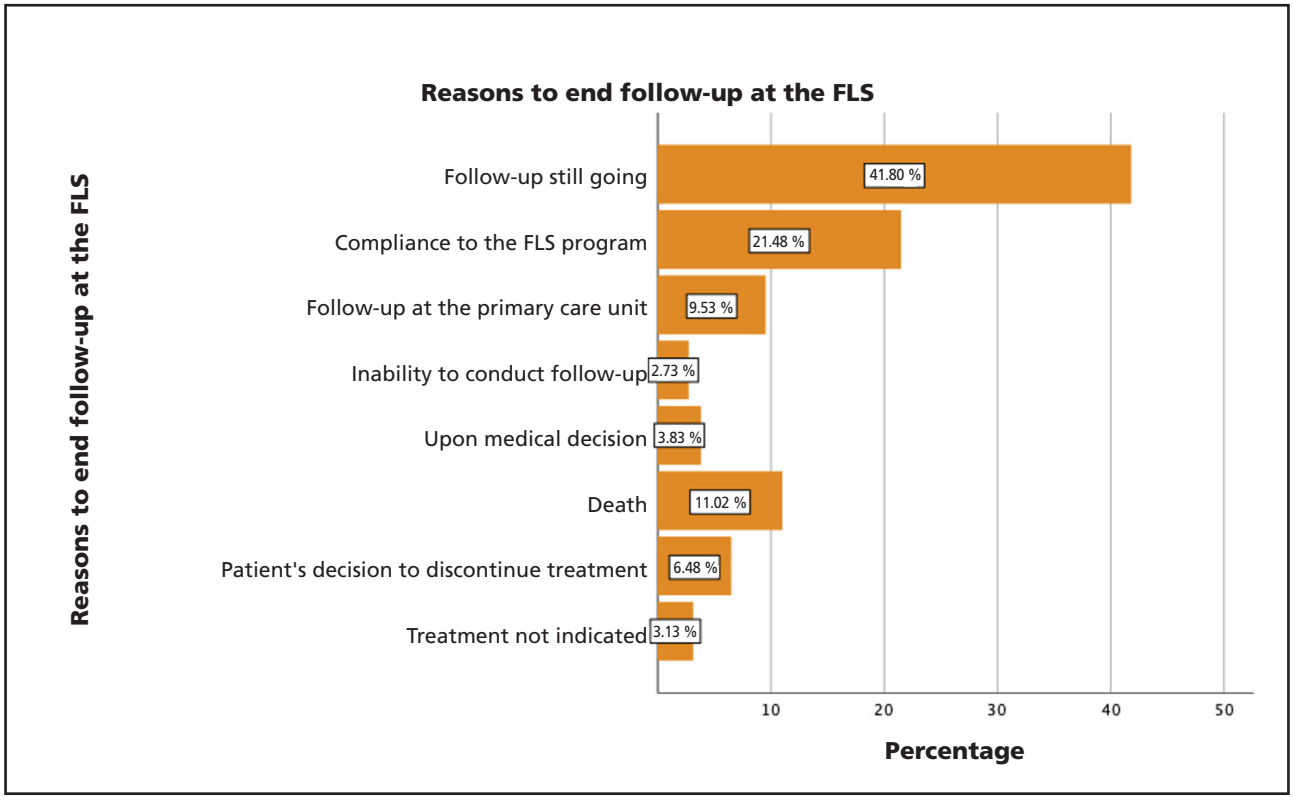

Figure 2. Causes to end of follow-up among users treated at the FLS (in percentages) (FLS: fracture liaison service).

DISCUSSION

This study reports the experience gained from implementing an FLS in our hospital to determine the basic profile of patients treated in the unit and shows data on follow-up and compliance to anti-osteoporotic treatment and calcium and vitamin D supplements.

Regarding the presence of previous fractures before the index fracture as the reason why patients are enrolled in our FLS, 39.1 % had sustained a previous fracture (in some cases, up to 3 previous fractures), which is higher compared the findings reported by Ojeda (23), where only 19 % of users had sustained a previous fracture before.

We should mention that the mean BMI of our sample was 27.21 kg/m^2 similar to the results obtained by Azagra et al. (27). Furthermore, only 1.7 % of our patients had BMIs < 18.5 kg/m^2, which is similar to the figures provided by the study conducted by Naranjo et al[28] who found a 1.4 % rate in that BMI range. In our case, 64.7 % of patients were overweight or obese.

A total of 81.7 % of the sample had a high risk of fracture, which is consistent with the profile of patients treated in an FLS who have already sustained, at least, 1 fracture. This stresses the importance of previous fracture when it comes to determining the risk of future fractures and reinforces the importance of initiating anti-osteoporotic treatment as soon as possible. These are conclusions also reached in the studies conducted by Walters et al. (6), De Bruin et al. (12), Borgström et al. (13), and Wu et al. (11).

Therefore, the profile of patient who is often examined in our unit would is that of an approximately 82-year-old woman who has sustained an osteoporotic index fracture, who is overweight, and who has relevant past medical history of osteoporosis with a high risk of fracture according to FRAX and is not on anti-osteoporotic treatment despite nearly 40 % have sustained a previous fracture before the index fracture.

Our study shows a high percentage of hip fractures as index fractures (44.5 %). This could be due, on the one hand, to the presence of an orthogeriatric unit at the hospital where our FLS is located, with which we work collaboratively for secondary fracture prevention. On the other hand, it could be a common finding in most FLSs that capturing hip fractures is easier since these patients require hospital admission. Our study shows a high number of hip fractures as the index fracture. In our case, within the first year of unit activity, the focus of patient recruitment was almost exclusively on hip fractures. Our results differ from former studies that present different FLS models and rates of patient recruitment. For example, in the study conducted by Luc et al. (8), they only had 9 % of the patients with hip index fractures, and in the study conducted by Borrgström et al. (13) they obtained only 19.6 % among their participants.

The mean number of falls sustained by our patients in the year prior to inclusion in the FLS (including the one that caused the index fracture) was 1.7. We believe that this data greatly underestimates the actual number of previous falls since it is collected retrospectively from the patients themselves or their relatives/caregivers who often do not remember falls that did not have the clinical significance of a fracture.

Regarding the type of treatment initiated, a high percentage of patients received alendronate (44.9 %), which is consistent with the national clinical guidelines during that period of time, and with the study conducted by Walters et al. (6). The high percentage of patients who received denosumab (40.6 %) is consistent with the fact that a high percentage of patients are very old, have a high prevalence of hip fractures, are on multiple drugs, and have significant comorbidities. Additionally, in our FLS, IV zoledronic acid was not administered except in some exceptional cases.

On the other hand, studies such as those conducted by Gómez-Navarro et al. (4), Walters et al. (6), and Ojeda (23) highlight the high percentage of patients who were not on any pharmacological treatment prior to being included in the FLS. Our study shows similar figures, specifically, 73.3 % of our patients were not on any prior pharmacological treatment. These data are similar compared to those from other studies that assessed treatment gap like the one conducted by Hiligsmann et al. (14) that estimated that treatment gap in 2019 went from 25 % to 95 % in European countries. In addition, according to the IOF report from 2021, treatment gap in high risk patients was a mean 71 %. However, when adjusting for sex, 71.5 % of women and 84.2 % of men were not on prior treatment, data somehow slightly different from the findings reported by Borgström et al. (13) who saw, back in 2020, that treatment gap in Europe was greater in women (73 %) compared men (63 %). Our study highlights a low percentage of patients receiving prior anti-osteoporotic treatment when the index fracture was sustained (26.7 %). However, reliable information regarding the prescription of such treatment in patients with or without a previous fracture could not be obtained to determine if the difference between the two groups was significant.

The data obtained show a higher number of patients receiving anti-osteoporotic treatment and supplements after being assisted at our FLS. Specifically, there was a 59.6 % increase in the use of anti-osteoporotic treatment and a 42.6 % increase in the use of supplements. This increase in the percentage of patients on treatment after FLS enrollment is also found in the results obtained by Axelsson et al. (29).

Regarding the follow-ups conducted, it was found that they reasonably abided by the initial protocol, although time ranges were too wide compared to the mean.

Regarding treatment compliance and supplements evaluated in each follow-up, we believe that the follow-up program of our FLS was crucial to achieve good compliance to anti-osteoporotic treatment (TAO) and supplements. Data on good compliance obtained throughout the entire follow-up period are satisfactory compared to the treatment gap discussed earlier in patients not included in FLS programs. At the first follow-up, good compliance was seen in 72.1 % of the patients, a percentage that went up in subsequent follow-ups. Within the second follow-up, the rate of good compliance to TAO was 80.6 %, within the third follow-up it was 83.1 %, and within the fourth follow-up, it went up to 83.7 %. This progressive increase in compliance over time was also reported by Walters et al. (6). However, these results differ from those found by Ojeda (23) and Naranjo et al. (28) as these studies did not find significant difference in the percentages of good compliance among follow-ups. We should mention that the study sample shows the number of patients included in the FLS from 2016 through 2020. Therefore, a percentage of patients included in 2019 and 2020 would not have reached 2 years since their inclusion and never had a third or fourth follow-up. The lower compliance rate seen within the 1st follow-up compared to the subsequent ones is striking. We do not know the exact reason for this, but it could be that adverse effects and treatment intolerances typically appear at the beginning of treatment, and until the patients are visited (in-person or by phone) at the 1st follow-up and treatment is adjusted, they may not fully comply to it.

Finally, regarding the causes for ending the follow-up in our FLS, the most common cause was completion of the follow-up program (21.48 % of patients), which in our case is typically 2 years, with some exceptions as previously mentioned. The second cause was mortality at the study period (11 % of the cases). Similar findings have been reported by Kanis et al. (26) who found a 15 % mortality rate among their participants. Only 6.48 % of patients discontinued treatment and, therefore, ended their follow-up by their own decision. We believe that this data probably does not reflect the reality as a whole, and the rate of treatment discontinuation is underestimated since the data obtained are self-reported by the patients and may not accurately represent the actual situation. At the end of the follow-up program, patients transition to being monitored by their primary care physician. The limited duration of follow-up in an FLS underscores the importance of a good relationship and communication with primary care units to maintain treatment compliance beyond the temporal scope of an FLS. Despite the optimistic results regarding compliance in our study, we should mention a free limitations. Firstly, it is a retrospective study, which limits the amount and quality of information available. Additionally, the patient sample is treated as a homogeneous group, which is not consistent with reality as different comorbidities, cognitive status, and social support can influence the patients' compliance capabilities. Secondly, regarding the assessment of treatment compliance, we should mention that information comes from the patients themselves and may differ to some extent from reality. In addition, there was no information available on effective drug withdrawal from pharmacies until the implementation of electronic health records within the final months of the study period, thus making it impossible to draw conclusions on long-term treatment persistence. Finally, there was no control group of patients not included in an FLS for comparison purposes.

On the other hand, one of the strengths of the study is the large number of patients included in the sample and each follow-up (despite the progressive loss of patients) with a structured follow-up program for a minimum period of up to 2 years, which allows obtaining relevant data on the impact of an FLS on treatment initiation and compliance.

In conclusion, this study describes the impact of our FLS on the initiation of anti-osteoporotic treatment and compliance to such treatment over the course of follow-up. The inclusion of these patients in an FLS appears to improve the percentage of patients with a previous osteoporotic fracture who receive preventive treatment significantly. In addition, the active follow-up conducted by the case manager nurse could be a determinant factor to improve treatment compliance in the early years after the fracture, thus reducing the risk of re-fracture.

REFERENCES

1. Pennestrì F, Corbetta S, Favero V, Banfi G. Fragility Fracture Prevention-Implementing a Fracture Liaison Service in a High Volume Orthopedic Hospital. Int J Environ Res Public Health 2019;16(24):4902. DOI: 10.3390/ijerph16244902
2. Garcia E, Fuentes JE. Adherencia y cumplimiento al tratamiento de la osteoporosis. Farma Journal 2016;1(2):125-32.
3. Noordin S, Allana S, Masri BA. Establishing a hospital based fracture liaison service to prevent secondary insufficiency fractures. Int J Surg 2018;54(Pt B):328-32. DOI: 10.1016/j.ijsu.2017.09.010
4. Gómez Navarro R, González García P, Martín Hernández C, Castro Sauras A, Valdearcos Enguídanos S. Prevención primaria y secundaria de la fractura de cadera por fragilidad ósea en la población del sector sanitario Teruel. Rev Esp Salud Pública 2017;9.
5. Amphansap T, Stitkitti N, Dumrongwanich P. Evaluation of Police General Hospital's Fracture Liaison Service (PGH's FLS): The first study of a Fracture Liaison Service in Thailand. Osteoporos Sarcopenia 2016;2(4):238-43. DOI: 10.1016/j.afos.2016.09.002.
6. Walters S, Khan T, Ong T, Sahota O. Fracture liaison services: improving outcomes for patients with osteoporosis. Clin Interv Aging 2017;12:117-27. DOI: 10.2147/CIA.S85551
7. Jaleel A, Saag KG, Danila MI. Improving drug adherence in osteoporosis: an update on more recent studies. Ther Adv Musculoskelet Dis 2018;10 (7):141-9. DOI: 10.1177/1759720X18785539

8. Luc M, Corriveau H, Boire G, Filiatrault J, Beaulieu MC, Gaboury I. Patient-Related Factors Associated with Adherence to Recommendations Made by a Fracture Liaison Service: A Mixed-Method Prospective Study. Int J Environ Res Public Health 2018;15(5):944. DOI: 10.3390/ijerph15050944
9. Nakayama A, Major G, Holliday E, Attia J, Bogduk N. Evidence of effectiveness of a fracture liaison service to reduce the re-fracture rate. Osteoporos Int 2016;27(3):873-9. DOI: 10.1007/s00198-015-3443-0
10. Skjødt MK, Khalid S, Ernst M, Rubin KH, Martinez-Laguna D, Delmestri A, et al. Secular trends in the initiation of therapy in secondary fracture prevention in Europe: a multi-national cohort study including data from Denmark, Catalonia, and the United Kingdom. Osteoporos Int 2020;31(8):1535-44. DOI: 10.1007/s00198-020-05358-4
11. Wu CH, Tu ST, Chang YF, Chang DC, Chien JT, Lin CH, et al. Fracture liaison services improve outcomes of patients with osteoporosis-related fractures: A systematic literature review and meta-analysis. Bone 2018;111:92-100. DOI: 10.1016/j.bone.2018.03.018
12. De Bruin IJA, Wyers CE, van den Bergh JPW, Geusens PPMM. Fracture liaison services: do they reduce fracture rates? Ther Adv Musculoskelet Dis 2017;9(7):157-64. DOI: 10.1177/1759720X17706464
13. Borgström F, Karlsson L, Ortsäter G, Norton N, Halbout P, Cooper C, et al. Fragility fractures in Europe: burden, management and opportunities. Arch Osteoporos 2020;15(1):59. DOI: 10.1007/s11657-020-0706-y
14. Hiligsmann M, Cornelissen D, Vrijens B, Abrahamsen B, Al-Daghri N, Biver E, et al. Determinants, consequences and potential solutions to poor adherence to anti-osteoporosis treatment: results of an expert group meeting organized by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the International Osteoporosis Foundation (IOF). Osteoporosis International 2019;30(11):2155-65. DOI: 10.1007/s00198-019-05104-5
15. Heyman N, Etzion I, Ben Natan M. A coordination project for improvement of osteoporosis medication use among patients who sustained an osteoporotic fracture: The Israeli experience. Osteoporos Sarcopenia 2018;4(4):134-9. DOI: 10.1016/j.afos.2018.11.084
16. Yeap SS, Nur Fazirah MFR, Nur Aisyah C, Zahari Sham SY, Samsudin IN, C Thambiah S, et al. Trends in post osteoporotic hip fracture care from 2010 to 2014 in a private hospital in Malaysia. Osteoporos Sarcopenia 2017;3(2):112-6. DOI: DOI: 10.1016/j.afos.2017.05.001
17. García-Sempere A, Hurtado I, Sanfélix-Genovés J, Rodríguez-Bernal CL, Gil Orozco R, Peiró S, et al. Primary and secondary non-adherence to osteoporotic medications after hip fracture in Spain. The PREV2FO population-based retrospective cohort study. Sci Rep 2017;7(1):11784. DOI: 10.1038/s41598-017-10899-6
18. Orimo H, Sato M, Kimura S, Wada K, Chen X, Yoshida S, et al. Understanding the factors associated with initiation and adherence of osteoporosis medication in Japan: An analysis of patient perceptions. Osteoporos Sarcopenia 2017;3(4):174-84. DOI: 10.1016/j.afos.2017.10.002
19. Fatoye F, Smith P, Gebrye T, Yeowell G. Real-world persistence and adherence with oral bisphosphonates for osteoporosis: a systematic review. BMJ Open 2019;9(4)::e027049. DOI: 10.1136/bmjopen-2018-027049
20. Swart KMA, van Vilsteren M, van Hout W, Draak E, van der Zwaard BC, van der Horst HE, et al. Factors related to intentional non-initiation of bisphosphonate treatment in patients with a high fracture risk in primary care: a qualitative study. BMC Family Practice 2018;19(1):141. DOI: 10.1186/s12875-018-0828-0
21. Blanch J, Casado E, González J, Valdés C, Ruiz-Baena J, Palomino R, Nogués X. Percepción de los profesionales médicos respecto la adherencia terapéutica de los pacientes con osteoporosis. Rev Osteoporos Metab Miner 2016;8(1): 5-23. DOI: 10.4321/S1889-836X2016000100003
22. Axelsson KF, Johansson H, Lundh D, Möller M, Lorentzon M. Association Between Recurrent Fracture Risk and Implementation of Fracture Liaison Services in Four Swedish Hospitals: A Cohort Study. J Bone Miner Res 2020;35(7):1216-23. DOI: 10.1002/jbmr.3990
23. Ojeda PS. Programa para la prevención secundaria de la fractura por fragilidad en el Área Norte de Gran Canaria, coordinado por reumatólogos, con la participación de enfermería y en colaboración con atención primaria. [Tesis doctoral]. Gran Canaria: Universidad de Las Palmas de Gran Canaria; 2016. Recuperado a partir de: http://hdl.handle.net/10553/19367
24. Ganda K, Puch M, Chen JS, Speerin R, Bleasel J, Center JR, et al. Models of care for the secondary prevention of osteoporotic fractures: a systematic review and meta-analysis. Osteoporosis Int 2013;24:393-406. DOI: 10.1007/ss00198-012-2090-y
25. Willers C, Norton N, Harvey NC, Jacobson T, Johansson H, Lorentzon M, et al. Osteoporosis in Europe: a compendium of country-specific reports. Arch Osteoporos 2012;17(23):1-129. DOI: 10.1007/s11657-021-00969-8
26. Kanis JA, Norton N, Harvey NC, Jacobson T, Johansson H, Lorentzon M, et al. SOPE 2021: a new scorecard for osteoporis in Europe. Arch Osteoporos 2021;16(82):1-82. DOI: 10.1007/s11657-020-00871-9
27. Azagra R, Roca G, Martín-Sánchez JC, Casado E, Encabo G, Zwart M, et al. Umbrales de FRAX® para identificar personas con alto o bajo riesgo de fractura osteoporótica en población femenina española. Med Clin (Barc) 2015;144(1):1-8.
28. Naranjo A, Ojeda-Bruno S, Bilbao-Cantarero A, Quevedo-Abeledo JC, Diaz-González BV, Rodríguez-Lozano C. Two-year adherence to treatment and associated factors in a fracture liaison service in Spain. Osteoporos Int 2015;26:2579-85. DOI: 10.1007/s00198-015-3185-z
29. Axelsson KF, Jacobsson R, Lund D, Lorentzon M. Effectiveness of a minimal resource fracture liaison service. Osteoporos Int 2016;27(11):3165-75. DOI: 10.1007/s00198-016-3643-2

ROMM
Revista de Osteoporosis y Metabolismo Mineral

Special Article

Romosozumab: confusion regarding its indications

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Abstract

Romosozumab is undoubtedly an excellent drug to treat osteoporosis. However, its high price—much higher than antiresorptive drugs—initially led to accepting that its indication should be limited to patients with particularly high risk of fracture. However, the implementation of this idea into the routine clinical practice has been challenging. Firstly, different terms ("very high risk", "high risk", "severe osteoporosis") have been used to describe such indications, and the specific meaning of each term changes from one author to the next. On the other hand, without enough scientific basis, concepts have been introduced to expand the drug indications to the point of proposing its universal or near-universal use ("imminent risk", initiation of anabolic treatment for osteoporosis universally or quasi-universally). All this has created confusion among prescribing physicians and led to overly restrictive regulations imposed by health authorities regarding its use. This manuscript delves into these and other ideas in detail.

Keywords:
Romosozumab. Trials. Osteoporotic fracture.

Received: 11/04/2023 • Accepted: 12/04/2023

Conflicts of interest: JGM has received honoraria for lectures and travel grants from Amgen and Theramex. JM Olmos has received travel grants and honoraria for presentations on behalf of UCB-Amgen, Eli Lilly, Stada, Gedeon-Richter, and Grunenthal, participated in clinical trials sponsored by UCB-Amgen and FAES, and served on the advisory boards of UCB, Stada, and Gedeon-Richter.

González Macías J, Olmos Martínez JM. Romosozumab: confusion regarding its indications Rev Osteoporos Metab Miner 2023;15(2):81-87

DOI: 10.20960/RevOsteoporosMetabMiner.00011

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INTRODUCTION

The marketing of romosozumab has been accompanied by a certain degree of confusion regarding the type of patient for whom it is indicated. Several factors contribute to this confusion. For example, the fact that the ARCH study (1) found a higher rate of serious cardiovascular events has led to its contraindication in patients who had previously experienced acute myocardial infarctions or strokes. Understandably, it is also advised to avoid it in patients with an equivalent cardiovascular risk. This raises the problem of how to define and determine this risk equivalence. Logically, it has been suggested to take into account the usual risk factors, but it has not been specified how to do so (whether risk scales should be used, which ones in particular, what values should be taken into consideration...). However, we will not dwell on this aspect now.

We do however, wish to emphasize the interest that discrepancy seen between the efficacy results in fracture prevention from the aforementioned ARCH trial and those from a previously published trial, the FRAME trial, (2) may have. In the first 12 months of the ARCH trial, romosozumab reduced non-vertebral fractures compared to alendronate approaching statistical significance ($p = 0.06$). In contrast, in the first 12 months of FRAME trial, romosozumab did not significantly reduce the incidence rate of this same type of fracture compared to placebo ($p = 0.10$). The explanation for this paradoxical difference (greater efficacy vs active comparator than vs placebo) is that the fracture risk of the patients included in the ARCH trial was considerably higher than that of those included in the FRAME trial. Information about this is provided by the comparison of the incidence rate of fractures in patients treated with romosozumab in the two studies; in the FRAME trial, the incidence rate of non-vertebral fractures in patients treated with romosozumab within the first year was 1.6 % while in the ARCH trial, it was 3.4 %. Hence, romosozumab demonstrates greater efficacy when the risk of fracture is higher. The overall results of the FRAME trial point out the same thing. At 24 months, there was no significant difference in the incidence rate of vertebral fractures or clinical fractures between the two study arms. However, the difference became statistically significant when patients recruited from Latin America (43 % of the overall study population), mainly from Colombia and Brazil (2,3), were excluded from the analysis. In these countries the risk of osteoporotic fracture is lower compared to the remaining countries that had included patients in the study. Once again, it is observed that the efficacy of romosozumab in the prevention of non-vertebral fractures varies with the risk level of the patient, showing greater efficacy when the risk is higher. All in all, these findings lead to the conclusion that the drug will be particularly useful in individuals with a higher risk, which should be taken into account when establishing its indications.

A third factor that has contributed to the confusion mentioned at the beginning —perhaps the main one in practice— is the price of the drug. Although it is of a similar order compared to the other anabolic drug marketed in Europe, teriparatide, it is notably higher compared to antiresorptive drugs. This has led health authorities in different countries to consider imposing conditions for its prescription and dispensation. These conditions often are not consistent with the indications proposed by the experts who have investigated the drug, which logically leaves the prescribing physician in a situation of uncertainty and confusion. Therefore, it is worth analyzing the underlying factors in this situation. We consider the following 3 factors to be the most relevant ones: a) confusion in the terminology describing the severity of the risk of fracture for which the drug may be indicated (sometimes referred to as "severe" osteoporosis, other times as "very high" risk of fracture or simply as "high" risk of fracture...); b) the addition of the notion that the risk of fracture in the period immediately following a previous fracture is "very high" (and use of the term "imminent" to refer to it, which is semantically questionable in this context and, therefore, misleading); and c) introduction of the idea that anabolic drugs are more effective when administered to patients who have not previously received an antiresorptive drug.

AMBIGUITY REGARDING TERMINOLOGY: HIGH RISK OF FRACTURE, VERY HIGH RISK OF FRACTURE, AND SEVERE OSTEOPOROSIS

Recently (April 2022), the National Institute for Health and Care Excellence (NICE), after a previous period of opposition, gave its approval regarding the use of romosozumab to treat postmenopausal osteoporosis (4). The corresponding document literally says that: romosozumab is recommended as an option for treating severe osteoporosis in peopleafter menopause who are at high risk of fracture only if they have a major osteoporotic fracture (MOF) within 24 months. It adds that the pharmaceutical company proposes that romosozumab should be used only in cases of imminent risk of fracture, defined as the risk associated with a person with severe osteoporosis who has had a MOF over the last 24 months (interestingly, NICE also states that its recommendation is broader than that of the pharmaceutical company, although in reality the difference is not as easy to see). In these comments, several terms need clarification: a) what does NICE mean by "high" risk of fracture; b) what do NICE and the pharmaceutical company mean by "severe" osteoporosis. Regarding the latter, we should mention that the World Health Organization (WHO) calls severe (or established) osteoporosis as having a T-score of ≤ -2.5 plus 1 or more fragility fractures (5). However, we should remember that the WHO's sole intention

when using this term was to distinguish densitometric osteoporosis with fractures from osteoporosis without fractures without trying to establish a specific therapeutic indication (it is well-known that the WHO classification was primarily formulated with epidemiological purposes in mind). We should also mention, regarding the scope of these concepts, that when the WHO speaks of severe osteoporosis, it does not specify the location of the fracture or the time elapsed since it happened unlike what NICE and the pharmaceutical company do when they limit the use of romosozumab to MOFs occurred over the past 24 months. In other words, they limit the indication of the drug to a narrower field compared to what the WHO understands as severe osteoporosis.

After the position of NICE regarding the use of romosozumab, the National Osteoporosis Guideline Group (NOGG) and the Royal Osteoporosis Society (ROS) in the United Kingdom, that had pressured NICE to modify its initial opposition to accepting the drug, drafted a consensus document in May 2022 (6). In it, they literally say that "treatment with romosozumab, is prioritised in postmenopausal women who have had a MOF within 24 months, with any one of the following: a) a BMD T-Score ≤ -3.5 (at the hip or spine), or b) a BMD T-score ≤ -2.5 (at the hip or spine) and either i/ vertebral fractures (either a vertebral fracture within 24 months or a history of ≥ 2 osteoporotic vertebral fractures), or ii/ very high fracture risk (e.g., as quantified by FRAX". The proposal, which is somewhat unclear and presents some differences with respect to what NICE suggests (e.g., T-score ≤ -3.5) introduces a new term: "very high risk." The document does not define it, but we know that the NOGG, in a previous publication, gives an accurate definition: it is the risk that corresponds, in the British version of FRAX, to the value resulting from multiplying the therapeutic threshold by 1.6 once BMD has been taken into account. A clear limitation of this definition is that it is associated with the use of that version of FRAX.

Unlike NOGG's approach, the term "very high risk" has been used in several American guidelines without a precise definition. For example, the American Association of Clinical Endocrinologists (AACE) (7) refers to "very high risk" patients as those with any of the following characteristics: "a recent fracture (e.g., within the past 12 months), fractures while on approved osteoporosis therapy, multiple fractures, fractures while on drugs causing skeletal harm (e.g., long-term glucocorticoids), very low T-score (e.g., less than -3.0), high risk for falls or history of injurious falls, and very high fracture probability by FRAX® (fracture risk assessment tool) (e.g., major osteoporosis fracture > 30 %, hip fracture > 4.5 %) or other validated fracture risk algorithm)." Aside from the high number of situations considered, we should mention the inaccuracy and questionable reliability and relevance of several of them: how many doctors actually believe that a T-score < -3.0 should be considered as "very low" risk?; what should we understand by "high risk of falls"? (patients with Parkinson's disease or stroke tend to fall: should they be treated with romosozumab only because they have these conditions?). In response to a letter asking the authors of these guidelines why they chose fracture probabilities of 30 % and 4.5 % (8), they answered (9) that they are "simple examples" and "not based on published evidence."

Other American endocrine guidelines like those from the Endocrine Society (10) define "extremely high risk" in a much easier and concise way though perhaps not sufficiently precise. Regarding the type of patient for whom they recommend the use of romosozumab, they state that they do so "in postmenopausal women with osteoporosis at very high risk of fracture, such as those with severe osteoporosis (*ie,* low T-score < -2.5 and fractures) or multiple vertebral fractures". As observed, they don't seem to establish restrictions regarding the type of fracture when the patient also has a T-score < -2.5. However, in another section of the document —in the footnote of the algorithm where they explain it— they literally say: "In postmenopausal women with osteoporosis at very high risk of fracture, such as those with severe osteoporosis *(ie,* low T-score < –2.5 and fractures) or multiple vertebral fractures". Since in this second case only vertebral fractures are mentioned, after reading these guidelines there is a feeling of inaccuracy left.

The guidelines from the Bone Health and Osteoporosis Foundation (BHOF, former National Osteoporosis Foundation, NOF) (11), although recognized based on the guidelines published by the Endocrine Society, introduce a few changes. They define "very high risk" as that patients with multiple vertebral or hip fractures and a T-score ≤ -2.5 in the lumbar spine or hip have. This is a very accurate definition. However, they, then, add that anabolic drugs are also advised in patients with recent fractures and/or a T-score < -3.0, situations that, precisely because anabolic drugs are recommended for them, can be included in the concept of extremely high risk. This time, the type of fracture is not specified, it is not said what is considered a recent fracture, and most importantly, the term "and/or" is introduced adding ambiguity to the profiles of the risk that should be taken into considered. It is not the same to require the coexistence of 2 different phenomena (recent fractures plus a T-score ≤ -3.0, as indicated by the "and" of the "and/or") as to accept the presence of either one of them (as indicated by the "or").

To conclude with the American proposals, we should note that the American College of Physicians (ACP) recently published its guidelines (12) also recommending the use of romosozumab in patients at "extremely high risk," but once again without precisely defining the boundaries of this concept. It simply states that it is "based on" "on older age, a recent fracture (for example, within the past 12 months), history of multiple

clinical osteoporotic fractures, multiple risk factors for fracture, or failure of other available osteoporosis therapy."

In conclusion, although what has been discussed so far indicates agreement that romosozumab is indicated for patients with a particularly high risk of fracture, in the definition of this degree of risk terms are used whose specific meaning is not specified, and whose scope is conceived differently by different authors. This complicates having a clear understanding of the problem, and also hinders reaching consensus.

CONSIDERATION THAT THE RISK OF FRACTURE IMMEDIATELY AFTER THE OCCURRENCE OF A PREVIOUS FRACTURE "IS EXTREMELY HIGH", AND ADDITION OF THE TERM "IMMINENT RISK" TO REFER TO IT

Various epidemiological studies conducted over the past few decades have indicated that the risk of fracture within the first few years following a previous fracture is greater than in subsequent years (13-15). Based on this, but without demonstrating that the initial risk is necessarily very high in absolute terms (although in relative terms it may be greater than the subsequent risk), it was decided to classify this risk of the early years as "very high." It is evident that accepting this approach implies that all women diagnosed with a fracture when it happens (in practice, all patients who suffer a fracture, except for those who are asymptomatic —morphometric vertebral fractures—) should be treated with romosozumab (or alternatively, with teriparatide).

To reinforce this idea, its advocates have gone further and agreed to label this initial risk with an pressing term: "imminent" (16-18). From a semantic point of view, its suitability is questionable so it is worth making a linguistic comment about it. In the world of communication, it is a common thing to apply a term to a specific concept that does not truly correspond to it, at least not fully, to persuade a certain audience and shape their way of thinking. This creates a distortion of the concept, creating what some describe as a "new reality," which leads to a change in the way the issue at stake is actually perceived (these inappropriately used terms act as "thought-creating elements" and are known as "linguistic framing"). The term "imminent" behaves this way when applied to the risk that follows the occurrence of a fracture initially. "Imminent" means "something that is about to happen" (according to the Royal Spanish Academy-DRAE). However, here this word is being used to describe something that may or may not happen and, in any case, even if it does happen, it does not have to happen immediately (that is, it is not "about to" happen). By using it in this particular circumstance a "new reality" is created with connotations of immediacy that do not correspond to the actual reality. This term is, therefore, misleading.

Semantics aside, it is important to know to what extent risk within the first few years after a fracture is truly higher compared to the following years. Two studies (19,20) —conducted with the goal of adding this aspect to FRAX— have quantified this difference. They are too complex to go into detail here, but the conclusion has been that the difference varies depending on the circumstances at stake (age, sex, type of fracture) that may not even be present, and that generally is not large. The authors themselves indicate that current knowledge is not enough to reach a definitive conclusión, and that further studies are needed to better understand the phenomenon. Accordingly, inferring that a recent fracture, simply because of being recent (without considering the absolute risk it represents based on its characteristics) should be treated with an anabolic drug is an unjustified generalization. As a matter of fact, a study published by Kanis et al. (21) that evaluates intervention thresholds for very high risk of fracture applied to NOGG guidelines explicitly states that "recent fracture alone did not invariably give rise to very high risk and depended in part on the site of the sentinel fracture." Similarly, in a recent editorial published in *Lancet Rheumatology*, Dr. R. Eastell is quoted in response to a question posed by the author of the editorial saying that "many patients with a major fracture in the previous 2 years will not have a high risk of subsequent fracture" (22).

In conclusion, the risk of fracture in the immediate period that follows a previous fracture does not have to be "very high" *per se*. Therefore, it does not necessarily require treatment with anabolic drugs. We should mention that this does not mean that patients should not be treated early. They should be. There is no sense in delaying the treatment of a patient who has had an osteoporotic fracture. However, it is crucial to understand the distinction between these 2 concepts: one thing is that early treatment should be initiated immediately after a fracture, and a totally different thing is that it must necessarily be done with an anabolic drug.

Regarding the confusion surrounding the use of romosozumab (or anabolic drugs in general) in the period following a fracture, we should mention that there is not agreement either on the duration of this period. For example, the NICE technology assessment document and the previously mentioned NOGG-ROS consensus document refer to a period of 24 months. However, the AACE and ACP guidelines mention a period of 12 months. In a recent conference (Budapest, Hungary, March 2023), Dr. B. Langdahl commented that Danish guidelines —seemingly still unpublished— mention a 3-year period.

There are also discrepancies regarding the type of fracture to which different authors believe that the idea of increased risk after the occurrence of a previous fracture is aplicable. As mentioned before, the AACE and ACP guidelines do not specify any particular type of fracture, therefore suggesting that they consider it applicable to any fragility fracture. In contrast, the NICE document and the NOGG-ROS consensus document limit it to major osteoporotic fractures (FOM). Other authors focus on vertebral and hip fractures (23). Some even propose more complex scenarios. For example, in the aforementioned Danish guidelines, romosozumab is considered for the management of both FOM and pelvic fractures while teriparatide is considered for vertebral fractures alone.

RECOMMENDATION FOR USING ANABOLIC DRUGS TO START TREATMENT IN ANY OSTEOPOROTIC PATIENT

Several studies (24,25) conducted with bone mineral density as the efficacy variable seem to indicate that the effect of anabolic drugs is lower when administered to patients who have previously received an antiresorptive drug compared to those who have not. Based on this, some authors argue (26,27) that osteoporotic patients should generally be initially treated with anabolic drugs because, should the patient not respond well, starting with an antiresorptive drug and then changing to a bone-forming drug, would reduce its efficacy. This approach seems to disregard the degree of the risk of fracture. As a matter of fact, if we were to apply this approach, the concepts of very high risk and increased risk in the initial post-fracture period would lose their meaning, since both define specific subpopulations of patients with osteoporosis obviously included in the overall osteoporotic population. The approach of treating all osteoporotic women in general is incompatible with treating only a portion of them.

Not only does this proposal disregard the degree of risk of fracture, but it also fails to consider the associated cost. Even if it were truly beneficial —which we'll discuss shortly— one must consider to what extent the increased benefit exceeds the higher cost involved. It is known that in any curve that relates the resources used to achieve a certain benefit with the actual benefit obtained, there's an "optimal zone" beyond which further benefit (including the "maximum" benefit) does not justify any additional expenses. This search for the optimal therapeutic zone is also applicable to the treatment of fractures with anabolic drugs, so it is of paramount importance to try to identify it.

But, above all, this approach ignores the fact that the only evidence we have regarding the efficacy of anabolic drugs on the management of fracture outcomes when administered after an antiresorptive drug is not indicative of a loss of efficacy. The VERO study [28], that compared the efficacy of teriparatide to risedronate in patients who had previously received an antiresorptive drug in approximately two-thirds of the cases, demonstrated that the anabolic drug retained its full anti-fracture efficacy in these patients.

We must remain attentive to studies conducted with romosozumab and how they vary from what we just mentioned regarding teriparatide. Because if the former does not behave similarly to the latter and loses efficacy when administered after an antiresorptive drug, it would clearly be a point where teriparatide would come out as the preferred option in the comparison.

CONCLUSIONS

The introduction of romosozumab to the market has led to a review of the drug selection criteria for the treatment of osteoporosis. Previously, there was a general agreement that the anabolic drug available in Europe, teriparatide, should be spared for cases of osteoporosis with a higher risk of fracture. While this condition was never precisely defined, its use did not pose significant problems because prescribing physicians often used it appropriately. Romosozumab, however, sought to have its indications clearly defined from the beginning. It immediately claimed a therapeutic niche defined as "very high risk" osteoporosis, and this expression appeared in the updates that various societies quickly made of their clinical practice guidelines to accommodate it. Due to its nature as an anabolic drug, this way of thinking was also applied to teriparatide (and abaloparatide), and we started talking, generically, about the indications of bone-forming treatment. However, the definition of "very high risk" remained unclear. The initial clinical practice guidelines that addressed this issue (AACE [7], Endocrine Society [10], IOF [29]) were far from offering a uniform criteria. As a result, clinicians did not have concrete and consensus-based rules on how to use these drugs. Things have not improved since then, quite the opposite. The introduction of debatable concepts (imminent risk, generalized initial anabolic treatment —"anabolics for everyone—") has generated more confusion. There are currently no signs that the problem will be solved in the short term. It is not surprising that health authorities from different countries are issuing restrictive regulations on this situation. How should we approach the problem? First, I believe we should ask experts to provide recommendations based on solid scientific evidence, free from conjecture, wishful thinking (in the sense of thinking guided by desire) or commercial interests. Second, we should remind prescribing

physicians that in medicine, when there is confusion, it is often preferable to exercise moderation regarding decision-making. And that, furthemore, it is desirable to adhere to indications that are formulated in a precise way, so as to leave no room for doubt.

ADDENDUM

Across this manuscript, we deliberately did not mention the clinical guidelines from the Spanish Society for Bone and Mineral Metabolism Research (SEIOMM) (30). The reason is that we wanted to make sure that the reasoning developed therein was not influenced by any desire to defend our guidelines. However, upon reading it after completion, we were under the impression that not referring to them could be interpreted as a lack of interest or even dismissal of the guidelines. Therefore, we believe it is necessary to make this final comment, placing them in relation to the issues raised above.

First, our guidelines do not mention the advisability of administering anabolic drugs as the initial drug. The reasons why we disagree with the "anabolics for everyone" strategy have been explained in the aforementioned discussion. We remain committed to classifying patients based on their level of risk, and starting with anabolics in "very high risk" patients only. We will not dwell on this point.

Secondly, our guidelines do not refer either to treating patients who have sustained a fracture over the past 2 years with anabolic drugs. As a matter of fact, there are no problems in adding this aspect to the guidelines. However, we don't believe it is beneficial for all patients, as we will discuss later on. Let's consider, for example, the proposal from NICE, a well-accredited organization. They suggest treating with romosozumab women who have had a MOF over the past 2 years. This idea can be easily added to our algorithm regarding the 2 most important fractures, vertebral and hip fractures, simply by modifying the wording of the second criterion we mentioned to identify very high risk patients. Instead of saying "patients with vertebral or hip fracture and a T-score < -3.0," we can say "patients with vertebral or hip fracture sustained over the past 24 months and a T-score < -3.0." Obviously, this excludes the other 2 major osteoporotic fractures. However, we should say on this regard that we share the opinion of those who do not attribute the same importance to wrist fractures as to vertebral and hip fractures. Wrist fractures do not exhibit a significantly higher risk within the 2 years following the fracture compared to later periods (as a matter of fact, it may be lower depending on age [19,20]), and the morbidity and mortality rates associated with wrist fractures are not comparable to those of vertebral and hip fractures. Therefore, the same therapeutic approach would not be justified. Humeral fractures are also quite different from vertebral and hip fractures. It is understandable that they are not considered eligible for anabolic treatment unless they are associated with other factors.

In conclusion, our clinical practice guidelines can add the temporal concept by simply redefining the second criterion of "very high risk" as mentioned earlier. Personally, we would not introduce such a modification because it essentially represents a restrictive change (we would no longer be treating patients who had a vertebral or hip fracture prior to the 24-month timeframe). Finally, regarding this temporal issue (the much talked about "imminent" risk), we should mention that when the last iteration of the guidelines was drafted, a survey was submitted to members of the committee responsible for drafting them. They were asked whether they supported or opposed the administration of an anabolic drug to patients who had had a fracture in the previous year based only on the fact that it had occurred within this timeframe. A total of 70 % of the responses were contrary to this.

REFERENCES

1. Saag KG, Petersen J, Brandi ML, Karaplis AC, Lorentzon M, Thomas T, et al. Romosozumab or alendronate for fracture prevention in women with osteoporosis. N Engl J Med 2017;377:1417-27. DOI: 10.1056/NEJMoa1708322
2. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Romosozumab treatment in postmenopausal women with osteoporosis N Engl J Med 2016;375:1532-43. DOI: 10.1056/NEJMoa1607948
3. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Romosozumab FRAME Study: A Post hoc analysis of the role of regional background fracture risk on nonvertebral fracture outcome. J Bone Miner Res 2018;33:1407-16. DOI: 10.1002/jbmr.3439
4. National Institute for Health and Care Excellence (NICE). Romosozumab for treating severe osteoporosis. Technology appraisal guidance. TA791. Published: 25 May 2022. Available from: https:// www.nice.org.uk/guidance/ TA791
5. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994;9:1137. DOI: 10.1002/jbmr.5650090802
6. National Osteoporosis Guideline Group and Royal Osteoporosis Society. Consensus Advisory Statement on the use of romosozumab following the 2022 NICE appraisal. Available from: https:// www.nogg.org.uk/sites/nogg/ download/NOGG-ROSRomosozumab-statementMay-2022.pdf
7. Camacho PM, Petak SM, Binkley N, Diab DL, Eldeiry LS, Farooki A, et al. American Association of Clinical Endocrinologists/American College of Endocrinology. Clinical practice guidelines for the diagnosis and treatment of postmenopausal osteoporosis-2020 Update. Endocr Pract 2020;26(Suppl):1-46. DOI: 10.4158/GL-2020-0524SUPPL

8. Chandran M. American Association of Clinical Endocrinologists/American College of Endocrinology. Clinical practice guidelines for the diagnosis and treatment of postmenopausal osteoporosis- 2020 Update. Endocr Pract 2021;27:378. DOI: 10.1016/j.eprac.2021.02.001
9. Watts NB, Camacho PM, Lewiecki EM, Petak SM. American Association of Clinical Endocrinologists/American College of Endocrinology. Clinical practice guidelines for the diagnosis and treatment of postmenopausal osteoporosis- 2020 Update. Endocr Pract 2021;27:379-80. DOI: 10.1016/j.eprac.2021.02.001
10. Shoback D, Rosen CJ, Black DM, Cheung AM, Murad MH, Eastell R. Pharmacological management of osteoporosis in postmenopausal women: An Endocrine Society guideline update. J Clin Endocrinol Metab 2020;105:dgaa048 DOI: 10.1210/clinem/dgaa048
11. LeBoff MS, Greenspan SL, Insogna KL, Lewiecki EM, Saag KG, Singer AJ, et al. The clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int 2022;33:2049-102. DOI: 10.1007/s00198-021-05900-y
12. Qaseem A, Hicks LA, Etxeandia-Ikobaltzeta I, Shamliyan T, Cooney TG; Clinical Guidelines Committee of the American College of Physicians; Cross JT Jr, Fitterman N, Lin JS, Maroto M, Obley AJ, Tice JA, Tufte JE. Pharmacologic treatment of primary osteoporosis or low bone mass to prevent fractures in adults: a living clinical guideline from the American College of Physicians. Ann Intern Med 2023;176:224-38. DOI: 10.7326/M22-1034
13. Johnell O, Oden A, Caulin F, Kanis JA. Acute and long-term increase in fracture risk after hospitalization for vertebral fracture. Osteoporos Int 2001; 12:207-14. DOI: 10.1007/s001980170131
14. van Geel TACM, van Helden S, Geusens PP, Winkens B, Dinant G-J. Clinical subsequent fractures cluster in time after first fractures. Ann Rheum Dis 2016;68:99-102. DOI: 10.1136/ard.2008.092775
15. Kanis JA, Johansson H, Odén A, Harvey NC, Gudnason V, Sanders KM, et al. Characteristics of recurrent fractures. Osteoporos Int 2018;29:1747-57. DOI: 10.1007/s00198-018-4502-0
16. Roux C, Briot K. Imminent fracture risk. Osteoporos Int. 2017;28:1765-9. DOI: 10.1007/s00198-017-3976-5
17. Johansson H, Siggeirsdóttir K, Harvey NC, Odén A, Gudnason V, McCloskey E, et al. Imminent risk of fracture after fracture. Osteoporos Int 2017;28:775-80. DOI: 10.1007/s00198-016-3868-0
18. Banefelt J, Åkesson KE, Spångéus A, Ljunggren O, Karlsson L, Ström O, et al. Risk of imminent fracture following a previous fracture in a Swedish database study. Osteoporos Int 2019;30:601-9. DOI: 10.1007/s00198-019-04852-8
19. Kanis JA, Johansson H, Harvey NC, Gudnason V, Sigurdsson G, Siggeirsdottir K, et al. Adjusting conventional FRAX estimates of fracture probability according to the recency of sentinel fractures.Osteoporos Int 2020;31:1817-28. DOI: 10.1007/s00198-020-05517-7
20. Leslie WD, Morin SN, Lix LM, McCloskey EV, Johansson H, Harvey NC, et al. The effect of fracture recency on observed 10-year fracture probability: a registry-based cohort study. J Bone Miner Res 2022;37:848-55. DOI: 10.1002/jbmr.4526
21. Kanis JA, Johansson H, Harvey NC, Lorentzon M, Liu E, Vandenput L, et al. An assessment of intervention thresholds for very high fracture risk applied to the NOGG guidelines: A report for the National Osteoporosis Guideline Group (NOGG). Osteoporos Int 2021;32:1951-60. DOI: 10.1007/s00198-021-05942-2
22. Thorley J. NICE approves romosozumab for postmenopausal osteoporosis. The Lancet Rheumatology 2022;4:e532. DOI: 10.1016/S2665-9913(22)00188-6
23. McClung MR, Rothman MS, Lewiecki EM, Hanley DA, Harris ST, Miller PD, et al. The role of osteoanabolic agents in the management of patients with osteoporosis. Postgrad Med 2022;134:541-55. DOI: 10.1080/00325481.2022.2069582
24. Ettinger B, San Martin J, Crans G, Pavo I. Differential effects of teriparatide on BMD after treatment with raloxifene or alendronate. J Bone Miner Res 2004;19:745-51. DOI: 10.1359/jbmr.040117
25. Leder BZ, Tsai JN, Uihlein AV, Wallace PM, Lee H, Neer RM, et al. Denosumab and teriparatide transitions in postmenopausal osteoporosis (the DATA-Switch study): extension of a randomised controlled trial. Lancet 2015;386:1147-55. DOI: 10.1016/S0140-6736(15)61120-5
26. Cosman F, Nieves JW, Dempster DW. Treatment sequence matters: anabolic and antiresorptive therapy for osteoporosis. J Bone Miner Res 2017;32:198-202. DOI: 10.1002/jbmr.3051
27. Cosman F, Kendler DL, Langdahl BL, Leder BZ, Lewiecki EM, Miyauchi A, et al. Romosozumab and antiresorptive treatment: the importance of treatment sequence. Osteoporos Int 2022;33:1243-56. DOI: 10.1007/s00198-021-06174-0
28. Kendler DL, Marin F, Zerbini CAF, Russo LA, Greenspan SL, Zikan V, et al. Effects of teriparatide and risedronate on new fractures in post-menopausal women with severe osteoporosis (VERO): a multicentre, double-blind, double-dummy, randomised controlled trial. Lancet 2018;391:230. DOI: 10.1016/S0140-6736(17)32137-2
29. Kanis JA, Harvey NC, McCloskey E, Bruyère O, Veronese N, Lorentzon M, et al. Algorithm for the management of patients at low, high and very high risk of osteoporotic fractures. Osteoporos Int 2020;31:797-8. DOI: 10.1007/s00198-020-05297-0
30. Riancho JA, Peris P, González-Macías G, Pérez-Castrillón JL; en nombre de la Comisión de Redacción de las Guías de Osteoporosis de la SEIOMM. Guías de práctica clínica en la osteoporosis posmenopáusica, glucocorticoidea y del varón (actualización 2022). Sociedad Española de Investigación Ósea y del Metabolismo Mineral (SEIOMM). Rev Osteoporos Metab Miner 2022;14:13-33. DOI: 10.4321/S1889-836X2022000100003

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Clinical Setting and Decision-Making

Postmenopausal osteoporosis with vertebral fracture: teriparatide vs romosozumab

Case report:

This is the case of a 66-year-old woman who went to her doctor complaining of lower back pain that appeared right after jumping while playing volleyball on the beach. The X-ray of the dorsal spine reveals the presence of 2 vertebral fractures. The dual-energy X-ray absorptiometry (DXA) showed a T-score of -3 in the femoral neck. The patient had a past medical history of breast cancer 15 years ago, which is why it was decided to prescribe chemotherapy but no local radiation therapy. She has experienced zero relapses since then.

She has a history of smoking 20 packs/year. However, she quit over a year ago when her sister had a myocardial infarction at the age of 50. The patient does not do any exercise but walks daily for an hour. She has hypertension, is not a diabetic, and her blood test results shows show elevated total cholesterol levels (250 mg/dL). Her HDL cholesterol levels are 30 mg/dL.

During the physical examination, the patient's vital signs are stable, her body mass index (BMI - the weight in kilograms divided by the square of the height in meters) is 24.4, and apart from a previous mastectomy scar, no particular findings are reported on her physical examination.

The risk of fracture estimated using the FRAX tool shows a 25 % and 8.6 % rate of suffering major osteoporotic and hip fractures, respectively, within the next 10 years. The patient has not had any cardiovascular events yet. However, the cardiovascular risk estimated using the Systematic Coronary Risk Evaluation (SCORE2) tool shows a 6.8 % risk within the next 10 years.

Treatment options:

You need to decide what the best initial treatment option for osteoporosis in this clinical setting would be taking the balance between the risk of fracture and cardiovascular risk into consideration:

Based on your own clinical experience, the medical literature available, the guidelines published, and other sources of information, which would be your approach with this patient?

1. *Start treatment with romosozumab is advised.*
2. *Start treatment with teriparatide is advised.*

To help in the decision-making process, we have asked 2 experts in the field of mineral bone metabolism to discuss the position held by the editors. At the end, we will also publish a comment from a cardiologist who has studied the patient's cardiovascular profile.

Recibido: 31/05/2023 • Aceptado: 13/06/2023

Conflicto de intereses: los autores declaran no tener conflicto de interés.

Ferrari S, Muñoz Torres M, González-Juanatey JR. Postmenopausal osteoporosis with vertebral fracture: teriparatide vs romosozumab. Rev Osteoporos Metab Miner 2023;15(2):88-91

DOI: 10.20960/RevOsteoporosMetabMiner.00022

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OPTION #1.
START EARLY TREATMENT WITH ROMOSOZUMAB IS ADVISED

Dr. Serge Ferrari

Full Professor of Medicine at the Geneva Faculty of Medicine. Head of the Service of Bone Diseases of the Geneva University Hospital. Switzerland

This 66-yr-old woman with two recent vertebral fractures is at "imminent" risk of another fragility fracture. Indeed, up to 25% of women with a recent vertebral osteoporotic fracture refracture within 1 year, particularly after 65 years of age (1). In addition, her low BMD at the femoral neck (-3.0 T-scores) indicates a generalized bone fragility also affecting the cortical compartment. Her high fracture risk is confirmed by the FRAX score, namely a 10-year risk of major osteoporotic fracture and a hip fracture at 25 % and 8.6 %, respectively, although in this case it might underestimate her actual fracture risk in the next couple of years since FRAX does not yet take into account the multiplicity nor the recency of the prevalent fractures.

Treatment must therefore be introduced promptly, not only to quickly reduce her risk of a subsequent fracture, but also to improve hip BMD in order to prevent peripheral, and notably hip, fractures. In her case I would prescribe romosozumab as first line therapy for one year, followed by an antiresorptive. Indeed romosozumab has been shown to significantly decrease the incidence of new vertebral and clinical fractures within one year as compared to alendronate (the ARCH trial [2] in women at high risk similar to this patient (i.e. with prevalent vertebral fractures, T-scores around -3.0 and average FRAX scores of 20 %). In addition, romosozumab has been shown to significantly increase hip BMD compared to alendronate (2) and also teriparatide (3,4), which in the case of our patient is a very relevant issue. Regarding her CV risk, the patient is not known for suffering from ischemic disease, which would contra-indicate the use of romosozumab. Her moderate CV risk can be improved by the appropriate use of anti-hypertensive medication, hypocholesterolemic agents, and by maintaining a healthy lifestyle (refraining from smoking). Finally, the history of breast cancer is not a contra-indication for romosozumab either.

I would therefore strongly recommend romosozumab therapy for one year, with a re-evaluation of BMD before switching to an anti-resorptive, either a BP or denosumab (5).

OPTION #2.
START TREATMENT WITH TERIPARATIDE IS ADVISED

Dr. Manuel Muñoz Torres

Professor of Medicine. Specialist Physician at the Department of Endocrinology and Nutrition. Hospital Universitario Clínico San Cecilio. Granada, Spain

The case presented here is representative of an "extremely high risk of fracture". Although this denomination does not have a universally agreed definition, it is included in the most recent clinical practice guidelines. Therefore, the pharmacological treatment of postmenopausal osteoporosis according to the Endocrine Society (6) states that anybody with multiple vertebral fractures and a BMD T-score < -2.5 in the spine or hip meets this criterion. More recently, the latest version of the clinical practice guidelines of the Spanish Society of Bone Research and Mineral Metabolism (SEIOMM) (7) spares this risk category for individuals with 2 or more vertebral fractures, 1 vertebral or hip fracture with a T-score < -3.0 or a T-score < -3.5. In these cases, early treatment with a bone-forming drug followed by an antiresorptive agent is considered the preferred approach (8). Teriparatide and romosozumab are the 2 bone-forming drugs currently available in Spain. To decide which of these 2 drugs is more appropriate for this patient we should take into account which are her most significant comorbidities in addition to the efficacy of each option.

According to a meta-analysis, a 2-year course of teriparatide has shown 65 %, 50 %, and 50 % drops of the risk of vertebral, non-vertebral, and hip fractures, respectively (9). Contraindications for its use include unexplained elevations of alkaline phosphatase levels, patients who have previously been treated with external radiation or localized radiotherapy to the skeleton, and the presence of tumors or bone metastases. There is extensive experience on this drug, and recently, biosimilars have been developed that have reduced costs.

Romosozumab is a monoclonal antibody that inhibits sclerostin with a beneficial effect on bone homeostasis as it stimulates bone formation while inhibiting resorption simultaneously. Romosozumab rapidly and significantly decreases the risk of vertebral fractures and clinical fractures in 12-month courses of monotherapy. In addition, it minimizes the risk of all types of fractures in sequential treatment both with denosumab and long-term alendronate (10). The drug is well-tolerated, but in one of the studies, there was a slight imbalance in the rate of cardiovascular events occurred. Differences were small (1.3 % vs 0.9 % in the control group), and although there is no plausible biological explanation for this finding, it is contrain-

dicated in individuals with a past medical history of myocardial infarction or stroke.

In our case, the patient's significant past medical history is the diagnosis of breast cancer —currently in remission— 15 years ago that was not treated with radiation therapy and no evidence of bone metastasis. Although a complete blood test including alkaline phosphatase levels would be necessary, there is not such a thing as a formal contraindication for the use of teriparatide. On the other hand, no previous cardiovascular events have ever been reported. However, the past medical history does identify several cardiovascular risk factors like smoking until a year ago, dyslipidemia, hypertension, and a family history of early coronary artery disease. Therefore, the cardiovascular risk index (SCORE) showed values of 6.8 %. The National Spanish Health Service has established specific conditions for its funding including, among other, low or moderate cardiovascular risk (SCORE < 5 %).

In conclusion, this patient on an extremely high risk of fracture should receive early treatment with a bone-forming drug as first-line therapy. Considering the comorbidities reported and funding limitations in Spain, the option recommended would be teriparatide after discussion and agreement with the patient.

COMMENTARY ON ASPECTS RELATED TO CARDIOVASCULAR RISK

Dr. José R. González-Juanatey

Director. Department of Cardiology. Hospital Clínico Universitario de Santiago de Compostela. CIBERCV, IDIS. Head of Cardiology Unit and CCU. Professor of Cardiology. Former President of the Spanish Society of Cardiology. Santiago de Compostela, A Coruña. Spain

The cardiovascular risk profile should be estimated routinely in individuals over 40 years (11) of age during any contacts with their healthcare providers, especially women like the one reported in this case who has a medical history of breast cancer and has received chemotherapy in the past. Therefore, recent data from the Spanish CARDIOTOX Registry (12) indicate that the risk of cardiotoxicity (defined as a reduction of left ventricular ejection fraction < 40 %) or progression into clinical heart failure, is associated, among other factors, with the patients' baseline cardiovascular risk.

In this case, the patient has a SCORE2 cardiovascular risk of 6.8 % that falls into the moderate-risk group. According to the recent European clinical practice guidelines on prevention, interventions on the patient's lifestyle would be advised including smoking cessation, exercise recommendations, and dietary changes. The guidelines also state that LDL cholesterol levels should be < 100 mg/dL and blood pressure kept under 140-130 mmHg. Other factors that should be taken into consideration — spared by the SCORE2 estimate— include the patient's risk profile, her sister's previous history of myocardial infarction at the age of 50, low HDL cholesterol levels, and having been received breast cancer treatment with chemotherapy. Although the specific LDL level is not provided, it is mentioned that her total cholesterol levels were 250 mg/dL with low HDL, indicative that the LDL levels are likely well above the 100 mg/dL mark. Therefore, the patient should receive treatment, at least, with a powerful enough statin to bring LDL down to < 100 mg/dL, and if necessary, consider adding ezetimibe to statin therapy. The patient's blood pressure levels are not described either. If they fall within the hypertension range (> 140 mmHg and/or 90 mmHg), apart from lifestyle recommendations, which should also include reducing salt intake, initiating antihypertensive treatment with an ACE inhibitor, ARB, thiazide or dihydropyridine calcium channel blocker should be considered too. If achieving blood pressure control requires bringing systolic pressure down by > 20 mmHg and/or diastolic pressure down by > 10 mmHg, combined therapy with a low-dose ACE inhibitor or ARB plus a thiazide or calcium channel blocker is advised.

In conclusion, this patient requires cardiovascular risk assessment by the healthcare professional involved in her management. Also, instructions on lifestyle changes and specific therapeutic interventions should be provided.

REFERENCES

1. Balasubramanian A, Zhang J, Chen L, Wenkert D, Daigle SG, Grauer A, et al. Risk of subsequent fracture after prior fracture among older women. Osteoporos Int 2019;30(1):79-92. DOI: 10.1007/s00198-018-4732-1
2. Saag KG, Petersen J, Brandi ML, Karaplis AC, Lorentzon M, Thomas T, et al. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. N Engl J Med 2017;377(15):1417-27. DOI: 10.1056/NEJMoa1708322
3. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med 2014;370(5):412-20. DOI: 10.1056/NEJMoa1305224
4. Langdahl BL, Libanati C, Crittenden DB, Bolognese MA, Brown JP, Daizadeh NS, et al. Romosozumab (sclerostin monoclonal antibody) versus teriparatide in postmenopausal women with osteoporosis transitioning from oral bisphosphonate therapy: a randomised, open-label, phase 3 trial. Lancet 2017;390(10102):1585-94. DOI: 10.1016/S0140-6736(17)31613-6
5. Cosman F, Crittenden DB, Grauer A. Romosozumab Treatment in Postmenopausal Osteoporosis. N Engl J Med 2017;376(4):396-7. DOI: 10.1056/NEJMc1615367

6. Shoback D, Rosen CJ, Black DM, Cheung AM, Murad MH, Eastell R. Pharmacological Management of Osteoporosis in Postmenopausal Women: An Endocrine Society Guideline Update. J Clin Endocrinol Metab 2020;105(3):dgaa048. DOI: 10.1210/clinem/dgaa048
7. Riancho JA, Peris P, González-Macías J, Pérez-Castrillón JL. Guías de práctica clínica en la osteoporosis postmenopáusica, glucocorticoidea y del varón (actualización 2022). Rev Osteoporos Metab Miner 2022;14(1):13-33. DOI: 10.4321/S1889-836X2022000100002
8. Guañabens N, Moro-Álvarez MJ, Casado E, Blanch-Rubió J, Gómez-Alonso C, Díaz-Guerra GM, et al.; SEIOMM Working Group. The next step after anti-osteoporotic drug discontinuation: an up-to-date review of sequential treatment. Endocrine 2019;64(3):441-55. DOI: 10.1007/s12020-019-01919-8
9. Díez-Pérez A, Marin F, Eriksen EF, Kendler DL, Krege JH, Delgado-Rodríguez M. Effects of teriparatide on hip and upper limb fractures in patients with osteoporosis: A systematic review and meta-analysis. Bone 2019;120:1-8. DOI: 10.1016/j.bone.2018.09.02
10. Castañeda S, Gómez-Alonso C, Graña J, Guañabens N, Muñoz-Torres M, Peris P, et al. Position of the Spanish Society of Rheumatology (SER) and the Spanish Society for Bone Research and Mineral Metabolism (SEIOMM) on romosozumab. Reumatol Clin (Engl Ed) 2022;18(7):383-6. DOI: 10.1016/j.reumae.2021.10.001
11. Authors/Task Force Members & ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Euro Heart J 2021;42:3227-337.
12. Caro-Codón J, López-Fernández T, Álvarez-Ortega C, Zamora Auñón P, Rodríguez IR, Gómez Prieto P, et al; CARDIOTOX registry investigators. Cardiovascular risk factors during cancer treatment. Prevalence and prognostic relevance: insights from the CARDIOTOX registry Eur J Prev Cardiol 2022;29 (6):859-68. DOI: 10.1093/eurjpc/zwaa034