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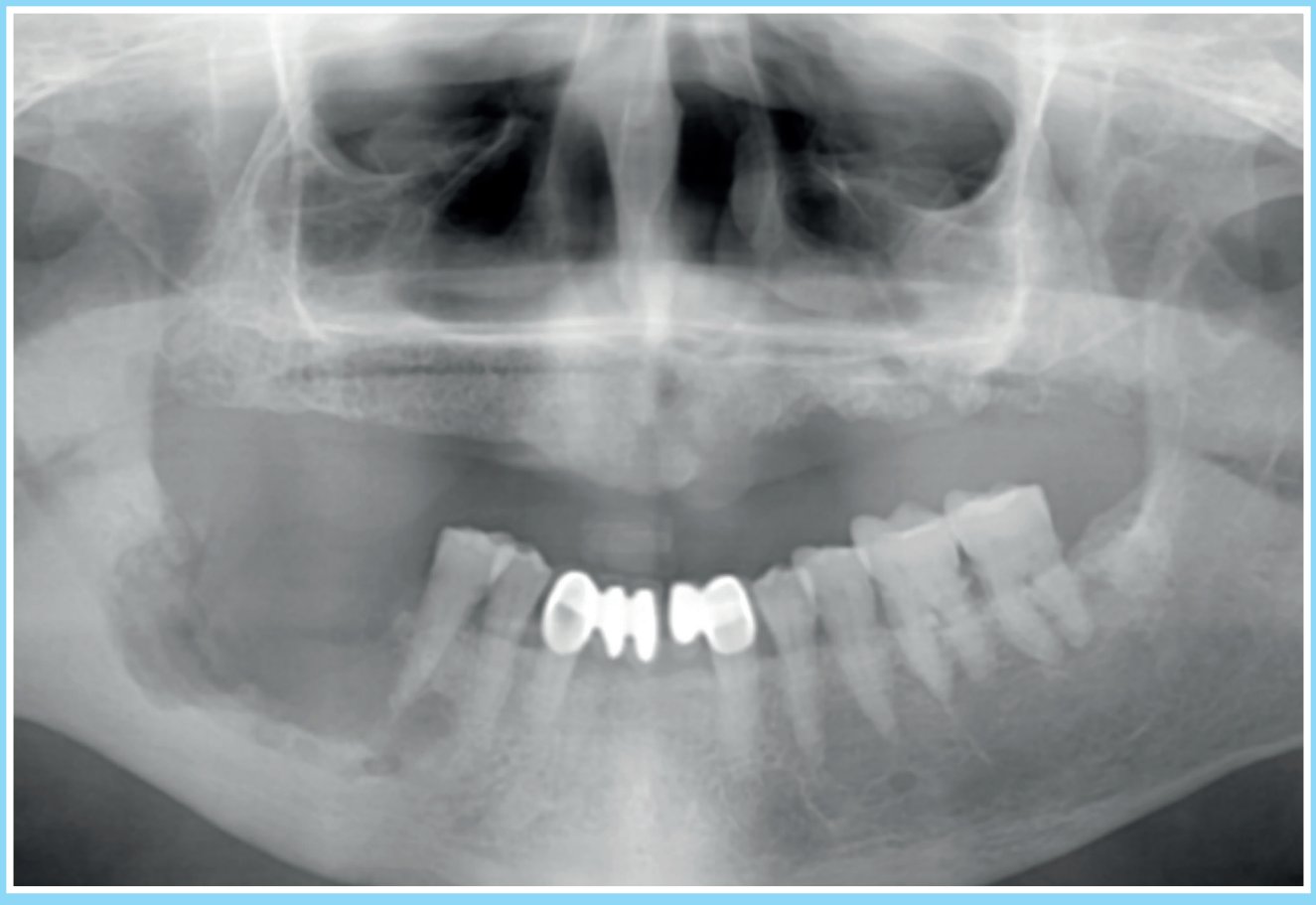

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Summary

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Originals

Special Document

Cover image:

Orthopantomogram of a patient with right mandibular osteonecrosis

Provided by Dr. José Luis Cebrián. Head of the Maxillofacial Surgery Department. La Paz University Hospital. Madrid

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Original

Vitamin D and nutritional support in patients with heart failure: effect on circulating cytokines

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Abstract

Background: heart failure (HF) is recognized as a highly state of inflammation. Increased circulating levels of cytokines have been previously reported and generally associated with worse clinical outcomes. In this context, the modulation of inflammation-related parameters seems to be a reasonable therapeutic option for improving the clinical course of the disease.

Aim: to compare changes in circulating cytokines and clinical progression of patients with HF when calcifediol supplementation is administered along with Mediterranean diet alone or with Mediterranean diet and 2 hypercaloric, hyperproteic oral nutritional supplements (ONS) enriched with eicosapentaenoic acid (EPA) docosahexaenoic acid (DHA) fatty acids.

Patients and methods: 25-hydoxi-vitamin D (25OHvitD) and circulating cytokines (IL-6, IL-8, IL-10, IP-10, MCP-1) were determined at baseline and after 24 weeks of nutritional support in a cohort of 38 patients previously included in an open label, controlled clinical tiral; briefly patients were randomized to receive calcifediol plus Mediterranean Diet (control group) vs calcifediol plus Mediterranean Diet and ONS (intervention group). Epidemiological, clinical, anthropometric, and biochemical evaluation was also performed.

Results: 25OHVitD insufficiency was observed in 58.3 % of patients. Patients from the intervention group exhibited higher increase in serum 25OHVitD, higher decrease in ferritin, C-reactive protein (C-RP), IL-8, IL-6 and IP-10; although 25OHVitD levels positively correlated at baseline with body cell mass and the phase angle ($p < 0.05$) they did not correlate with serum ferritin, C-RP or the circulating evaluated interleukins. Any associations were observed between serum 25OHVitD and left ventricular ejection fraction (LVEF) or the N-terminal pro-brain natriuretic peptide (NT-proBNP). An age-, sex- and 25OHvitD adjusted multivariate analysis showed that the only cytokine associated with increased mortality in patients with HF was MCP-1 (OR 1.01, 95 %CI, 1.01-1.02), which was not modulated in the intervention or the control group after 24-weeks of treatment.

Conclusion: although the combination of calcifediol, Mediterranean diet and hypercaloric, hyperproteic, EPA and DHA enriched ONS with decreased serum levels of inflammation related parameters (C-RP) and ferritin, as well as circulating cytokines, 25OHvitD levels were not correlated with these inflammation markers or the clinical progression of patients (mortality and new hospital admissions).

Keywords:
Oral supplements. Calcifediol. Heart failure. Cytokines. Mortality. Outcomes.

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INTRODUCTION

Vitamin D deficiency (VDD) is increasingly recognized as a significant factor in cardiovascular health, with associations identified in both coronary heart disease (CHD) and heart failure (HF) (1). Specifically, VDD, defined as 25-hydroxyvitamin D (25-OH-Vit D) levels < 20 ng/mL, has been associated with risk factors for cardiovascular disease and adverse outcomes (2) including all-cause mortality, cardiovascular mortality, and major adverse cardiovascular events (MACE) (1). Similarly, in patients with HF, multiple studies have revealed a striking correlation between VDD and worse prognosis (3). Furthermore, VDD in HF patients has been associated with reduced left ventricular ejection fraction (LVEF), increased natriuretic peptides, and increased mortality (4). Prospective studies have even indicated that the risk of developing HF is higher in patients with VDD (5). These associations highlight the potential role of vitamin D in the pathogenesis and progression of HF. Despite the strong epidemiological evidence implicating VDD in adverse cardiovascular outcomes, clinical trials involving vitamin D supplementation have largely failed to demonstrate consistent improvements in CVD outcomes; this discrepancy suggests that the relationship between vitamin D and cardiovascular disease is complex and may involve intermediary factors or specific patient subgroups that could benefit from intervention (1).

VDD has been involved in and may promote greater risk through inflammation; individuals with both VDD and elevated high-sensitivity C-reactive protein (hsCRP) levels exhibit an approximately 3-fold greater hazard of cardiovascular mortality vs those with normal vitamin D levels and low hsCRP (1). Similar findings have been observed for all-cause mortality and MACE, suggesting a synergistic detrimental effect of VDD and inflammation in cardiac heart disease (1).

Evidence from these sources consistently points towards a significant association between VDD and adverse cardiovascular outcomes, including HF. Inflammation seems to be a crucial mediator in this relationship, with lower vitamin D levels often correlating with higher levels of pro-inflammatory markers (5). Additionally, it has been described that vitamin D may exhibit immunomodulatory properties in reducing certain inflammatory cytokines like α necrosis tumor factor (TNF-α) in patients with HF, despite this, vitamin D supplementation trials have not consistently translated these effects into improved clinical outcomes (1).

HF is recognized as a systemic pro-inflammatory state that involves the activation of both innate and adaptive immunity mechanisms. Hemodynamic stress and volume overload in HF can lead to cardiomyocyte damage, stimulating the release of pro-inflammatory cytokines such as MCP-1, and IL-6 (5). These inflammatory signals can have effects on additional organs, which contributes to skeletal muscle inflammation, adipose tissue inflammation and atherogenesis. This pro-inflammatory state deteriorates ventricular function by inducing myocardial contractile dysfunction, hypertrophy, apoptosis, and fibrosis, ultimately leading to the progression of HF due con cardiac remodeling (6). Elevated concentrations of inflammatory markers in HF patients have been associated with adverse outcomes such as reduced LVEF, increased pro-BNP, and increased mortality (5). The complex interplay between inflammation and HF suggests that targeting inflammatory pathways could be a potential therapeutic strategy (1).

Several authors suggest that vitamin D possesses immunoregulatory functions, furthermore, *in vitro* and *in vivo* models have demonstrated protective roles through mechanisms involving various inflammatory pathways (5). Despite this, there is inconsistent evidence from the clinical trials about the use of vitamin D supplementation for decreasing inflammation in these patients (7,8).

In this context, we aimed to evaluate the correlation between vitamin D and circulating cytokines levels in patients with a recent admission due to HF, their relation with nutritional parameters (combining anthropometric, instrumental and biochemical measurements) and determine their progression after 24-weeks of vitamin D supplementation along with Mediterranean diet alone or vitamin D supplementation, Mediterranean diet and nutritional support with a hypercaloric, hyperproteic, omega 3 (*n*-3)- enriched oral nutritional supplement (ONS).

MATERIAL AND METHODS

PATIENTS

This study was approved by *Hospital Universitario Reina Sofía* Ethics Committee (Cordoba, Spain; reference No. 5164 approved on October 21st, 2021 and updated on May 30th, 2023). The study was conducted in full compliance with the criteria set forth in the Declaration of Helsinki and national and international clinical practice guidelines. We conducted a prospective open-label study in which written informed consent was obtained from each participant prior to their inclusion in the study. All patients received comprehensive information about the study before consenting to participate. Only those who agreed to participate were subsequently included. This cohort was initially evaluated in an open, randomized, controlled clinical trial (ClinicalTrials.gov number: NCT05848960) (9). The trial included patients of both sexes, aged between 18 and 85 years, with a left ventricular ejection fraction (LVEF) < 50 % and who had been hospitalized due to heart failure over the past 6 months.

NUTRITIONAL SUPPORT

In the clinical trial, patients received vitamin D supplementation with calcifediol at a different dose depending on the baseline levels of 25-OH-Vit D (25-OH-Vit D > 30 ng/mL: dose 0.266 mg every 30 days; 25-OH-Vit D 20-29 ng/mL: dose of 0.266 mg calcifediol every 21 days; 25-OH-Vit D 10-19 ng/mL: dose of 0.266 mg calcifediol every 15 days; 25-OH-Vit D < 10 ng/mL: dose of 0.266 mg calcifediol every 10 days. Additionally, patients were randomized by the clinical investigator to receive either Mediterranean diet alone or Mediterranean diet plus 2 hypercaloric, hyperproteic ONS per day, with a 1:1 allocation for 24 weeks. The ONS included slow-release carbohydrates, fiber mixture and a combination of *n*-3 and *n*-6 fatty acids. ONS were kindly donated by Vegenat Healthcare®, bottles were administered every 3 weeks. A total of 19 patients were included in each arm. All patients referred an adherence > 75 % to treatment. At baseline, all patients received general education and advice about nutritional support, Mediterranean diet and physical activity.

NUTRITIONAL EVALUATION

A morphofunctional nutritional evaluation was performed as previously described (10-12). Briefly, physical examination included body composition analysis (bioelectrical bioimpedance, abdominal, arm and calf circumferences), functional tests (up and go test and handgrip strength) and nutritional ultrasound of abdominal adipose tissue and rectus-femoris (RF) muscle of the quadriceps. Biochemical nutritional analysis was also performed (hemoglobin, lymphocytes, total cholesterol, total, high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, transferrin, albumin, prealbumin), heart-related markers (N-terminal pro-brain natriuretic peptide (NT-proBNP)) and inflammation markers (C-reactive protein (C-RP) and ferritin) were included. Left ventricular ejection fraction (LVEF) measured using transthoracic ultrasound was also evaluated.

CYTOKINE MEASUREMENT

Serum cytokines were quantified by Cytometry Bead Array (CBA, BD Cytometric Bead Array Human Soluble Protein Master, ref. 558264/558265; Becton Dickinson and Company, San Jose, CA, USA). The following cytokines were analysed based on the manufacturer´s instructions for use: IL-6 (ref. 558276), IL-8 (CXCL8, ref. 558277), IL-10 (ref. 558274), MCP-1 (CCL2, ref. 558287) and IP-10 (CXCL10, ref. 558280). For sample acquisition, a FACS Canto II was used, and a minimum of 300 events were recorded per each cytokine. Median Fluorescence Intensity (MFI) data was transformed in concentration (pg/mL) using a calibration curve as a reference.

STATISTICAL ANALYSIS

The Kolmogorov-Smirnov test was used to assess the data normal distribution. For the descriptive statistics, the mean and standard deviation of the continuous variables and the frequencies and percentages of the discrete variables were calculated. To assess differences across continuous variables, the Mann-Whitney U test was used (nonparametric data). Paired analysis was performed by Wilcoxon test (nonparametric data). For differences between the discrete variables, Pearson´s test was used. Statistical analyses were performed using SPSS statistical software version 20, and Graph Pad Prism version 6. Significance was defined as a *p*-value of < 0.05.

RESULTS

BASELINE CHARACTERISTICS OF THE GROUPS

A total of 38 patients were included in the study, 28.9 % were women, 42.1 % had type 2 diabetes mellitus and 34.2 % had ischemic cardiomyopathy; the median ejection fraction was 33 % and baseline NT-proBNP was 4225 pg/mL. A total of 73.7 %exhibited overweight or obesity. There were no differences in patients that underwent Mediterranean diet alone (control group) and those who received additional nutritional support (intervention group) (Table I). Median 25-OHvitD at baseline was 18 (11-26) ng/dL, 58.3 % of patients had 25OHvitD < 20 ng/dL. Specific baseline characteristics are shown in table I.

After 6 months of nutritional intervention, 25-OHvitD increased to 22 (16-31) ng/dL, this increase was higher in the intervention group [from 17 (9-29) to 25 (19-38), $p = 0.08$] than the control group [form 15 (11-21) to 17 (9-29)]. In parallel, serum ferritin and C-RP significantly decreased in the intervention group ($p < 0.01$) but not in the control group ($p>0.05$); additionally, also NT-proBNP levels significantly decreased in patients that received nutritional support with ONS (Table II).

CLINICAL ASSOCIATIONS AND CORRELATIONS BETWEEN SERUM 25-OHvitD AND NUTRITIONAL PARAMETERS

In this cohort, 25OHvitD at baseline was correlated with body cell mass (BCM) $r = 0.612$ ($p < 0,01$) and the phase angle (PA) $r = 0.349$ ($p < 0.05$). Baseline 25OHvitD < 20 ng/dL was associated with increased BMI (29.9 kg/m2 (IQR 5.55) vs 26.4 kg/m2 (IQR 7.9), any other association with anthropometric or biochemical parameters, including LVEF and NT-proBNP was observed.

Table I. Baseline clinical characteristics of the patients. Inter-group comparison based on nutritional intervention

Characteristics	Total (*n* = 38)	Calcidefiol plus Mediterranean diet (*n* = 19)	Calcidefiol plus Mediterranean diet and ONS (*n* = 19)	*p*
Sex (♂/♀)	71.1 %/28.9 % (11/27)	31.6/68.4 (6/13)	73.7/26.3 (14/5)	0.50
Age (years)	67.5 (61-78)	72 (64.5-80)	65 (56-72)	0.06
Tobacco exposure (%)				
No	57.9 (22/38)	42.1 (8/19)	73.7 (14/19)	
Active	18.4 (7/38)	15.8 (3/19)	21.1 (4/19)	
Previous exposure	23.7 (9/38)	42.1 (8/19)	5.3 (1/19)	
Type 2 diabetes *mellitus*	42.1 (16/38)	36.8 (7/19)	47.4 (9/19)	0.38
Previous ischemic cardiomyopathy	34.2 (13/38)	36.8 (7/19)	31.6 (6/19)	0.50
Ejection fraction (%)	33 (25-49.5)	40 (32.5-54)	38 (23-35)	0.46
NT-proBNP (pg/mL)	4225 (2001-7289)	3678 (1966-7203)	4412 (2177-7255)	0.59
Current weight (kg)	78 ± (70.3-89.5)	81 (75-90)	76 (70-85)	0.17
Overweight/obesity (%)	73.7 (28/38)	57.1 (16/19)	42.9 (12/19)	0.14
Mortality (%)	13.2 (5/38)	21.1 (4/19)	5.3 (1/19)	0.17

Categorical data are expressed as percentages and absolute number in brackets. Continuous variables are expressed as median with interquartile range n brackets. ONS: oral nutritional supplement.

Table II. Biochemical analysis at baseline and 6 months after nutritional support

	Total			Mediterranean diet			Mediterranean diet and OS		
Characteristics	Baseline (*n* = 38)	6 months (*n* = 33)	*p*1	Baseline (*n* = 19)	6 months (*n* = 15)	*p*2	Baseline (*n* = 19)	6 months (*n* = 18)	*p*3
Biochemical parameters									
Ferritin (mg/dL)	106 (35-176)	73 (32 - 111)	0.003	74 (32 - 171)	80 (37 - 113)	0.46	130 (104 - 169)	80 (37 - 113)	< 0.01
C-RP (mg/L)	2.1 (0.5-6.9)	1.0 (0.5-2.6)	0.02	2.2 (0.5-15)	2.1 (0.5-5.6)	0.79	1.4 (0.7-5.8)	0.7 (0.5-1.5)	< 0.01
NT-proBNP (pg/mL)	1855 (1080-4364)	741 (393-1992)	<0.01	1757 (557-6027)	489 (178-1676)	0.17	1952 (1179-3307)	1303 (741-2111)	0.02
Vitamin D (ng/dL)	18 (11-26)	22 (16-31)	0.08	15 (11-21)	17 (9-29)	0.51	17 (9-29)	25 (19-38)	0.08
Interleukin levels									
IL-6	0.8 (0-11)	0	0.001	2.4 (0 -14.97)	0	0.07	0 (0 - 7.55)	0	0.01
IL-8	116 (26-311)	8.84 (2.8-14.9)	< 0.0001	190 (38.1-621.7)	10.27 (2.41-14.56)	0.001	53.1 (20-160.5)	7.83 (5.63-20.65)	0.001
IP-10	314.5 (226-409)	196 (94-328)	0.002	288 (229-492)	208 (120-419)	0.25	319 (219-391)	193 (70-318)	0.002
IL-10	0	0		0	0		0	0	
MCP-1	154 (85-234)	163 (84-220)	0.30	173 (148-244)	163 (89-206)	0.18	126 (51- 205)	162 (83-277)	0.93

p1 refers to the comparison between all patients at baseline and after 24 weeks; p2 indicates the comparison between patients of the control group (Mediterranean diet) at baseline and after 24 weeks; p3 indicates the comparison between patients of the intervention group (Mediterranean diet plus oral nutritional supplementation) at baseline and after 24 weeks.

After 24-weeks of intervention, 25-OHvitD < 20 ng/mL was associated with higher serum ferritin ($p < 0.05$) and remarkably, 25-OHvitD > 30 ng/mL were associated with lower body cell mass (BCME), extracellular cell mass (ECME), lean mass, water and bone mass (Fig. 1).

CORRELATIONS AMONG 25OHvitD, BODY COMPOSITION AND CIRCULATING CYTOKINE LEVELS

Although, at baseline, 25OHvitD positively correlated with phase angle ($r = 0.428$; $p = 0.01$) and negatively with C-reactive protein serum levels (C-RP; $r = -0,378$; $p = 0.02$) serum 25OHvitD levels did not significantly correlate with circulating levels of IL6, IL8, IL10, MCP1 and IP10. After 24-weeks of intervention, serum 25OHvitD negatively correlated with extracellular mass ($r = -0.375$; $p = 0.03$) and IL6 levels $r = -0.390$ ($p < 0.05$).

CLINICAL ASSOCIATION BETWEEN HF-RELATED OUTCOMES AND CIRCULATING INTERLEUKINS

At the 24-week follow-up, 27.3 % of patients ($n = 9$) experienced at least 1 hospital readmission due to HF. Most (6/9) had a single readmission, while the remaining 3 patients had 2 or more. Among those readmitted, 55.6 % ($n = 5$) belonged to the control group and 44.4 % ($n = 4$) to the intervention group ($p = 0.3$). Despite mortality rate was higher in the control group (21.1 %) vs the intervention group (5.3 %), this difference was not statistically significant. An age-, sex- and 25OHvitD adjusted multivariate analysis showed that the only cytokine associated with increased mortality in patients with HF was MCP-1 (OR 1.01, 95 %CI, 1.01-1.02). In contrast, no circulating cytokine was associated with new hospital admissions due to HF at the 24-week follow-up.

DISCUSSION

Given the conflicting evidence on the anti-inflammatory effects of vitamin D supplementation in specific contexts, we evaluated the clinical impact of its combination with oral nutritional supplements (ONS) in patients recently hospitalized for HF. In this cohort, we observed that patients from the intervention group resulted in clinical improvements including lean mass gain, cell mass gain, decreased levels of serum ferritin and C-RP, and a more significant improvement in functionality, quality of life, LVEF and decrease in NT-proBNP serum levels after 24 weeks of intervention. In parallel, we observed that Mediterranean diet and vitamin D supplementation with calcifediol resulted in decreased IL-8 circulating levels in these patients, while its combination with an ONS (with slow-release carbohydrates, fiber mixture and enriched with EPA and DHA) resulted in an additional significant decrease in serum IL-6 and IP-10 (13). Clinically, as expected, lower 25OHvitD levels

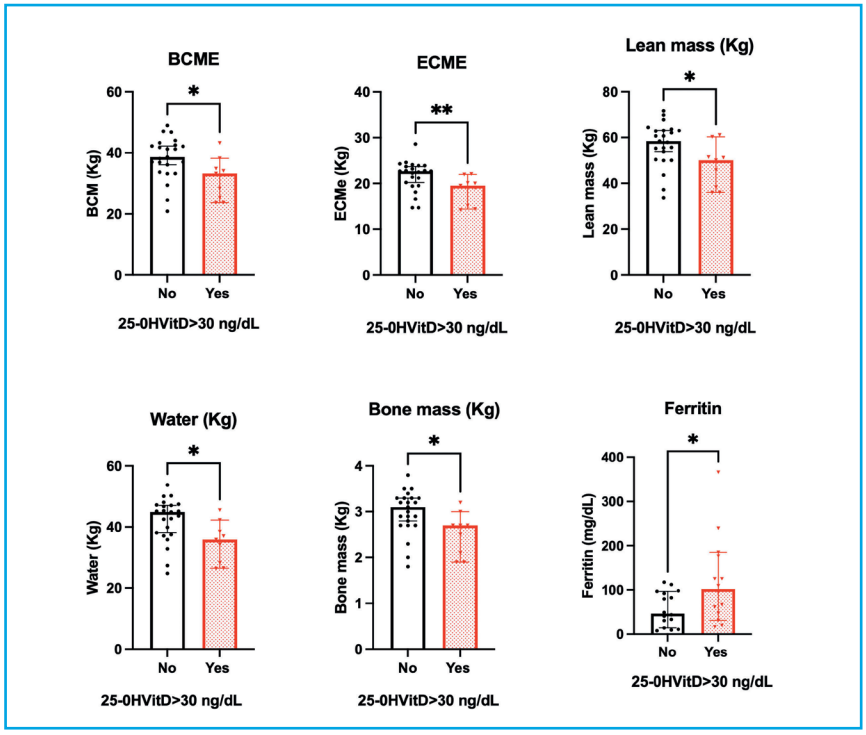

Figure 1. Clinical associations between 25OHvitD, clinical and biochemical parameters after 24-weeks of nutritional support and vitamin D supplementation. Serum 25-OHvitD levels tended to correlate with transferrin ($p = 0.05$). Additionally, serum25-OHvitD negatively correlated with body weight $r = -0.380$ ($p < 0.05$), BMI $r = -0.359$ ($p < 0.05$) and adipose tissue in the rectus femoris $r = -0.375$ ($p < 0.05$). Both, at baseline and at the end of the study, 25-OHvitD levels were not associated with circulating interleukins.

were observed in patients with increased C-RP and decreased phase angle as previously described in different cohorts of patients (14), suggesting decreased muscle quality and functionality in patients with decreased serum 25OHvitD levels (15). Despite this findings, serum 25OHvitD levels were not associated with mortality or additional hospital admissions due to HF. Furthermore, it did not correlate with circulating interleukin levels and only after 24-weeks of intervention it negatively correlated with IL-6 serum levels.

As in our cohort, former studies have reported a high prevalence of VDD in HF patients; however, in contrast to our study, they observed a significant inverse correlation between serum 25OHvitD levels and several pro-inflammatory cytokines, including IL1β, TNF-α, IL6, IL8, and IL17A (5). In line with our study, a few meta-analyses of randomized controlled trials have shown no significant differences in inflammation-related markers including C-RP (4, 16). In contrast, another metanalysis suggested a potential, anti-inflammatory effect of vitamin D supplementation on TNF-α (17), however, it is unknown whether this reduction in TNF-α turns into a significant improvement in the clinical evolution for HF (18).

Another area of interest is the clinical relationship between 25-hydroxyvitamin D (25OHvitD) and body composition parameters. In this regard, we observed positive clinical correlations with BCM and the phase angle, indicating that in HF patients, higher levels of 25OHvitD are associated with better clinical conditions (19,20).

This study has some limitations. First the number of participants; furthermore, we cannot determine a specific relation between the combination of the supplementation with calcifediol and ONS and the clinical benefit. Finally, underlying molecular mechanisms were not evaluated.

Overall, our results reveal a close relation between 25OHvitD, circulating cytokines and body composition parameters in patients with HF, but no specific relations between circulating cytokines and VDD have been observed at baseline. Remarkably, the combination of nutritional support with ONS and calcifediol produced significant decrease in serum IL-6 and IP-10, suggesting that nutritional interventions can affect the clinical evolution of the heart function patients with previous admissions due to HF. Our results do not allow to differentiate whether this effect was improved by the vitamin D supplementation or the composition of the ONS itself. Importantly, MCP-1 was the only parameter independently associated with mortality and it does not change after nutritional and calcifediol supplementation.

Importantly, according to former studies, the synergistic combination of vitamin D with hypercaloric/hyperproteic ONS demonstrates clinically significant benefits in specific populations, primarily through enhanced muscle protein synthesis and metabolic optimization (9,11,21). In this context, it is hypothesized that vitamin D potentiates the effect of the nutritional support downregulating myostatin, which is a muscle growth inhibitor and enhancing leptin sensitivity (redirecting calories toward muscle synthesis) (22).

This data suggests that the role of vitamin D as an anti-inflammatory drug in cardiovascular disease is complex, maybe some factors can mask the real effect, including the origin and evolution of the HF, the severity of the deficiency, the type and dosage of supplementation, and the presence of other comorbidities (4). Large-scale, well-designed clinical trials focusing on VDD individuals with HF and assessing both inflammatory markers and long-term clinical endpoints, are necessary to fully elucidate the therapeutic potential of vitamin D supplementation in these patients since it would help to develop effective strategies for improving clinical outcomes in these patients.

CORRESPONDING AUTHORS

Aura D. Herrera-Martínez, María José Molina Puertas, and Aurora Jurado Roger are the corresponding authors of this article.

AUTHORS´ CONTRIBUTIONS

Conceptualization: A. D. H. M., C. M. J., M. J. M.P., and A. J. R.; funding acquisition: A. D. H. M.; practical performance: S. C. P., A. S. S., J. L. A., A. D. H. M., G. M. G., C. M. J., and M. A. G. M.; formal and data analysis: A. D. H. M.; preparation manuscript: A. D. H. M., M. J. M. P., and C. M. J.; critical review of manuscript: A. D. H. M., M. J. M. P., and A. J. R. Final review of the manuscript: all authors.

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Original

Romosozumab in real-life practice – Efficacy and safety results in patients with severe osteoporosis in Castilla-La Mancha

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Abstract

Introduction: severe osteoporosis represents a significant challenge in clinical practice due to its impact on patients' quality of life and the high risk of fractures it entails. Romosozumab, a monoclonal antibody that inhibits sclerostin, has been shown to improve bone mineral density (BMD) through a dual mechanism by increasing bone formation and decreasing resorption. However, its effectiveness and safety in real-world clinical settings require detailed evaluation.

Objective: to assess the effectiveness and safety of romosozumab in patients with severe osteoporosis and high fracture risk in a real-world clinical environment.

Methods: multicenter, retrospective, observational study of patients with severe osteoporosis and/or high fracture risk who received romosozumab between May 2023 and November 2024 in various hospitals and departments across Castilla-La Mancha, with a 12-month follow-up to evaluate changes in BMD, fracture incidence, and adverse effects.

Results: after one year of treatment with romosozumab, the 58 postmenopausal women included in the study (mean age: 71.7 years) showed an increase in BMD of 11.4 % in the lumbar spine, 3.7 % in the femoral neck, and 2.6 % in the total hip. Two vertebral fractures were recorded, with no major cardiovascular events, and bone remodeling markers showed no significant numerical variations.

Conclusion: romosozumab significantly improves BMD in patients with severe osteoporosis, showing an adequate security profile and promising results in real clinical practice.

Keywords:
Osteoporosis. Real world evidence. Romosozumab.

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INTRODUCTION

Osteoporosis is a metabolic bone disease characterized by a progressive decrease in bone mineral density (BMD) and deterioration of bone microarchitecture, leading to increased fragility and, consequently, a higher risk of fractures (1,2). This condition predominantly affects postmenopausal women due to the reduction in estrogen levels, which accelerates bone loss. However, it can also occur in men and in individuals with comorbidities associated with bone mass loss (3).

Osteoporotic fractures are one of the main causes of disability in the elderly population, significantly affecting patients' mobility and functional independence. Among them, vertebral and hip fractures have particularly severe impacts, as they increase both mortality and the risk of future fractures. Therefore, the prevention and effective treatment of osteoporosis are of great importance to reduce the clinical and economic burden of the disease.

In this context, romosozumab has emerged as an innovative therapeutic alternative. It is a humanized monoclonal antibody that inhibits sclerostin, a glycoprotein produced by osteocytes that acts as a negative regulator of bone formation. By blocking sclerostin's action, romosozumab promotes osteoblast differentiation and activity, stimulating new bone formation. Additionally, it exerts an inhibitory effect on osteoclasts, reducing bone resorption. This dual mechanism differentiates it from other available osteoporosis treatments, such as bisphosphonates and denosumab, which primarily target bone resorption inhibition.

The efficacy profile of romosozumab has been widely evaluated in phase III clinical trials. In the FRAME study (Fracture Study in Postmenopausal Women with Osteoporosis), which included more than 7,000 postmenopausal women with osteoporosis, treatment with romosozumab for 12 months was associated with a significant increase in BMD at the lumbar spine (13.3 %) and total hip (6.9 %), as well as a 73 % reduction in the risk of new vertebral fractures vs placebo (4).

Similarly, the ARCH study (Active-Controlled Fracture Study in Postmenopausal Women with Osteoporosis at High Risk), which compared romosozumab with alendronate in women with previous fragility fractures, demonstrated that romosozumab followed by alendronate significantly reduced the risk of vertebral (-48 %), nonvertebral (-19 %), and clinical (-27 %) fractures vs alendronate alone (5). These results establish romosozumab as an effective therapeutic option, particularly in women at high fracture risk.

However, since both studies were conducted under controlled conditions, it is essential to confirm these findings through real-world studies evaluating its effectiveness, tolerability, and safety in more heterogeneous populations and routine clinical settings.

MATERIALS AND METHODS

STUDY DESIGN

We conducted a retrospective, multicenter, and multidisciplinary observational study, involving the Rheumatology and Endocrinology departments of several hospitals in Castile-La Mancha (Spain). Patients with a diagnosis of severe osteoporosis and/or high fracture risk who initiated treatment with romosozumab between May 2023 and November 2024 were included, with a 12-month follow-up. The study was carried out in a real-world clinical practice context, without external intervention in treatment selection by investigators.

INCLUSION AND EXCLUSION CRITERIA

Postmenopausal women with severe osteoporosis confirmed by dual-energy X-ray absorptiometry (DXA) and a past medical history of prior fractures were included. Patients with recent major cardiovascular events (myocardial infarction or stroke) or uncontrolled hypocalcemia were excluded, in line with previously described safety warnings for romosozumab.

VARIABLES ANALYZED

The *main variables* analyzed were:

- *Age, body mass index (BMI), and relevant comorbidities* (such as hypertension, dyslipidemia, diabetes *mellitus,* and history of osteoporotic fracture).
- *Bone mineral density (BMD):* measured at the lumbar spine, femoral neck, and total hip using DXA, with assessments at baseline and at 12 months.
- *Laboratory parameters:* serum creatinine, corrected calcium, phosphorus, alkaline phosphatase, parathyroid hormone (PTH), and vitamin D levels, measured at baseline and at 12 months.
- *Bone turnover markers:* serum levels of P1NP (N-terminal propeptide of type I procollagen) as a bone formation marker and CTX (C-terminal telopeptide of type I collagen) as a bone resorption marker, measured at baseline and at 12 months.
- *Incidence rate of new fractures:* vertebral and nonvertebral fractures were documented during follow-up.
- *Adverse events:* treatment-related adverse events, including injection-site reactions, hypocalcemia,

and cardiovascular events, were recorded during follow-up.

Secondary variables included:
- Age at menopause onset and type of menopause (natural or surgical).
- Previous cardiovascular risk: estimated using the REGICOR tool.

STATISTICAL ANALYSIS

Data cleaning was performed by removing outliers, followed by a descriptive analysis of numerical variables, including minimum and maximum values, quartiles, mean, and standard deviation (SD) (Table I).

On the other hand, to evaluate changes in BMD after one year of romosozumab treatment, a comparative boxplot between baseline and one-year measurements was generated (Fig. 1), along with a hypothesis test for mean differences (Table II). Normality was assessed using the Shapiro–Wilk test; if normality was met, Student's t-test was applied, otherwise, the non-parametric Wilcoxon test was used. A separate hypothesis test was conducted to assess differences in mean baseline and one-year values for bone and renal biomarkers (Table III).

All analyses were performed using Python and the numpy, matplotlib, seaborn, and scipy.stats libraries.

ETHICAL COMMITTEE APPROVAL

The study was approved by the ethics committees of the participating hospitals, as well as by Hospital General

Table I. Statistical summary of clinical variables in women treated with romosozumab

Variables	Mean	SD	Minimum	Percentile 25	Median	Percentile 75	Maximum
Age	71.74	10	34	66	73	78	92
Body mass index	26.11	4.82	18.79	22.32	24.85	29.05	37.30
Age at menopause	47.26	6	30	45	48	51	56
Surgical menopause	0.10	0	0	0	0	0	1
Previous cardiovascular risk	3.06	3.18	0.00	1.00	2.00	5.00	15.00
Number of treatment days	365.47	51.20	28.00	364.00	365.00	369.75	486.00
Lumbar BMD (g/cm^2) baseline	0.78	0.18	0.51	0.67	0.74	0.85	1.32
Femoral neck BMD (g/cm^2) baseline	0.60	0.13	0.30	0.52	0.62	0.69	0.85
Total hip BMD (g/cm^2) baseline	0.66	0.13	0.32	0.56	0.67	0.74	1.00
Lumbar BMD (g/cm^2) after 1 year of tx.	0.90	0.18	0.67	0.77	0.85	1.00	1.40
Femoral neck BMD (g/cm^2) after 1 year of tx.	0.66	0.10	0.47	0.59	0.65	0.74	0.87
Total hip BMD (g/cm^2) after 1 year of tx.	0.71	0.11	0.50	0.62	0.72	0.80	0.89
Phosphorus baseline	3.54	0.66	1.70	3.20	3.50	3.80	5.00
Alkaline phosphatase baseline	82.38	35.12	37.00	60.00	73.00	93.00	223.00
PTH baseline	68.52	33.23	30.30	40.18	60.15	91.15	153.00
Vitamin D baseline	35.73	15.48	6.00	25.00	32.00	44.70	77.30
P1NP baseline	46.20	29.96	0.17	24.50	36.20	63.60	102.00
CTX baseline	35.05	183.43	0.04	0.16	0.29	0.60	971.00
Creatinine at 6 months	0.76	0.16	0.49	0.67	0.72	0.86	1.17
Corrected calcium at 6 months	9.43	0.36	8.70	9.10	9.40	9.70	10.30
Phosphorus at 6 months	3.52	0.62	1.90	3.18	3.45	4.00	5.00
Alkaline phosphatase at 6 months	98.30	36.55	51.00	75.50	87.00	108.00	199.00

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Table I (cont.). Statistical summary of clinical variables in women treated with romosozumab

Variables	Mean	SD	Minimum	Percentile 25	Median	Percentile 75	Maximum
PTH at 6 months + BD1	74.19	45.09	26.20	41.58	63.30	88.85	202.00
Vitamin D at 6 months	37.82	11.79	7.00	31.50	38.00	43.60	75.00
P1NP at 6 months	100.13	121.97	11.10	45.80	58.40	105.50	610.00
CTX at 6 months	0.47	0.50	0.08	0.16	0.37	0.52	2.57
Creatinine at 12 months	0.79	0.19	0.54	0.66	0.75	0.90	1.34
Corrected calcium at 12 months	9.48	0.48	8.72	9.13	9.40	9.90	11.06
Phosphorus at 12 months	3.38	0.80	1.90	3.00	3.30	3.70	6.80
Alkaline phosphatase at 12 months	87.76	31.45	38.00	68.00	74.00	101.00	176.00
PTH at 12 months	77.43	43.32	29.00	48.00	63.70	95.30	212.00
Vitamin D at 12 months	35.68	19.62	0.00	25.90	35.70	43.00	101.00
P1NP at 12 months	53.12	40.45	15.30	26.50	37.75	61.13	191.00
CTX at 12 months	0.67	0.96	0.10	0.17	0.29	0.64	3.75
Fractures with romosozumab	0.02	0	0	0	0	0	1

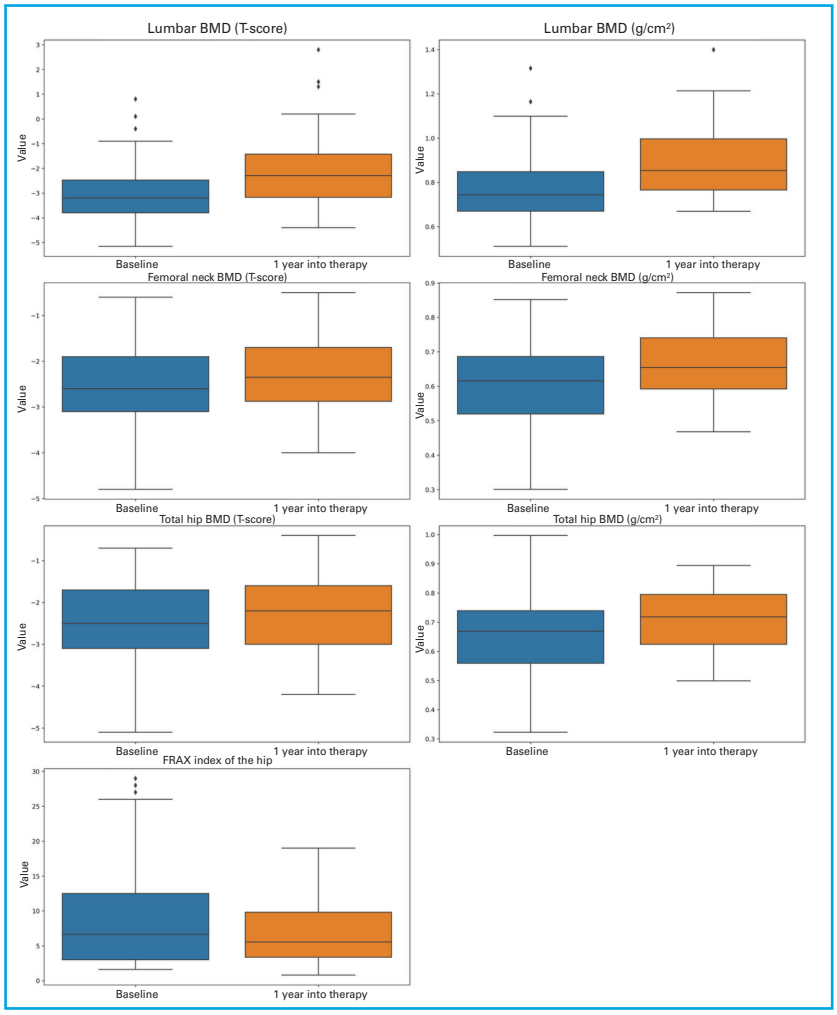

Figure 1. Evolution of BMD from baseline to 1-year treatment values.

Table II. Hypothesis testing for the difference in means between lumbar, femoral neck, and total hip BMD at baseline and 1 year into therapy

Variable	Test used	Statistic	*p*-value	Significant difference
Lumbar BMD (T-score)	Wilcoxon	24	0	Yes
Lumbar BMD (g/cm^2)	Paired Student's t-test	-6.4351	0	Yes
Femoral neck BMD (T-score)	Paired Student's t-test	-2.1984	0.0336	Yes
Femoral neck BMD (g/cm^2)	Wilcoxon	204	0.0094	Yes
Total hip BMD (T-score)	Paired Student's t-test	-2.8122	0.0091	Yes
Total hip BMD (g/cm^2)	Paired Student's t-test	-4.0553	0.0002	Yes

Universitario de Ciudad Real (Ciudad Real, Spain) which led the study (Act No. 12/2023), and was conducted in full compliance with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from patients for publication of the material prior to the procedure (available upon justified request to the principal investigator). Patient data confidentiality was ensured in compliance with current data protection regulations.

RESULTS

The cohort included 58 women with a mean age of 71.74 years (SD ± 10) and an average BMI of 26.11 kg/m^2. The mean age at menopause onset was 47.26 years, with surgical menopause being uncommon (10 %). The mean baseline cardiovascular risk was 3.06 (SD ± 3.18), and the average treatment duration with romosozumab was 1 year (Table I). The main comorbidities were hypertension (16 %) and dyslipidemia (12 %), followed by diabetes *mellitus* (3 %), smoking (2 %), and intestinal malabsorption (1 %).

When comparing BMD T-scores before and after 1 year of treatment, a significant improvement was observed across all assessed regions. Estimated BMD increased by approximately 11.4 % at the lumbar spine, 3.7 % at the femoral neck, and 2.6 % at the total hip, corresponding to improvements in T-score from -2.97 (SD ± 1.37) to -2.02 (SD ± 1.66), from -2.61 (SD ± 0.91) to -2.30 (SD ± 0.83), and from -2.46 (SD ± 0.97) to -2.24 (SD ± 0.94), respectively.

In all DXA-measured regions, a consistent increase was observed in both mean values and quartiles after one year of treatment, as shown in the boxplots (Fig. 1). Exploratory analysis revealed that patients with higher baseline femoral neck values had smaller BMD gains at one year, although this difference was not statistically significant and no multivariate analysis was performed. The hypothesis tests in table II confirmed that differences between baseline and one-year BMD means were statistically significant, with p values < 0.05.

Regarding bone and renal biomarkers, baseline mean values were: creatinine 0.76 mg/dL, corrected calcium 9.42 mg/dL, phosphorus 3.54 mg/dL, alkaline phosphatase 82.38 U/L, PTH 68.52 pg/mL, and vitamin D 35.73 ng/mL.

Table III. Hypothesis testing of bone and renal biomarkers for mean differences between baseline and 1 year into therapy

Variable	Test used	Statistic	*p*-value	Significant difference
Creatinine (mg/dL)	Wilcoxon	313,5	0.1945	No
Corrected calcium (mg/dL)	Paired Student's t-test	-0.9334	0.3561	No
Phosphorus (mg/dL)	Paired Student's t-test	1.7521	0.0876	No
Alkaline phosphatase (U/L)	Wilcoxon	264	0.1866	No
PTH (pg/mL)	Paired Student's t-test	-0.797	0.4301	No
Vitamin D (ng/mL)	Paired Student's t-test	0.0384	0.9696	No
P1NP (ng/mL)	Wilcoxon	20	0.8203	No
CTX (ng/mL)	Wilcoxon	61	0.4874	No

At 6 and 12 months of follow-up, these parameters remained relatively stable, with minor fluctuations lacking clinical relevance. Bone turnover markers CTX and P1NP showed wide dispersion, particularly at baseline, reflecting substantial interindividual variability. Hypothesis tests evaluating mean differences at various follow-up points did not show statistically significant results for any biomarker (Table III).

For the incidence rate of fractures, 2 patients experienced a vertebral fracture during treatment, representing a substantially lower percentage than in historical non-anabolic cohorts.

In terms of safety, six patients reported mild adverse events, including headache and local injection-site reactions. Treatment was discontinued in two cases due to side effects. No major cardiovascular events were recorded.

DISCUSSION

The results of this study are consistent with former clinical trials such as FRAME and ARCH, which demonstrated significant improvements in BMD and reduced fracture risk with romosozumab (4,5). In FRAME, a 13 % increase in lumbar spine BMD was observed after 12 months of treatment, while ARCH reported a 50 % reduction in vertebral fracture risk. Our findings regarding fracture incidence, although limited by the lack of a control group, are consistent with these clinical trial data that confirmed a significant reduction of the risk of vertebral fracture with romosozumab (1,2).

The rate of adverse events in our cohort was relatively low. However, without a direct comparator or subgroup analysis, it is not possible to determine whether this reflects better tolerability or differences in patient selection.

In our cohort, the bone turnover markers CTX and P1NP showed wide variability without statistically significant differences during follow-up. This variability suggests heterogeneous responses among patients, possibly influenced by previous treatments. The dual effect of romosozumab on bone formation and resorption has been well established in controlled trials. For example, in the study by Mineta et al., a significant increase in P1NP was observed at 6 (58.9 %; $p < 0.01$) and 12 months (55.9 %; $p < 0.01$), along with a marked decrease in TRACP-5b —a bone resorption marker— at 6 months (-14.7 %; $p < 0.001$) and 12 months (-18.8 %; $p < 0.001$) *vs* baseline (7). These findings confirm the anabolic and antiresorptive profile of romosozumab under controlled conditions. However, our results did not reproduce this pattern conclusively, likely due to the influence of prior antiresorptive therapy, which may have modulated the biochemical response. In this regard, studies in postmenopausal women with osteoporosis have shown that the response to romosozumab or denosumab may vary according to prior treatment. In treatment-naïve patients, denosumab administration is associated with an early increase in P1NP and a significant reduction in CTX from the first few months, whereas previously bisphosphonate-treated patients show a weaker or delayed response, likely due to residual effects on bone remodeling (8).

These results suggest that prior therapy type may significantly influence romosozumab's clinical and densitometric response. In a prospective Swiss cohort of 99 patients (9), treatment-naïve individuals showed significantly greater BMD responses at both the lumbar spine and hip compared with pretreated patients. In our real-world experience, most romosozumab candidates had previously received antiosteoporotic therapy. In our cohort, only 6 patients were treatment-naïve, and no statistical comparison with the pretreated group was performed due to the small subsample size. Thus, previous treatment heterogeneity may have partially influenced the observed outcomes.

Moreover, our data reinforce evidence supporting the drug's safety. Compared with the STRUCTURE study (6), which reported cardiovascular events in 2 % of patients, no major events occurred in our study, suggesting a potentially more favorable safety profile in the evaluated population.

Of note, this study focused exclusively on postmenopausal women, consistent with romosozumab's approved indication. However, the BRIDGE trial —a phase III randomized, placebo-controlled, double-blind study— showed similar efficacy in men (10), suggesting potential benefit beyond the female population. Nevertheless, more evidence is needed before routine use in men, given the numerical imbalance in confirmed major cardiovascular events [romosozumab: 8 (4.9 %) vs placebo: 2 (2.5 %)].

Furthermore, phase II extension studies have evaluated romosozumab's 24-month safety and efficacy profile, showing continued BMD gains > year 1, particularly with monthly 210 mg dosing, and that transition to subsequent treatments such as denosumab may maintain or even enhance these benefits (11). Our findings suggest that treatment effects may be more pronounced in patients with lower baseline BMD, as also noted in McClung's study (11). However, as this was an unadjusted exploratory analysis, results should be interpreted with caution.

Overall, these results reinforce the utility of romosozumab in patients with severe osteoporosis and high fracture risk, highlighting its positive impact on BMD and appropriate safety profile in clinical practice.

Nonetheless, therapeutic response appears modulated by previous treatment history; future research should focus on identifying specific clinical profiles predictive of efficacy and on evaluating optimal therapeutic sequencing strategies with other antiosteoporotic agents to maximize long-term benefits.

CONCLUSIONS

Romosozumab is an effective treatment for improving BMD in patients with severe osteoporosis and/or high fracture risk. The results obtained in real-world clinical practice confirm its efficacy and safety, aligning with previous findings. Its use should be considered in selected patients, particularly those with low baseline BMD and high fracture risk.

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Original

Early-onset osteoporosis associated with variants in the *WNT1* gene

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Abstract

Introduction: early-onset osteoporosis is a poorly recognized condition that affects young men and premenopausal women. In some cases, it may have a monogenic origin, involving variants in genes related to bone homeostasis. *WNT1* encodes a ligand of the Wnt signaling pathway, which plays a crucial role in osteoblastic differentiation. This study describes a series of patients with early-onset osteoporosis associated with heterozygous variants in the *WNT1* gene.

Patients and methods: patients with early-onset osteoporosis and no identifiable secondary cause referred to our Bone Metabolism Unit were reviewed. Biochemical and densitometric studies were performed, as well as next-generation sequencing using a gene panel related to bone metabolism. Variants were analyzed using bioinformatic tools and assessed for their potential clinical impact.

Results: a total of 6 patients (2 men and 4 women) with *WNT1* gene variants were identified. The mean age at diagnosis was 36 years. Four patients presented osteoporosis with vertebral and peripheral fractures, while 1 had multiple peripheral fractures. One patient also showed joint hypermobility and was found to carry a heterozygous variant in the FLNA gene. Another exhibited vertebral fractures during lactation. All received treatment for osteoporosis, with variable responses.

Conclusions: heterozygous variants in the *WNT1* gene may be associated with early-onset osteoporosis and an increased fracture risk. Genetic sequencing enables improved etiological diagnosis, although additional studies are needed to optimize therapeutic management, including the potential benefit of drugs that modulate Wnt pathway activity.

Keywords:
Monogenic bone disorders. *WNT1*. Early-onset osteoporosis.

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INTRODUCTION

Osteoporosis is a metabolic bone disease characterized by reduced bone mass and alterations in bone microarchitecture, resulting in an increased risk of fracture following low-impact trauma. It predominantly affects postmenopausal women and men older than 50 years. However, early-onset forms of the disease exist in young men and premenopausal women, though their incidence rate remains poorly established (1). These younger individuals have a longer life expectancy and therefore may accumulate a higher lifetime risk of fragility fractures.

During the initial evaluation of these patients, it is essential to rule out potential secondary causes of osteoporosis. In certain cases, a fragility fracture may represent the first sign of monogenic osteoporosis, making it important to identify variants in genes related to skeletal homeostasis. The best-known example is osteogenesis imperfecta, usually caused by pathogenic variants in the genes encoding type I collagen chains (*COL1A1* and *COL1A2*) and, more rarely, by variants in other genes involved in bone formation and osteoid matrix production (2). However, other patients develop early-onset osteoporosis, detected in childhood or early adulthood, without features typical of osteogenesis imperfecta. Although the pathogenesis of these cases is not well understood, some involve variants in genes associated with bone formation, such as those encoding Wnt ligands—a signaling pathway essential for osteoblast formation (3). The aim of this study was to describe the characteristics of a series of patients with early-onset osteoporosis associated with *WNT1* gene variants, which encodes one such Wnt ligand.

PATIENTS AND METHODS

All patients referred to our Bone Metabolism Unit, Internal Medicine Department, Hospital Universitario Marqués de Valdecilla (Santander, Spain) (reference center for the autonomous community of Cantabria), for early-onset osteoporosis (premenopausal women and men under 50 years old) with no evidence of secondary causes were reviewed.

In addition to standard biochemical and radiological studies, next-generation sequencing was performed in all patients, analyzing a total of 49 individuals.

DNA extracted from peripheral blood was analyzed using a custom SureSelect QXT (Agilent) gene panel including genes related to bone metabolism: *ABCC6, ABL1, ADAMTS10, ADAMTS17, ADAMTSL4, ALDH18A1, ALPL, AMER1, ANKH, AP2S1, ATP6V0A2, ATP7A, B3GAT2, BMP1, CA2, CASR, CHST14, CBS, CDKN1C, CLCN5, CLCN7, COL11A1, COL11A2, COL1A1, COL1A2, COL2A1, COL3A1, COL5A1, COL5A2, COMP, CREB3L1, CRTAP, CTSK, CYP27B1, DHCR7, DMP1, DVL1, ELN, ENPP1, FAM20C, FBLN5, FBN1, FDPS, FGF23, FGFR1, FGFR3, FKBP10, FLNA, FUCA1, GAA, GALNS, GALNT3, GBA, GDF3, GDF6, GLA, GLB1, GNA11, GNAS, IDS, IDUA, IFITM5, LEMD3, LGR4, LMX1B, LRP4, LRP5, LRP6, MED12, MEOX1, P3H1, PHEX, PLOD1, PLOD2, PLS3, PPIB, PTH1R, ROR2, RUNX2, SEC24D, SERPINF1, SERPINH1, SGSH, SHOX, SLC29A3, SLC2A10, SLC34A3, SLCO2A1, SMAD3, SMAD6, SMPD1, SOST, SOX9, SP7, SPARC, TCIRG1, TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, TMEM38B, TNFRSF11A, TNFRSF11B, TNFSF11, TNXB, VDR, WNT1, WNT16, WNT5A, BMS1, XYLT2, ZNF469.* Library quality and concentration were quantified using a chip-based capillary electrophoresis system (TapeStation, Agilent) and sequenced on the MiSeq platform (Illumina). Variant filtering and analysis were conducted using the Alissa (Agilent) bioinformatics platform, considering variants with clinical relevance according to ClinVar 20180401 and pathogenic predictions from PolyPhen, SIFT, Mutation Taster, and Human Splice Finder. Copy number variation analysis was performed using DECoN software. Genetic variant classification followed the American College of Medical Genetics and Genomics (ACMG) guidelines (4).

All patients gave their prior informed consent for genetic analysis. The study was approved by the Ethics Committee (Internal Code: 2022.156).

RESULTS

We identified a total of 6 patients with heterozygous *WNT1* variants (pathogenic, likely pathogenic, or of uncertain significance; 2 men and 4 women, with a median age at genetic diagnosis of 49 years [range, 37-66]). The median age at the index fracture was 40 years (range, 5-49).

Main clinical and densitometric characteristics are shown in table I. Genetic alterations are detailed in table II.

Cases 1, 3, 4, and 6 had low BMD with vertebral and peripheral fractures. All but cases 2 and 4 had sustained > 2 vertebral fractures at diagnosis. Case 2 presented multiple peripheral fractures following low-energy trauma.

Case 3 had a hypermobility syndrome with recurrent patellar dislocation (> 10 episodes) and a combined patellar and femoral condyle fracture. A heterozygous variant in *FLNA*—which encodes filamin A—was also identified and could explain the joint hypermobility.

Table I. Phenotypic and densitometric characteristics of the patients

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age at onset / sex	47 / Male	41 / Male	49 / Female	37 / Female	39 / Female	5 / Female
Fractures	Yes	Yes	Yes	Yes	Yes	Yes
Type of fractures	5th and 7th ribs; T8 and T9; ulna; radius; metacarpal	Fibula; metacarpal; elbow; acetabular labrum	T11, T12, L1 and L2; patella; femoral condyle; hip fracture	T9, T11, L2 and L5	Metatarsal	Femur; radius; T11-T12
Lumbar spine	Z: -2.9 T: -3.9	Z: -1.8 T: -1.8	Z: -1.8 T: -3.4	Z: -3.4 T: -3.5	Z: -0.5 T: -1.6	Z: -1.6 T: -3.7
Femoral neck	Z: -1.7 T: -2.7	Z: -1.3 T: -1.8	Z: -2.0 T: -3.4	Z: -1.2 T: -1.5	Z: -1.6 T: -2.6	Z: 0.4 T: -1.4
Total hip	Z: -1.7 T: -2.7	Z: -0.9 T: -1.1	Z: -1.9 T: -3.0	Z: -0.9 T: -1.0	Z: -2.1 T: -2.8	Z: 0.3 T: -1.2
Trabecular Bone Score	Not available	L1-L4: 1.280	L1-L4: 1.110	Not available	L1-L4: 1.175	L1-L4: 1.012

Table II. Genotypic characteristics of variants in *WNT1*. In all cases, the variants were heterozygous

Case	Reference no.	DNA	Protein	Type of variant	Effect	Other variants
Case 1	NM_005430.4	c.1005_1027del23	p.Thr336Alafs*125	Frameshift	Probably pathogenic	DVL1 (VUS) p.Ala658Thr
Case 2	NM_005430.4	c.541G>A	p.Gly181Ser	Missense	Uncertain significance	–
Case 3	NM_005430.4	c.105-4C>T	p.?	Splice-site	Uncertain significance	FLNA (VUS) p.Ile2115Val
Case 4	NM_005430.4	c.506dupG	p.Cys170Leufs*6	Frameshift	Pathogenic	–
Case 5	NM_005430.4	c.105-6C>T	p.?	Splice-site	Uncertain significance	BMS1 (VUS) p.Arg1068Lys
Case 6	NM_005430.4	c.308A>C	p.Asn103Thr	Missense	Uncertain significance	CREB3L1 (VUS) p.Leu390Serfs*15

Case 4 presented early-onset osteoporosis during the postpartum period while breastfeeding. She had four vertebral fractures at diagnosis with severe low back pain and achieved satisfactory clinical recovery after completing lactation and starting anabolic therapy.

Case 5 had a past medical history of transient regional osteoporosis of both feet and a metatarsophalangeal stress fracture years before densitometric diagnosis.

Case 6 presented a low-impact femoral fracture during childhood, followed by peripheral fractures during premenopause and BMD values in the osteoporotic range. She was sequentially treated with raloxifene, aminobisphosphonates, teriparatide, and denosumab.

Treatment varied according to the attending clinician. All patients received osteoporosis therapy, at least with teriparatide, except case 5, who was trea-

ted only with aminobisphosphonates. Cases 3 and 6 experienced new fragility fractures despite treatment. Case 3 sustained a hip fracture eight years after diagnosis, while on zoledronic acid therapy, despite improved BMD. Case 6 developed a vertebral fracture during ibandronate treatment but had no further fractures after completing sequential courses of zoledronate, teriparatide, and finally denosumab.

DISCUSSION

It is well known that loss of function in the canonical Wnt pathway, and the resulting decrease in Wnt/β-catenin signaling, leads to impaired osteoblastic differentiation and thus reduced bone formation. Likewise, lower Wnt activity decreases osteoprotegerin expression and increases RANKL, leading to enhanced bone resorption (5,6).

Wnt1 is a soluble ligand that binds to LRP5/6 and Frizzled receptor complexes on osteoblast precursor cells. Biallelic defects (homozygous or compound heterozygous variants) in *WNT1* cause a rare form of osteogenesis imperfecta, classified as type XV (2). Patients exhibit short stature, multiple vertebral and peripheral fractures, and frequent deformities. About half present cranial abnormalities such as microcephaly, cranial asymmetry, ptosis, hydrocephalus, cerebellar hypoplasia, or Chiari type I malformation, and approximately 40 % have neurodevelopmental delay or severe intellectual disability (7). In contrast, monoallelic *WNT1* defects (heterozygous variants) appear to be associated with early-onset osteoporosis.

Early-onset osteoporosis has various etiologies. Many cases are due to diseases (celiac disease, hyperthyroidism, malnutrition, mastocytosis, etc.) or drugs (glucocorticoids, sex hormone antagonists, etc.). Other early-onset forms are genetic, some associated with recognizable syndromes (e.g., Turner syndrome). In others, skeletal involvement is the sole or predominant manifestation. New massive sequencing techniques allow identification of a causal variant in approximately 20 % of patients (8). These represent "primary" osteoporoses, whereas the rest may be considered "idiopathic," likely resulting from a combination of factors including multiple low-impact variants, environmental influences, and epigenetic mechanisms.

Among genes associated with primary early-onset osteoporosis are those related to the Wnt pathway, including receptors LRP5/6 (9) and ligands such as *WNT1* (10). In addition to our series, other reports have described heterozygous *WNT1* variants (Table III). Although we lack a precise estimate of their prevalence among early-onset osteoporosis cases, some studies suggest they may represent up to 10 % (11). In total, approximately 160 mutations have been documented in this gene (https://databases.lovd.nl/shared/genes/*WNT1*).

To provide context for the genetic variants identified in this study, we present figure 1 that illustrates the location of heterozygous *WNT1* variants identified in our patients and previously published cases. Table III summarizes published series of patients with *WNT1* variants, and figure 2 shows homozygous *WNT1* mutations associated with osteogenesis imperfecta type XV.

In the absence of functional studies of the variants to demonstrate their pathogenic nature, it is impossible to determine the extent to which these *WNT1* variants contribute to the pathogenesis of skeletal fragility in these patients. Nevertheless, it is reasonable to consider that they may represent an important factor, in addition to the role of other genetic variants—whether identified or not in our analysis. One of the patients presented a variant in DVL1, a gene that encodes another component of the Wnt signaling pathway (12). However, variants in this gene have not been described as being associated with osteoporosis. Variants in FLNA have also not been linked to early-onset osteoporosis, although they have been associated with vascular and connective tissue abnormalities, including joint hypermobility, as observed in case 3 (13). Some cases of familial osteoporosis and osteogenesis imperfecta related to variants in the BMS1 and CREB3L1 genes, respectively, have been reported (14,15). Therefore, the variants of uncertain significance found in these genes might contribute to the osteoporosis observed in these patients. In fact, contrary to the traditional view that common diseases result from common variants with low functional impact and Mendelian diseases from rare variants with major effects, recent years have seen the emerging concept that both common and rare variants may influence the phenotype of patients with prevalent diseases (16,17).

There are few data regarding the treatment of patients carrying pathogenic variants in genes of the Wnt pathway. Some publications have suggested a poor response to bisphosphonates (18), whereas other authors have reported that antiresorptive drugs such as denosumab or aminobisphosphonates exert a beneficial effect in terms of bone mass gain (19). In our patients, treatment with an anabolic agent such as teriparatide was often chosen to promote osteoblastogenesis (20). The potential role of antibodies that block inhibitors of the Wnt pathway, such as sclerostin or DKK1, remains unclear.

In conclusion, biallelic defects in *WNT1* cause a form of osteogenesis imperfecta. Monoallelic defects may be associated with early-onset osteoporosis and a tendency to present vertebral and peripheral fractures.

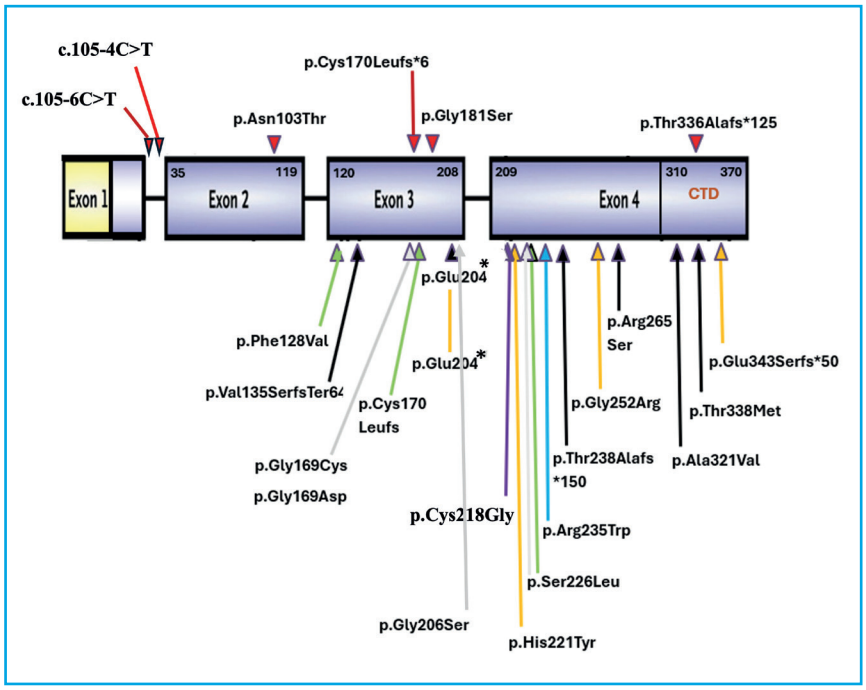

Figure 1. Heterozygous *WNT1* variants in patients with early-onset osteoporosis. Red arrows indicate mutations found in our case series. Yellow arrows show variants published by Perís et al. (11). Green arrows indicate those reported by Hu et al. (22). The blue arrow indicates those published by Palomo et al. (23). Violet arrows correspond to the regions affected in patients from Mäkitie et al. (24). Black arrows represent the regions of Rouleau et al. (26). Gray arrows correspond to the work published by Wang et al. (27). CTD: C-terminal domain. Yellow indicates the exon region transcribed but not translated; violet indicates the portion translated into protein.

Table III. Comparative descriptive table of published patient series with *WNT1* mutation*

Authors	Population	*WNT1* mutation in homozygosis (No. of patients)	*WNT1* mutation in heterozygosis (No. of patients)	Mean age** and range (years)	Fractures	Treatment
Perís et al. (11)	Early-onset OP	0	5	27 (2-48)	3 vertebral, 11 peripheral, 1 femur, 1 pelvis	Teriparatide Denosumab
Hu et al. (22)	Early-onset OP, Osteogenesis imperfecta	12	4	31 (7.3-47)	8 vertebral, 9 peripheral	Bisphosphonates Denosumab
Palomo et al. (23)	Early-onset OP, Osteogenesis imperfecta	4	6	35 (10-45)	8 vertebral, 3 peripheral	Not specified
Mäkitie et al. (24,25)	Early-onset OP	0	18	22 (8-42)	9 vertebral, 60 peripheral	Bisphosphonates Teriparatide Denosumab
Välimäki et a. (20)	Early-onset OP	0	3	No especifican	11 vertebral, 2 peripheral, 1 femur	Bisphosphonates
Rouleau et al. (26)	Idiopathic primary OP	0	6	23 (7-52)	3 vertebral, 5 peripheral, 3 femur	Not specified
Wang et al. (27)	Early-onset OP	0	4	8 (2-12)	8 vertebral, 4 peripheral	Bisphosphonates

**The table does not represent an exhaustive review of all published cases in the literature.*
***The diagnostic age is defined by the index clinical fracture.*

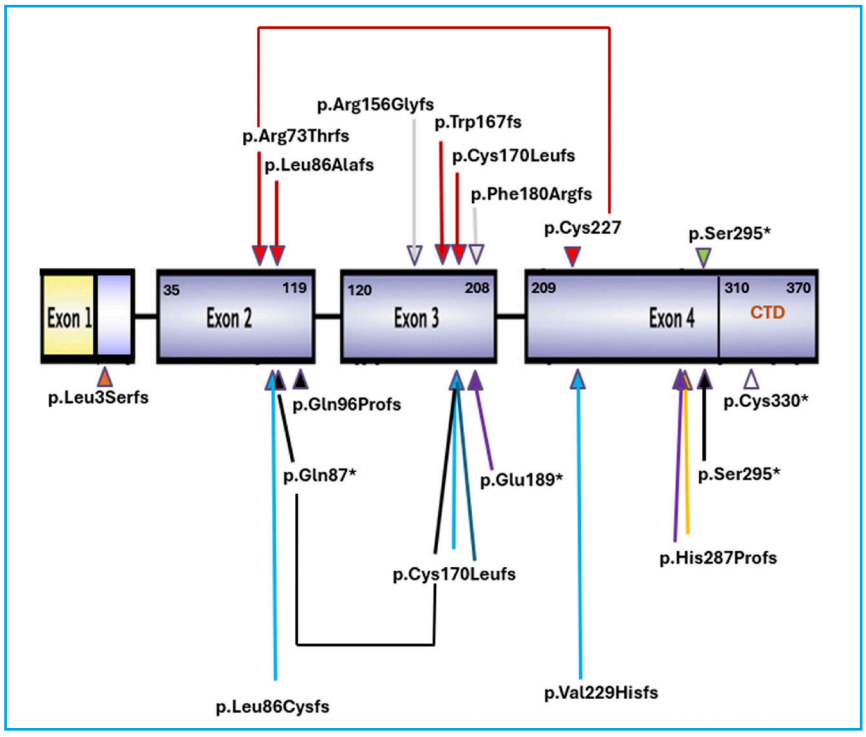

Figure 2. Biallelic *WNT1* variations in patients with osteogenesis imperfecta type XV. Blue arrows correspond to those published by Nampoothiri et al. (7). Violet arrows show affected positions in patients from Keupp et al. (21). Red arrows show mutations reported by Hu et al. (22). The green arrow indicates those published by Laine et al. (28). Gray arrows correspond to Lu et al. (29). Black arrows indicate regions described by Pyott et al. (30). The yellow arrow shows the mutation published by Kantaputra et al. (31). The orange arrow corresponds to Kuptanon et al.[32], the white arrow to Faqeih et al. (33), and the navy blue arrow to Mrosk et al. (34). Colored lines connect compound heterozygous mutations. CTD: C-terminal domain. Yellow indicates the exon region transcribed but not translated; violet indicates the portion translated into protein.

It would be of great interest to develop clinical trials aimed at identifying the optimal treatment for this population and, in particular, to assess the potential benefit of using Wnt pathway inhibitor drugs.

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Original

mir-199b-5p as a potential biomarker of bone fragility in type 2 diabetes *mellitus* – Dual role in musculoskeletal pathophysiology

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Abstract

Type 2 diabetes *mellitus* (T2DM) is associated with increased risk of bone fragility and fractures, yet early diagnostic tools for musculoskeletal complications remain lacking. This study aimed to identify microRNAs (miRNAs) differentially expressed in T2DM patients and assess their potential as biomarkers for bone fragility. Serum samples from 8 T2DM patients and 8 matched healthy controls were analyzed using high-throughput sequencing and RT-qPCR. Among the miRNAs examined, hsa-miR-199b-5p was significantly under-expressed in T2DM patients, particularly in those with degraded trabecular bone score (TBS) and lower bone mineral density (BMD).

Statistical analyses revealed strong positive correlations between miR-199b-5p expression and indicators of bone integrity, including cortical and trabecular volumetric BMD, and serum periostin levels. Conversely, negative correlations were found with fracture risk (FRAX), TBS-adjusted FRAX, and CTX levels, supporting its role in bone metabolism. Literature suggests miR-199b-5p promotes osteogenesis via the GSK-3β/β-catenin and periostin-mediated Wnt/β-catenin signaling pathways. Thus, its reduced expression may contribute to impaired bone remodeling in T2DM. Interestingly, miR-199b-5p shows contrasting effects in osteoarthritis (OA), where it is upregulated and contributes to cartilage degradation. Periostin, similarly, promotes bone formation in T2DM but may exacerbate inflammation in OA. These findings underscore the context-dependent roles of miR-199b-5p and highlight its potential as a dual biomarker: protective in T2DM bone loss, yet detrimental in OA. Further research is needed to clarify its therapeutic relevance and ensure disease-specific targeting strategies.

Keywords:
Type 2 diabetes *mellitus*. microRNAs. Bone fragility. Osteoarthritis. Biomarkers.

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INTRODUCTION

Diabetes *mellitus* is a leading cause of morbidity and mortality worldwide (1), with type 2 diabetes *mellitus* (T2DM) accounting for 90% % of all diabetes cases (2). The recent surge in the diagnosis rates of these and other lifestyle-related metabolic disorders can largely be attributed to the widespread adoption of sedentary lifestyles and excessive energy consumption in industrialized nations (3). Additional factors such as advanced age, hypertension, and family history further increase the predisposition to develop T2DM (4).

Currently, 13.8% % of the Spanish population suffers from T2DM, with an estimated 6% % of individuals unaware of their condition. Furthermore, approximately 12.6% % of the Spanish population is at risk of developing T2DM due to impaired glucose tolerance or abnormal fasting glucose levels (5). According to the di@bet.es study, 5.98 million Spaniards have issues with glucose metabolism (5). The International Diabetes Federation (IDF) projects that the number of diabetic patients globally will reach 643 million by 2030, making diabetes one of the "epidemics of the 21st century" (6,7).

In the early stages of T2DM, metabolic disturbances such as hyperglycemia, insulin resistance, dyslipidemia, and hyperinsulinemia contribute to cellular and organ-level damage, resulting in both microvascular complications (e. g, retinopathy, nephropathy, neuropathy) and macrovascular complications (e. g, coronary artery disease, cerebrovascular disease, peripheral artery disease). These vascular issues account for approximately 70–80% % of diabetes-related mortality (8,9). In addition to these vascular issues, growing evidence has highlighted that bone fragility is also prevalent in T2DM (10). The risk of fractures in patients with T2DM has significantly increased due to changes in bone remodeling and microarchitecture. Several studies have demonstrated a higher risk of fractures, particularly at the hip and vertebrae (11,12). This fragility is driven by chronic hyperglycemia, insulin resistance, and the accumulation of advanced glycation end products (AGEs), which impair bone quality and disrupt osteoblast-osteoclast balance (10). Indeed, multiple meta-analyses have confirmed an increased risk of incident hip, vertebral, and non-vertebral fractures in individuals with T2DM, with major consequences for quality of life (QoL) and long-term disability (13,14). On the other hand, recent studies have also drawn a connection between osteoarthritis (OA) and metabolic syndrome, a cluster including insulin resistance, dyslipidemia, and hypertension, further linking diabetes to musculoskeletal deterioration (15,16). The presence of diabetes is believed to accelerate the progression of OA and complicate its management, leading to the proposed diabetes-induced osteoarthritis (DM-OA) phenotype (17). This phenotype suggests that systemic inflammation and oxidative stress in diabetes predispose patients to OA, with chronic hyperglycemia promoting cartilage degradation, joint inflammation, AGEs accumulation, and matrix stiffening, all of which impair joint cushioning and function (18). Recognizing musculoskeletal complications of type 2 2 diabetes mellitus*mellitus* as clinically significant outcomes, comparable with traditional vascular complications, underscores the urgent need for early detection strategies and targeted therapies to preserve bone and joint health in this growing patient population.

However, there is currently no tool available for the early diagnosis of these musculoskeletal complications. In this context, evaluating the differential expression of molecules such as microRNAs (miRNAs) may offer valuable insights into the development of diagnostic techniques for this population. The potential of miRNAs as biomarkers for musculoskeletal complications is supported by a growing body of scientific literature, which reports differential miRNA responses to various diseases (19,20), further highlighting the importance of investigating their role in T2DM-associated musculoskeletal deterioration.

In addition to miRNAs, proteins such as periostin have also emerged as important biomarkers in T2DM-related bone fragility. Periostin, a protein originally identified as osteoblast-specific factor-2, is involved in bone formation and remodeling but also plays a role in other complications of T2DM, such as cardiovascular disease (21). Th interaction of periostin with pathways such as Wnt/β-catenin, which regulate osteoblast differentiation and bone metabolism, makes it a potential therapeutic target for improving bone health in diabetes (22). Its levels are altered in various musculoskeletal disorders, including those seen in T2DM, making it a promising biomarker for osteoporosis and bone fragility in these patients.

MicroRNAs (miRNAs) are small endogenous RNA molecules that post-transcriptionally regulate gene expression. They have been shown to play key roles in a wide range of biological processes. Recent advancements in transcriptomic technologies, especially next-generation sequencing and advanced bioinformatics tools, enable more in-depth exploration of messenger RNAs (mRNAs) and non-coding RNAs (ncRNAs), including miRNAs (23). Over 2,000 miRNAs have currently been identified, and it is estimated that they regulate approximately 30% % of all human genes. miRNAs are present in circulating blood, representing an opportunity to use these disease-related circulating miRNAs as potential biomarkers (24). In this study, the miRNome of a group of healthy individuals and patients with T2DM was analyzed to identify differentially expressed miRNAs between the 2 2 groups. The differential expression of selected miRNAs was subsequently validated using RT-qPCR. Following validation, the potential role of these miRNAs, particularly miR-199b-5p, as biomarkers in T2DM was further investigated through statistical analysis of their correlation with determinants of bone fragility and fracture risk.

MATERIALS AND METHODS

STUDY POPULATION

This study included a total of 16 participants, 8 T2DM patients (males with a mean age of 60 ± 5 years) and 8 control subjects, sex and aged matched. T2DM was diagnosed according to the American Diabetes Association criteria of 2017. T2DM patients were recruited during 2015 from the Endocrinology and Nutrition Unit of *Hospital Universitario Clínico San Cecilio* (Granada, Spain). Samples from healthy controls were managed and provided by the Andalusian Biobank.

Inclusion criteria for patients with T2DM were absence of cardiovascular disease, history of cardiovascular events, or renal, hepatic, GI, or thyroid disease. All patients were on diabetes drugs, such as metformin, sulfonylureas, insulin, or a combination of these drugs.

Venous blood samples were obtained at the Clinical Analysis Unit of Hospital Universitario Clínico San Cecilio.

The study was approved by the Ethics Committee of Jaén, Andalucía (ID 1630-M1-18/PI-0514-2018; December 20, 2018), and conducted in accordance with relevant ethical guidelines for human and animal research. Written informed consent was obtained from all participants.

CLINICAL EVALUATION, BIOCHEMICAL AND BONE PARAMETERS OF THE STUDY POPULATION

Anthropometric and biochemical measurements

Height, weight, and waist circumference were recorded following standard protocols. BMI was calculated as weight (kg) divided by height squared (m^2). Fasting venous blood samples were collected in the morning, with serum stored at 80 °C until analysis. Biochemical parameters measured included HbA1c, total cholesterol, HDL-c, LDL-c, triglycerides, creatinine, calcium, phosphorus, and vitamin D, using routine automated methods. Serum bioactive periostin was quantified in duplicate by ELISA (Biomedica Medizinprodukte GmbH), with detection ranges of 20-4,000 pmol/L and intra-/inter-assay variation ≤ 6% % and ≤ 3% %, respectively. Average periostin levels in healthy individuals are ~864 pmol/L.

Hypertension and Dyslipidemia Assessment: Blood pressure was measured using an automated sphygmomanometer after 5 minutes of rest, with 2 2 readings 1-2 minutes apart; additional readings were taken if differences exceeded 10 mmHg. The mean of the last 2 2 readings was used. Hypertension was defined as systolic/diastolic ≥ 140/90 mmHg or antihypertensive treatment. Dyslipidemia was defined by HDL-c < 50 mg/dL, LDL-c > 100 mg/dL, triglycerides > 150 mg/dL, and/or lipid-lowering drug use.

Areal bone mineral density (aBMD)

aBMD of the left hip was measured using a Hologic QDR 4500 densitometer. The shaft region was located 2 cm distal to the midpoint of the lesser trochanter along the shaft axis. Osteoporosis and osteopenia were categorized based on World Health Organization (WHO) criteria. Scans were performed by an experienced operator following ISCD guidelines; the device was calibrated daily with a spine phantom. Laboratory coefficients of variation were 1.8% % (femoral neck) and 1.5% % (total hip), with prior studies reporting 2.13% % and 3.14% % for shaft and trochanter, respectively.

Trabecular Bone Score (TBS)

TBS was measured at the LS using the latest version of TBS iNsight (version 3.0.2.0, Medimaps, Merignac, France). TBS was calculated as the mean value of the individual measurements for vertebrae L1-L4, based on a grey-level analysis of the DXA images. The TBS precision error (percentage of the coefficient of variation) was 1.82% %. Diagnosis of preserved and degraded microarchitecture was based on the following TBS ranges: patients with TBS values ≥ 1.31 were categorized as preserved TBS and patients with TBS values < 1.31 were categorized as degraded TBS (25).

3D-DXA modeling

Trabecular macrostructure, cortical thickness, and femoral shape were assessed using 3D-Shaper software (v2.2), which generates participant-specific QCT-like models by registering a statistical shape and density model from QCT scans onto DXA images. Cortical thickness and density were derived from fitted mathematical functions along the femur surface. Measures included volumetric BMD (vBMD) of trabecular, cortical, and integral compartments, and cortical surface BMD (sBMD). Strong correlations with Quantitative Computed Tomography (QCT) (r = 0.86-0.95) and low coefficients of variation demonstrated high accuracy and precision.

Fracture Risk Assessment (FRAX)

FRAX estimates the 10-year probability of hip and major osteoporotic fractures by integrating clinical risk factors such as age, sex, BMI, fracture history, parental

hip fracture, smoking, glucocorticoid use, rheumatoid arthritis, secondary osteoporosis, alcohol intake adjusted by BMD and TBS values.

RNA EXTRACTION

miRNeasy Serum/Plasma kit (QIAGEN) was used to extract total RNA from 200 µL of serum samples following the protocol in full compliance with the manufacturer's instructions for use. UniSp2, UniSp4 and UniSp5 sequences were added using RNA Spike-in mix (vial 1 of the miRNeasy Serum/Plasma kit) to be used as a control for this step. The extracted RNA was used to carry out the sequencing and validation steps.

SEQUENCING

Library development and sequencing

The extracted total RNA samples were sequenced through Illumina technology. miRNA libraries development was performed using the QIAseq miRNA Library Kit (QIAGEN). Adapters were ligated with molecular unique identifiers to subsequently back-transcribe the samples to cDNA, which was amplified by PCR. To guarantee the quality of the amplified samples, these were purified by capillary electrophoresis analysis (Alignent DNA 1000 Chip). Libraries were pooled equimolar and quantified by qPCR. Libraries were sequenced using a NextSeq sequencer (Illumina Inc). FASTAQ files of the sequences were created using bcl2fastq software.

Differential gene expression

Differential expression of miRNAs was analyzed using the Empirical Analysis of Differential Gene Expression (DGE) algorithm implemented in CLC Genomics Workbench v20.0.4. The NormFinder program, developed by Andersen et al. (26), was used to identify endogenous control miRNAs and to generate the volcano plot. Visualization of the volcano plot was performed using the ggplot2 and ggrepel packages in RStudio. For miRNAs selected based on the volcano plot criteria, Mann-Whitney tests were conducted on variance stabilizing transformation (VST)-normalized data, retaining those with p-values < 0.05.

VALIDATION

Polyadenylation and reverse transcription

miRNA molecules were reverse transcribed to cDNA with a poly(A) tail at the 3′ end using the miRCURY LNA RT Kit (Qiagen), according to the manufacturer's protocol. UniSp6 6 RNA spike-in was included as a control.

Quality control

RNA sequences added in the total RNA extraction step (UniSp2, UniSp4 and UniSp5) and in reverse transcription step (UniSp6) were amplified to make sure that the process had been conducted correctly. In addition, hemolysis-related sequences (has-miR-451a and has-miR-23a-3p) and endogenous miRNA sequences (hsa-miR-26a-5p and has-miR-30d-5p) were amplified to determine the quality of starting samples. All reactions were performed in triplicate. miRCURY LNA SYBR Green PCR kit was used to perform RT-qPCR following the protocol indicated by the manufacturer. According to the miRCURY LNA miRNA PCR assay protocol, the PCR conditions consisted of 2 2 steps: first, a denaturation step at 95 °C for 10 seconds, followed by a combined annealing/extension step at 56 °C for 60 seconds. The reaction occurred for a total of 40 cycles.

RT-qPCR

First, miRCURY LNA miRNA Custom PCR Panels were designed (Fig. 1). These plates were designed to amplify: 1) miRNAs identified in the sequencing; 2) 3 endogenous miRNAs; and 3) 2 control miRNAs (UniSp3 and UniSp6) in triplicate. miRCURY LNA SYBR Green PCR kit as well as in the quality control step was used.

miRNA expression analysis

The ΔΔCt relative quantification method was used to evaluate the differential expression of miRNAs in the study groups. miRNA expression data are expressed as mean ± standard deviation (SD).

$$\Delta CT\ (patients) = CT\ (miRNA\ study) - CT\ (miRNA\ problem)$$
$$\Delta CT\ (controls) = CT\ (miRNA\ study) - CT\ (miRNA\ problem)$$
$$\Delta\Delta CT = \Delta CT\ (patients) - \Delta CT\ (controls)$$
$$FC = 2\text{-}\Delta\Delta CT$$

Statistical analysis

Student t tests were performed, with $p \leq .05$ 5 considered statistically significant. Binary logistic regression was conducted using the Wald forward stepwise method. Receiver operating characteristic (ROC) curves were generated from the regression results. Statistical analyses were performed with SPSS, version 25 5 (IBM Corp).

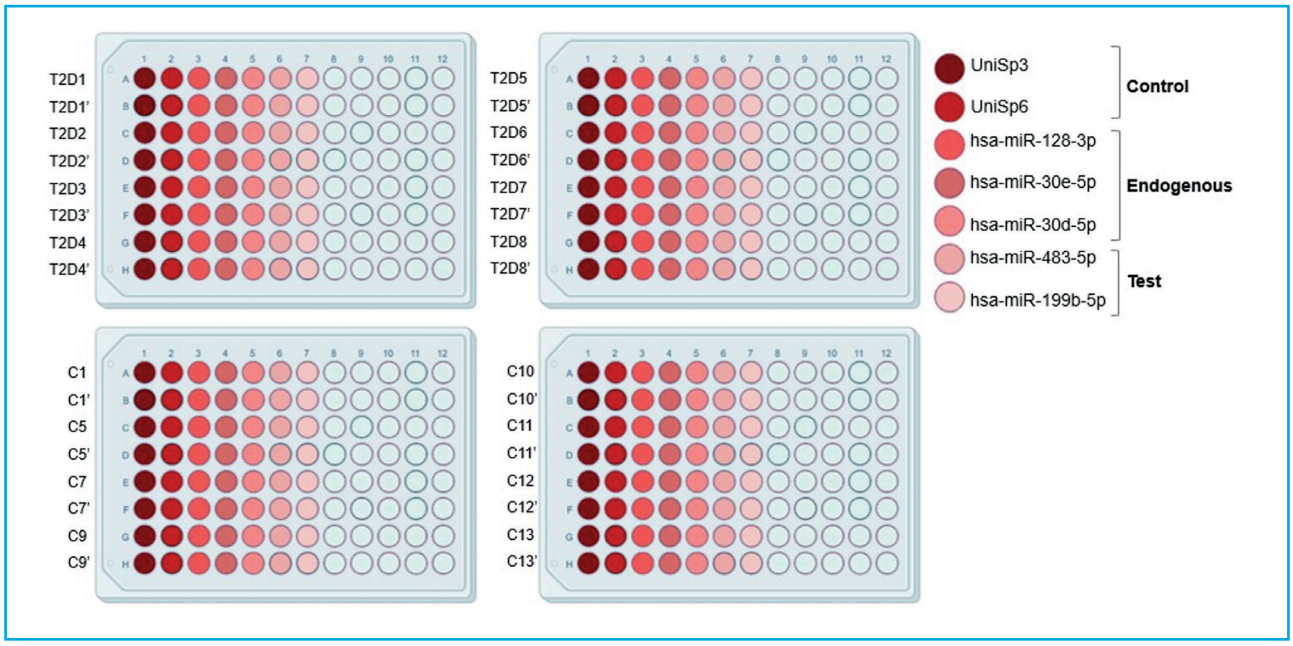

Figure 1. miRCURY LNA miRNA Custom PCR Panels scheme.

RESULTS

STUDY POPULATION

Tables I and II illustrate the clinical, biochemical, and bone-related parameters of T2DM patients stratified by preserved vs degraded TBS. Patients with degraded TBS tended to have higher BMI and waist circumference and lower levels of total cholesterol, LDL, and HDL. Regarding bone parameters, this group showed lower values of BMD (both volumetric and areal), low levels of periostin and CTX, and higher PTHi concentrations, suggesting a trend toward impaired bone quality in individuals with degraded TBS.

DIFFERENTIAL EXPRESSION ANALYSIS AND VALIDATION OF SEQUENCING DATA BY RT-QPCR

Differential expression analysis was conducted to identify miRNAs with significant and biologically relevant changes between T2DM patients and healthy control. A volcano plot was generated to visualize differential expression, with cut-off thresholds set at a false discovery rate (FDR) < 0.05 and an absolute log2 fold change |Log2FC| > 1. Based on these criteria, hsa-miR-199b-5p and hsa-miR-491-5p were found to be significantly downregulated in T2DM, while hsa-miR-483-5p, hsa-miR-122-5p, hsa-miR-5010-5p, hsa-miR-193b-5p, and hsa-miR-320c were upregulated (Fig. 2A).

To confirm these findings, raw count data were normalized using the variance stabilizing transformation (VST), and a non-parametric hypothesis test was applied to compare miRNA expression levels across groups. Significant differences were observed for all selected miRNAs except hsa-miR-193b-5p (Fig. 2B).

Table I. Biochemical characteristics of T2DM patients

	Preserved TBS	Degraded TBS
Age (years)	61.75 ± 2.75	60.50 ± 2.08
Weigh (kg)	85.83 ± 10.74	98.33 ± 14.87
Height(cm)	169.75 ± 9.00	170.25 ± 10.34
BMI (kg/m^2)	29.72 ± 2.28	33.96 ± 4.54
Waist (cm)	102.25 ± 6.59	112.25 ± 10.90
HbA1c (%)	7.95 ± 1.42	7.68 ± 1.89
Uric acid (mg/dL)	5.98 ± 1.54	5.93 ± 0.97
Cholesterol (mg/dL)	215 ± 29.92	148 ± 35.42
LDL (mg/dL)	158 ± 29.07	80.25 ± 30.89
HDL (mg/dL)	53.75 ± 9.64	45.50 ± 11.82
TGs (mg/dL)	207 ± 123.94	113 ± 38.03
Creatinine (mg/dL)	0.72 ± 0.12	0.84 ± 0.1
AHT (%)	50 %	50 %
Dyslipidemia (%)	100 %	75 %

BMI: body mass index; HBA1c: glycated hemoglobin; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TGs: triglycerides; AHT: arterial hypertension; T2DM: type 2 diabetes mellitus; TBS: trabecular bone score.

Table II. Bone and fracture risk parameters in T2DM patients

	Preserved TBS	Degraded TBS
TBS	1.28 ± 0.05	1.02 ± 0.10
Hip FRAX	0.30 ± 0.18	0.20 ± 0.22
Hip FRAX_TBS	0.38 ± 0.28	0.5 ± 0.68
Trabecular_vBMD (g/cm³)	193.29 ± 40.75	170.15 ± 91.13
Cortical_vBMD (g/cm³)	869.13 ± 72.35	860.24 ± 84.04
Cortical_sBMD (g/cm³)	186.42 ± 35.52	174.69 ± 21.79
Integral_vBMD (g/cm³)	339.17 ± 67.44	315.58 ± 107.29
TH_BMD (g/cm²)	1.205 ± 0.17	1.11 ± 0.25
Periostin (pmol/L)	1799.99 ± 346.31	1339.66 ± 245.23
iPTH (pg/mL)	25.35 ± 1.34	33.83 ± 9.77
VitD (ng/mL)	20.63 ± 5.98	19.08 ± 6.69
Calcemia (mg/dL)	9.98 ± 0.26	9.85 ± 0.24
Phosphorus (mg/dL)	3.20 ± 0.22	3.53 ± 0.50
CTX (ng/L)	0.46 ± 0.31	0.26 ± 0.12

FRAX: BMD-adjusted (with or without TBS) hip Fracture Risk Assessment Tool; vBMD: volumetric bone mineral density; sBMD: subchondral bone mineral density; TH_BMD: total hip bone mineral density; iPTH: intact parathyroid hormone; CTX: C-terminal telopeptide of type I collagen.

These miRNAs were subsequently selected for validation by RT-qPCR. Among them, only hsa-miR-199b-5p was successfully validated. Consistent with sequencing data, hsa-miR-199b-5p was found to be significantly downregulated in T2DM patients vs healthy controls (0.496 ± 0.043-fold, $p < 0.05$).

CORRELATION BETWEEN HSA-MIR-199B-5P EXPRESSION AND DETERMINANTS OF BONE FRAGILITY AND INCREASED RISK OF FRACTURE IN T2DM PATIENTS (Fig. 3)

Within the T2DM group, the expression of hsa-miR-199b-5p was correlated with several clinical markers of bone fragility and fracture risk. Spearman correlation analysis revealed significant negative correlations between hsa-miR-199b-5p expression and A) FRAX hip fracture risk (p = -0.74, 95% %CI, {-0.95, -0.05}, p = 0.036); B) FRAX hip fracture risk (TBS adjust) (p = -0.862, 95% %CI, {-0.98, -0.38}, p = 0.005); C) CTX (pg/ml) (p = -0.719, 95% %CI, {-0.95, 0}, p = 0.0446).

In contrast, positive correlations were observed between hsa-miR-199b-5p expression and D) serum levels of periostin (pmol/L) (p = 0.786, 95% %CI, {0.16, 0.96}, p = 0.0208); E) Cortical vBMD (g/cm³) (p = 0.893,

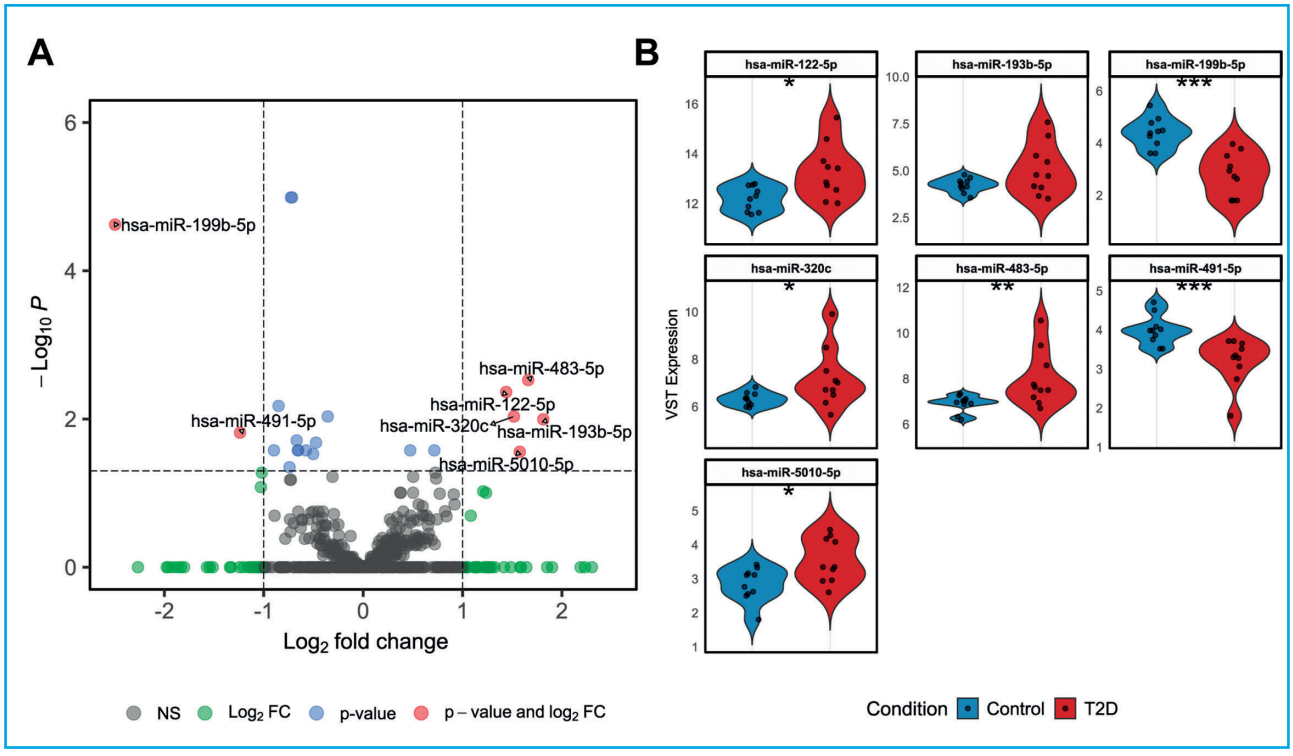

Figure 2. Differential expression of miRNAs. A. Volcano plot showing Log2 fold change (x-axis) vs –Log10 p-value (y-axis). Dashed lines indicate cut-offs for significance (FDR < 0.05) and expression change (|Log2FC| > 1). Labeled miRNAs passed both thresholds. B. Violin plots of selected miRNAs showing expression levels after variance stabilizing transformation (VST).

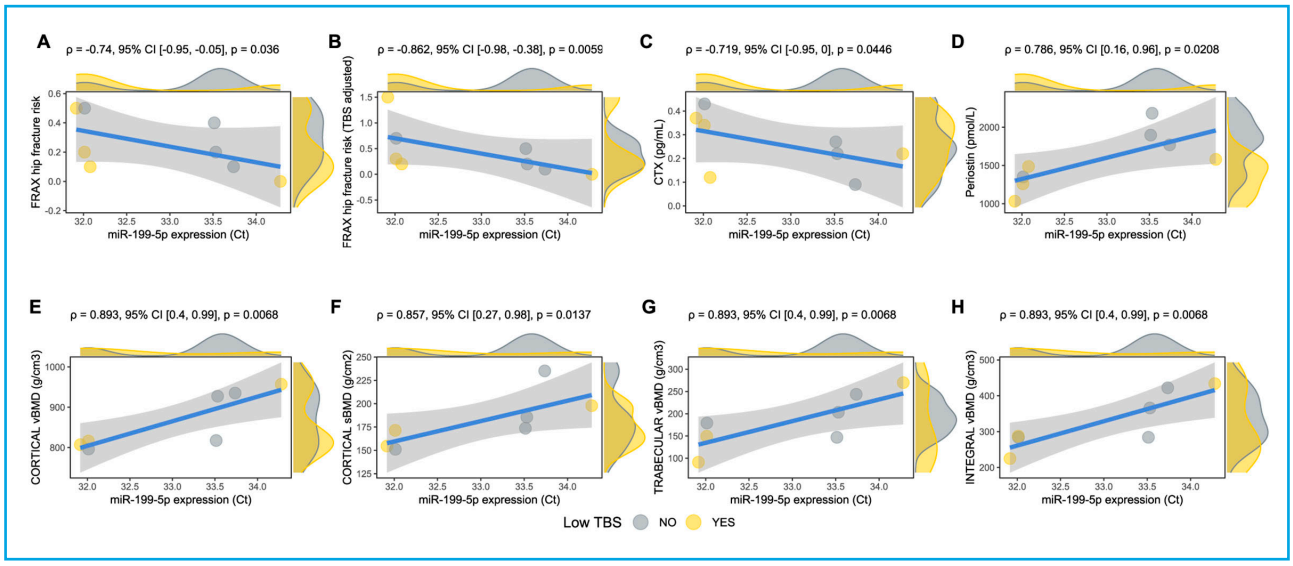

Figure 3. Scatter plots showing the correlation between hsa-miR-199b-5p expression and FRAX hip fracture risk (A); FRAX hip fracture risk (TBS adjust) (B); CTX (pg/mL) (C); serum levels of periostin (pmol/L) (D); Cortical vBMD (g/cm^3) (E); Cortical sBMD (g/cm^3) (F); Trabecular vBMD (g/cm^3) (G); Integral vBMD (g/cm^3) (H), in T2DM patients (n = 8). The p-values between the different associations were obtained via Spearman´s correlation coefficients (showing $p < 0.05$ in each scatter plot).

95% %CI, {0.4, 0.99}, p = 0.0068); F) Cortical sBMD (g/cm^3) (p = 0.857, 95% %CI, {0.27, 0.98}, p = 0.0137); G) Trabecular vBMD (g/cm^3) (p = 0.893, 95% %CI, {0.4, 0.99}, p = 0.0068), E) Integral vBMD (g/cm^3) (p = 0.893, 95% %CI, {0.4, 0.99}, p = 0.0068).

DISCUSSION

Our results obtained from sequencing and validation steps suggest differential expression of hsa-miR-199b-5p in T2DM patients vs the healthy group. With the aim of exploring the potential of miR-199b-5p as a novel therapeutic target for the prevention and management of bone fragility associated with T2DM, we conducted multiple correlation analyses in T2DM patients. Our work reveals a positive correlation between miR-199b-5p expression and bone mineral density parameters, including cortical vBMD (mg/cm^3), cortical sBMD (mg/cm^3), trabecular vBMD (mg/cm^3), and integral vBMD (mg/cm^3). Consistently, we also observed a negative correlation between hsa-miR-199b-5p expression and FRAX hip fracture risk, FRAX hip fracture risk adjusted for trabecular bone score (TBS), and serum C-terminal telopeptide of type I collagen (CTX) levels (pg/mL), all of which are established indicators of demineralization and bone fragility. These clinical findings are in line with emerging molecular evidence supporting the involvement of miRNA, particularly miR-199b-5p, in osteogenesis and bone metabolism. It has been demonstrated that primary mesenchymal stem cells (MSCs) secrete small RNAs via exosomes, which are increasingly recognized for their role in intercellular communication (27). Bioinformatics analysis using DIANA-mirPath revealed that the expression of exosomal miR-199b-5p, among other miRNAs, is impaired during osteogenic differentiation, suggesting that exosomal miR-199b-5p may play a key role in regulating osteoblast differentiation (28). The effects of knockdown and overexpression of miR-199b-5p on osteoblast differentiation have been studied by measuring alkaline phosphatase (ALP) expression and activity, as well as the expression of the osteogenic marker gene Runx2 (29). The findings indicated that miR-199b-5p enhanced the osteogenic potential of bone marrow-derived MSCs (BMSCs) by modulating the GSK-3β/β-catenin signaling pathway, suggesting that miR-199b-5p and its analogs could serve as promising therapeutic candidates for bone and musculoskeletal disorders. Furthermore, the screening and validation of miRNAs associated with osteoporosis, using high-throughput sequencing, revealed a reduced expression of miR-199b-5p (20). Based on all this evidence, our results suggest that reduced expression of miR-199b-5p in T2DM patients may disrupt the regulation of osteogenesis, potentially resulting in diminished bone mineralization and increased skeletal fragility.

Additionally, our results also demonstrated a positive correlation between miR-199b-5p expression and serum periostin levels (pmol/L). Periostin is a ubiquitous protein originally known as osteoblast-specific factor-2 and belongs to a group of nonstructural extracellular matrix (ECM) proteins. This protein not only plays a role in adverse cardiac remodeling, being associated with cardiovascular disease in T2DM according to the SCORE2-Diabetes algorithm (21), but also

promotes bone formation, regeneration, and repair (22). An experimental study on bone loss prevention demonstrated that periostin-induced downregulation of sclerostin can activate the Wnt/β-catenin signaling pathway, thereby inhibiting bone loss (30). This mechanism likely involves the interaction of Wnt proteins with the FZD and LRP6 receptors, leading to the inactivation of the cytoplasmic GSK-3β/Axin2/APC complex. As a result, β-catenin accumulates in the cytoplasm and translocates into the nucleus, where it promotes the transcription of target genes involved in bone formation, ultimately facilitating osteoblast differentiation (31). In this line, our findings suggest that reduced expression of miR-199b-5p in T2DM patients may contribute to impaired osteogenesis through its regulatory effect on periostin. The observed positive correlation between miR-199b-5p expression and serum periostin levels supports the hypothesis that miR-199b-5p may modulate bone metabolism, at least in part, via the periostin–Wnt/β-catenin signaling pathway.

Overall, our results suggest that miR-199b (with osteoprotective role in T2DM) could serve as a predictive biomarker, potentially enabling the early identification and monitoring of osteoporosis progression associated with T2DM.

Interestingly, while our findings support a protective role for miR-199b-5p in bone metabolism and osteogenesis, particularly in the context of T2DM-related osteoporosis, emerging evidence suggests that this microRNA may exert deleterious effects in osteoarthritis (OA). Multiple studies have highlighted the role of miR-199b-5p in chondrogenesis. A longitudinal bioinformatics analysis identified miR-199b-5p as a key pro-chondrogenic regulator (32). Experimental data from the same study showed that inhibition of miR-199b-5p during the early stages of chondrogenesis led to downregulation of chondrogenic markers. A deeper understanding of the regulatory mechanisms involved in chondrogenesis may offer valuable insights into OA pathogenesis. The mechanisms of differentiation differ between growth plate cartilage and articular cartilage. While the growth plate drives longitudinal bone growth via cartilage-to-bone substitution, articular cartilage maintenance depends on delayed chondrocyte maturation and hypertrophy (33). Therefore, it is plausible that, in OA, the cartilage maturation process becomes aberrantly activated, leading to a loss of biomechanical integrity. In this context, serum exosomal small RNA sequencing from clinical OA patients, along with gene expression data from serum and cartilage samples retrieved from the GEO database, revealed that miR-199b-5p is consistently upregulated (19). In vitro studies further demonstrated that miR-199b-5p negatively affects chondrocyte viability and promotes extracellular matrix degradation. Conversely, inhibition of miR-199b-5p under inflammatory conditions exerted protective effects against tissue damage. Additionally, in an OA model, blocking miR-199b-5p, alleviated disease progression, suggesting that, in contrast to its osteoprotective role in T2DM, miR-199b-5p may contribute to cartilage degeneration in OA. Of note, none of the patients included in our study had a diagnosis of osteoarthritis (OA), supporting the interpretation that the observed downregulation of miR-199b-5p is specifically linked to diabetic bone fragility rather than joint-related pathology.

Similarly, periostin, which was positively correlated with miR-199b-5p expression in our T2DM cohort, appears overexpressed in OA cartilage (34). Immunohistochemical analysis localized periostin to chondrocytes and their surrounding matrix, particularly near erosive regions. In vitro, periostin stimulation of chondrocytes induced the upregulation of MMPs, IL-6, and IL-8, supporting its involvement in OA-associated inflammatory responses.

Overall, these findings underscore the context-dependent nature of miR-199b-5p activity. While it may confer protection vs bone fragility in T2DM by promoting osteogenesis through periostin-mediated Wnt/β-catenin signaling, its upregulation in joint tissues under inflammatory conditions may exacerbate OA disease. Therefore, the development of miR-199b-5p-based therapies requires a nuanced understanding of its distinct effects in bone versus cartilage to ensure the safety and efficacy profile.

Our study has several strengths and limitations. One of the main limitations is its cross-sectional design, which does not allow us to establish causal relationships between miR-199b-5p expression and bone fragility parameters in T2DM. While our findings are consistent with the biological role of this microRNA in osteogenesis, longitudinal studies are required to determine if its downregulation precedes bone loss or results from it. In addition, although the sample size of our population was small, it is acceptable for a pilot study exploring novel molecular markers in a well-defined clinical context. However, caution is advised when extrapolating these findings to broader populations. Moreover, the study cohort included only male participants, which limits the assessment of potential sex-specific differences, so further studies including both, larger populations and women, especially postmenopausal who are at increased risk of osteoporosis, are needed to confirm these results and to enhance generalizability.

Despite these limitations, our study has notable strengths. As far as we know, this is the first study linking reduced serum miR-199b-5p expression to diabetic bone fragility offering novel insights into its potential role as a biomarker. The associations were robust and supported by correlations with multiple clinical parameters, including BMD, TBS-adjusted FRAX, serum periostin and CTX levels. Additionally, the study excluded patients with osteoarthritis, reducing the risk

of confounding from inflammatory joint disease and reinforcing the specificity of the observed associations with bone metabolism. The integration of bioinformatic analysis and molecular validation strengthens the biological plausibility of our findings.

While our study highlights the potential of miR-199b-5p as a biomarker for bone fragility in T2DM, we acknowledge that the use of circulating microRNAs in clinical practice remains limited. Nevertheless, significant progress has been made in recent years toward the development of robust and standardized assays, and circulating miRNAs are increasingly recognized as promising candidates for non-invasive diagnostics. In this context, our findings contribute to the growing foundation for the future incorporation of miRNA profiling into clinical decision-making as a complementary tool for fracture risk assessment.

CONCLUSIONS

This study identifies hsa-miR-199b-5p as a promising biomarker for bone fragility in T2DM patients. Its reduced expression is significantly associated with lower bone mineral density and increased fracture risk, suggesting a potential role in impaired osteogenesis. Furthermore, the strong correlation between miR-199b-5p and periostin levels highlights its involvement in the Wnt/β-catenin signaling pathway. However, its contrasting role in OA underscores the need for context-specific interpretation. These findings support the potential of miR-199b-5p as both a diagnostic tool and a therapeutic target for skeletal complications in T2DM.

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Special Document

Multidisciplinary expert position statement on the prevention and management of osteonecrosis of the jaws related to bone resorption inhibitors

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Abstract

Introduction: medication-related osteonecrosis of the jaw (ONMRJ) is a serious complication associated with the use of antiresorptive drugs, such as bisphosphonates and denosumab, indicated for osteoporosis and oncological diseases with bone involvement. Its clinical management continues to be controversial due to variability in diagnostic, prevention, and treatment strategies.

Objective: to develop a multidisciplinary position paper that summarizes the main international and national recommendations on the prevention and management of ONJ, as well as to propose clinical practice strategies that promote therapeutic adherence and minimize the risk of complications.

Materials and methods: a literature review and telematic meetings were held with specialists in oral and Maxillofacial Surgery, Dentistry, Rheumatology, Endocrinology, Hematology, and Medical Oncology to formulate recommendations based on scientific evidence and expert opinion.

Results: the main drugs involved in ONMRM, risk profiles, predisposing factors, diagnostic criteria, and clinical staging are identified. Clinical action algorithms are provided for prevention and treatment in patients with osteoporosis or cancer (bone metastases and/or myeloma), as well as guidelines for invasive dental procedures. The need for interdisciplinary coordination and the implementation of strategies that promote patient education and therapeutic adherence are also addressed.

Conclusion: ONMRM presents great variability in its diagnosis, prevention, and therapeutic approach. This document provides clinicians with recommendations for managing these patients from a multidisciplinary approach. In order to ensure proper prevention, the importance of prior dental evaluation, risk factor control, and patient education is emphasized. Treatment should be tailored to each case, prioritizing conservative options and promoting adherence to treatment.

Keywords:
Antiresorptive drugs. Antiangiogenic drugs. Bisphosphonates. Denosumab. Bone exposure in the jaws. Medication-related osteonecrosis of the jaw. Maxillary osteonecrosis.

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INTRODUCTION

Medication-related osteonecrosis of the jaws (MRONJ) is a severe adverse reaction that causes necrosis of the jawbone tissue, clinically manifesting as exposed necrotic maxillary bone in patients treated with certain medications associated with this complication (1). This term was first introduced in 2003, when Marx published the first series of 36 MRONJ cases (2). Later, in 2005, a series of 10 cases was reported in patients undergoing oncologic chemotherapy (3). Since then, the concept has evolved to include various drugs that regulate bone remodeling (4), especially antiresorptive agents.

TYPES OF DRUGS ASSOCIATED WITH MEDICATION-RELATED OSTEONECROSIS OF THE JAWS AND PATIENT PROFILE

Antiresorptive drugs associated with MRONJ are mainly used to treat osteoporosis and to prevent skeletal complications in patients with oncologic diseases affecting the skeletal system or those with multiple myeloma (5,6). They are also used in less common conditions such as fibrous dysplasia, Paget's disease, osteogenesis imperfecta, and giant cell tumors.

The most important antiresorptive drugs include bisphosphonates, which act by inhibiting the enzyme farnesyl pyrophosphate synthase, blocking osteoclast activity and therefore bone resorption, and denosumab, which prevents osteoclast differentiation and activation by interfering with RANK ligand (RANK-L) signaling. These mechanisms of action make both drugs highly effective in preventing severe skeletal complications (7).

Clinically, 2 main patient profiles can be identified: those with osteoporosis treated with antiresorptive agents and those with oncologic disease involving bone, such as metastases from solid tumors or multiple myeloma.

In patients with osteoporosis, bisphosphonates and denosumab are administered at standard doses and lower frequencies, usually orally and subcutaneously, respectively.1 Recently, romosozumab has been introduced—a monoclonal antibody with dual osteoanabolic and antiresorptive action—indicated for patients with severe osteoporosis and a high risk of fractures. However, given its transient effect, a subsequent consolidation phase with antiresorptive therapy is required to maintain the achieved benefits (8).

In cancer patients, bisphosphonates and denosumab are used at higher doses and frequencies, typically administered intravenously or subcutaneously (9). In addition, other drugs used in oncology have been linked to MRONJ, including antiangiogenic agents such as bevacizumab and tyrosine kinase inhibitors (cabozantinib, axitinib, sunitinib, and sorafenib) (10), as well as common adjuvant therapies such as corticosteroids, aromatase inhibitors (letrozole, anastrozole), selective estrogen receptor modulators (raloxifene), mTOR and CDK4/6 inhibitors, immunomodulatory agents (lenalidomide), cytotoxic agents, and monoclonal antibodies (11).

PREVALENCE AND INCIDENCE RATE

The prevalence and incidence rate of MRONJ depend on several factors, including patient profile, type of drug used, clinical indication, as well as dosage and frequency of administration. In patients treated for osteoporosis, the reported incidence is low, ranging between 0.001 % and 0.04 % (12). However, in cancer patients with bone metastases or multiple myeloma, the incidence increases to between 1.3 % and 1.8 % (13).

RISK-BENEFIT OF TREATMENT

In osteoporosis, bisphosphonates and denosumab have been shown to reduce the risk of vertebral fractures by up to 70 % and hip fractures by up to 40 % (13). These results not only demonstrate the therapeutic efficacy of antiresorptive agents but also their significant clinical benefits, as they help reduce morbidity and mortality associated with osteoporotic fractures and markedly improve patients' quality of life. Because the clinical benefit tends to increase with treatment duration, in some cases, a preventive "drug holiday" may be considered after several years of continuous bisphosphonate use (1). This strategy takes advantage of these drugs' ability to persist in bone tissue for long periods, maintaining partial antiresorptive effects after treatment discontinuation. However, the long-term effectiveness and safety of this practice remain under debate (14,15). It is also essential to clearly differentiate these "drug holidays" from definitive treatment cessation, which should only be considered when a valid clinical justification exists (16).

In oncology, because antiresorptive agents are administered at higher doses and frequencies than in osteoporosis, cumulative drug exposure is greater. However, this intensive use is fully justified by their proven ability to prevent serious skeletal complications such as pathologic fractures or spinal cord compression—conditions that have a decisive impact on patients' quality of life and survival (13,17,18).

CURRENT ISSUES IN THE MANAGEMENT OF ANTIRESORPTIVE AGENTS

The main issues associated with antiresorptive drug use include poor therapeutic adherence, variability in clinical management among medical specialties, and lack of consensus in recommendations for MRONJ prevention.

In osteoporosis, lack of adherence is one of the main barriers. It is estimated that up to 50 % of patients discontinue medication within the first year, significantly reducing the benefits in fracture prevention. The most common causes are lack of awareness of treatment benefits and fear of adverse effects, particularly MRONJ (12). Additionally, there is substantial variability in clinical management. While rheumatologists and endocrinologists prioritize efficacy in fracture prevention, dentists and oral and maxillofacial surgeons focus on minimizing MRONJ risk. These differing priorities may lead to conflicting decisions, especially when evaluating whether to continue or suspend treatment in patients scheduled for dental procedures (19). In cancer patients, heterogeneity is even greater, as recommendations for bisphosphonate and denosumab use vary depending on tumor type and the treating specialty (1).

Despite efforts by various scientific societies and clinical practice guidelines (CPGs), differences still exist regarding patient management (4). Therefore, it is crucial to have evidence-based, consensus-driven recommendations that promote a multidisciplinary approach to optimize drug efficacy and minimize MRONJ occurrence without compromising therapeutic benefits.

The objective of this article is to develop an updated multidisciplinary position paper on the prevention and management of MRONJ, integrating the most evidence-based international and national recommendations and proposing practical strategies based on expert opinion for patients with osteoporosis or bone metastases and/or multiple myeloma treated with antiresorptive agents.

MATERIALS AND METHODS

To develop this position paper, a multidisciplinary panel was convened, composed of specialists in Oral and Maxillofacial Surgery, Dentistry, Rheumatology, Endocrinology, Hematology, and Medical Oncology, selected based on their clinical experience, scientific output, and participation in the management of patients treated with antiresorptive bone agents.

The main objectives of the expert group were: a) to harmonize diagnostic and therapeutic criteria; b) to propose prevention strategies tailored to patient risk profiles and the type of agent involved; and c) to offer practical recommendations for health care professionals involved in patient care across different specialties.

We conducted a literature review across Medline/PubMed using the following search strategy: ("Medication-related osteonecrosis of the jaws" OR osteonecrosis OR MRONJ) AND (jaw OR jaws OR maxillary OR mandible OR mandibular) AND ("clinical guideline" OR "consensus" OR "clinical practice guideline"). The search was limited to publications between 2003 and 2025, in Spanish and English. Clinical practice guidelines, consensus statements, and systematic reviews on MRONJ were included, while letters to the editor and conference abstracts were excluded. This search was complemented by the selection of clinical guidelines by the multidisciplinary panel and by incorporating the accumulated clinical experience of the experts.

Through multiple online meetings and structured exchanges of documents and communications, the group collaboratively developed an updated and consensus-based position paper.

RESULTS

Based on the review of documents selected by the expert panel (1,4,12,13,19-23), the main recommendations for the diagnosis, prevention, and management of patients at risk of MRONJ or with established disease are outlined below.

DIAGNOSTIC CRITERIA

The diagnosis of MRONJ must be established thoroughly to rule out other forms of chronic osteomyelitis not induced by antiresorptive agents. Differential diagnoses include delayed healing, bone exposure, or bone sequestra due to infections, trauma, or chronic inflammatory processes (24). On this basis, clinical practice guidelines agree on three essential diagnostic criteria (25). First, current or previous exposure to an antiresorptive or antiangiogenic drug is required. Second, there must be exposed bone or an intraoral or extraoral fistula with bone involvement persisting for more than 8 weeks without healing. Third, there must be no history of radiotherapy to the maxillofacial region or evidence of metastatic disease in the jaws (1,12,19).

RISK FACTORS

Multiple risk factors contribute to the development of MRONJ. Among pharmacologic factors, the cumu-

lative drug dose, treatment duration exceeding three years, and frequency of administration are significant. The risk is higher in cancer patients receiving high-dose intravenous antiresorptives compared with those treated for osteoporosis with oral formulations (23). Similarly, concomitant use of antiangiogenic agents, corticosteroids, immunosuppressants, aromatase inhibitors, or chemotherapy increases susceptibility to MRONJ (23,26). The main drugs associated with this complication are summarized in table I.

Among local factors, invasive dental procedures such as extractions, surgical procedures, or implant placement involving osteotomy represent the most common triggers, occurring in at least half of all cases (23). Periodontal disease, oral infections, advanced caries, and mechanical factors (e. g., ill-fitting prostheses or mandibular tori) also contribute. Finally, tobacco and alcohol consumption, together with systemic diseases such as poorly controlled diabetes or immunodeficiency, further increase risk (23,27,28).

PATIENT STAGING

The staging of MRONJ (0-3) guides therapeutic decision-making and helps establish prognosis. The disease may present with or without bone exposure, as bone that is probeable through a fistula also meets diagnostic criteria.

Stage 0 is the most controversial. Up to 50 % of cases progress to stage 1, which is why the American Association of Oral and Maxillofacial Surgeons (AAOMS) considers it a potential precursor of MRONJ (1). However, its recognition is not universal among scientific societies, as some interpret it as a preclinical phase or a remission stage of stages 1-3, potentially causing confusion in management and diagnostic errors (28-31). In clinical practice, dentists are often the first to identify these cases, especially in patients with osteoporosis, who are referred to oral and maxillofacial surgeons once the condition becomes more severe.

Differential diagnosis is challenging because stage 0 symptoms are nonspecific and overlap with common oral pathologies. Manifestations such as diffuse pain, nonspecific odontalgia, or persistent mandibular discomfort may suggest stage 0 MRONJ but are more commonly due to other conditions such as temporomandibular dysfunction, occlusal trauma, poorly fitting prostheses, advanced generalized periodontitis (stages 3-4), caries, bruxism, or long-standing severe edentulism. According to the consensus experts, this clinical overlap explains why, in primary care, some patients are misdiagnosed with atypical facial neuralgia when they actually have functional disorders unrelated to MRONJ.

The recommended clinical approach in suspected stage 0 MRONJ is not to suspend antiresorptive treatment, to refer the patient early to a maxillofacial specialist,

Table I. Strength of the association between drugs and the occurrence of medication-related osteonecrosis of the jaws (MRONJ)

Drugs with strong signal intensity	Drugs with moderate signal intensity	Drugs with weak signal intensity
Zoledronic acid Alendronic acid Pamidronic acid Ibandronic acid Risedronic acid Alendronic acid + colecalciferol Denosumab Radium-223 chloride	Cabazitaxel Romosozumab Fulvestrant Raloxifene Letrozole	Etidronic acid Eribulin Exemestane Anastrozole Arsenic trioxide Prednisolone Everolimus Lenalidomide Daratumumab Bevacizumab Ramucirumab Trastuzumab Sunitinib Lenvatinib Cabozantinib Palbociclib Abemaciclib

Classification is based on signal intensity obtained from pharmacovigilance analysis of the FAERS (FDA Adverse Event Reporting System), using the Expected Information Component (EIC) and its 95 % confidence interval according to the Bayesian Confidence Propagation Neural Network (BCPNN) method. Strong signals (+++) are defined as CI-2SD > 3.0, moderate (++) if 1.5 < CI-2SD ≤ 3.0, and weak (+) if 0 < CI-2SD ≤ 1.5. Data adapted from Zhong et al., 2025.

and to maintain close follow-up to confirm or rule out the lesion and safely guide further management. Premature and unwarranted treatment discontinuation may compromise fracture prevention efficacy, especially in patients at high skeletal risk (1,4,22).

Table II summarizes the clinical stages, diagnostic criteria, and therapeutic proposals based on scientific evidence. In the complementary assessment, imaging studies are essential. Panoramic radiography remains the most widely used technique, though computed tomography (CT) has gained importance for its ability to detect early changes, precisely define lesion extent, and confirm clinical staging.

PREVENTION AND MULTIDISCIPLINARY MANAGEMENT

Prevention and multidisciplinary management of MRONJ are essential to reduce its incidence and improve prognosis in patients treated with antiresorptive agents. Preventive strategies should begin before treatment initiation and include identification of risk factors, comprehensive dental evaluation, elimination of infectious foci, and maintenance of adequate oral health (32).

The need for and timing of pre-treatment dental evaluation depend on the patient's clinical profile and in-

Table II. Clinical classification and therapeutic management by stages of medication-related osteonecrosis of the jaws (MRONJ)

Stage	Description	Symptoms	Clinical findings	Radiographic findings	Recommended management
At risk	No exposed bone, but the patient uses antiresorptive agents	—	—	—	Semiannual follow-up (non-oncologic) or quarterly (oncologic); strict oral hygiene
0	Patients without clinical evidence of necrotic bone but presenting nonspecific symptoms or clinical and radiographic findings	Unexplained odontalgia not due to an odontogenic cause. Dull bone pain in the mandible, possibly radiating to the temporomandibular joint region. Sinus pain, which may be associated with inflammation and thickening of the maxillary sinus wall. Altered neurosensory function	Tooth loosening not explained by chronic periodontal disease. Intraoral or extraoral inflammation	Alveolar bone loss or resorption not attributable to chronic periodontal disease Changes in trabecular pattern, sclerotic bone, and lack of new bone formation in extraction sockets. Regions of osteosclerosis affecting alveolar and/ or surrounding basal bone. Thickening/darkening of the periodontal ligament (thickened lamina dura, sclerosis, and narrowing of the periodontal ligament space)	Pain control, clinical monitoring, antibiotics if infectious signs are present
1	Exposed and necrotic bone or a fistula reaching bone in asymptomatic patients without evidence of infection/ inflammation	—	—	May present radiographic findings described for Stage 0, located in the alveolar bone region	Chlorhexidine mouth rinses, follow-up every 4-6 weeks. In surgical treatments, platelet-rich plasma (PRP) may be used if several months have passed and the socket remains unhealed
2	Exposed and necrotic bone, or fistula reaching bone, with evidence of infection/ inflammation	—	—	May present radiographic findings described for Stage 0, located in the alveolar bone region	Oral antibiotics (amoxicillin + metronidazole), analgesia, conservative debridement. I n surgical treatments, platelet-rich plasma (PRP) may be used if several months have passed and the socket remains unhealed

(Continues on next page)

Table II (cont.). Clinical classification and therapeutic management by stages of medication-related osteonecrosis of the jaws (MRONJ)

Stage	Description	Symptoms	Clinical findings	Radiographic findings	Recommended management
3	Exposed and necrotic bone or fistulas extending to bone	Evidence of infection and one or more of the following: – Exposed necrotic bone extending beyond the alveolar bone region (i.e., inferior border and ramus of the mandible, maxillary sinus, or zygoma) – Pathologic fracture – Extraoral fistula – Oroantral/oronasal communication – Osteolysis extending to the inferior border of the mandible or sinus floor	—	—	Conservative surgery or bone resection, interdisciplinary management

dividual risk. In patients with osteoporosis, pre-treatment dental evaluation is recommended when MRONJ risk factors, urgent dental needs, or pending invasive procedures are present. However, in low-risk patients—and to avoid delaying therapy initiation—oral evaluation may be performed concurrently with the start of treatment. Conversely, in cancer patients, pre-treatment oral evaluation is mandatory, and antiresorptive therapy should be delayed until complete healing of any oral lesions, whenever the patient's systemic condition allows (33-35).

Dental follow-up frequency should be tailored to MRONJ risk and clinical context. The expert panel recommends follow-up every 6-12 months for patients with osteoporosis and every 3-6 months for cancer patients (solid tumor bone metastases or multiple myeloma) treated with antiresorptives.

DENTAL PROCEDURES IN PATIENTS TREATED WITH ANTIRESORPTIVES

Dental management should be individualized according to drug type, clinical profile, and MRONJ risk. In all patients, noninvasive conservative treatments—such as fillings, nonsurgical endodontics, orthodontics, or prosthetic work—are considered safe, and suspension of antiresorptive therapy is not recommended.

PATIENTS WITH OSTEOPOROSIS AND LOW MEDICATION-RELATED OSTEONECROSIS OF THE JAW RISK

Low-risk procedures, such as minor dentoalveolar surgical procedures, simple extractions, or implant placement without osteotomy, can be safely performed with an atraumatic surgical technique, primary wound closure, and close clinical and radiologic monitoring. In these cases, bisphosphonate discontinuation is not recommended.

For patients treated with denosumab, the safest approach is to schedule the procedure 5-6 months after the last administration, avoiding excessive delay of the next dose to minimize the risk of skeletal complications and maintain therapeutic efficacy (22).

PATIENTS WITH OSTEOPOROSIS AND HIGH MEDICATION-RELATED OSTEONECROSIS OF THE JAW RISK

In this group, bisphosphonate discontinuation has not been shown to reduce MRONJ risk due to the drugs' long skeletal half-life (1,22,23). However, some authors suggest temporary interruption to promote mucosal healing, with recommendations ranging from 1 week to 2 months before surgery (22,36). The expert panel considers it reasonable to suspend treatment for up to 2 months, maintaining discontinuation until full mucosal healing, provided the patient's systemic condition allows and therapy reintroduction is ensured thereafter.

In contrast, for patients treated with denosumab, suspension is not recommended, as even brief delays may cause rapid bone loss and increased risk of multiple vertebral fractures (1,22,23) In these cases, procedures should be scheduled 5-6 months after the last dose, and treatment should be resumed 4-6 weeks after mucosal closure, avoiding delays longer than one month (22).

PATIENTS WITH ONCOLOGIC DISEASE (BONE METASTASES OR MULTIPLE MYELOMA)

Routine discontinuation of therapy before invasive dental procedures is not supported by scientific evidence,

as no significant risk reduction has been demonstrated (4,34). These patients should be considered permanently high-risk, even after treatment completion. Therefore, invasive surgical procedures should be avoided whenever possible, prioritizing conservative alternatives such as nonsurgical endodontics over extractions (1,4).

When surgery is unavoidable or urgent, it should be performed in a specialized surgical unit in coordination with Oncology and/or Hematology services. Atraumatic techniques with primary closure are recommended, accompanied by abundant irrigation with saline or chlorhexidine and, when possible, the use of platelet concentrates to promote tissue healing (4). Some observational studies suggest that temporary suspension of intravenous bisphosphonates for at least 3 months before surgery may reduce MRONJ risk, although evidence remains limited (37). In such cases, treatment reintroduction should occur after complete wound healing, possibly delayed up to 3 months depending on clinical progress.

For patients treated with denosumab, routine suspension is also not recommended due to rebound effects characterized by rapid bone loss and multiple vertebral fractures. The safest approach is to schedule surgery 3-4 months after the last dose and resume therapy 6-8 weeks postoperatively, once mucosal healing is confirmed, with close monitoring for potential "rebound effects" if denosumab interruption exceeds 4 weeks beyond the next scheduled dose (1).

PATIENTS WHO HAVE COMPLETED ANTIRESORPTIVE THERAPY

The optimal timing for invasive dental procedures depends on the drug type and clinical context. Generally, it is safer to operate at least 12 months after the last dose, provided adequate healing and absence of active lesions. This interval is particularly important for patients treated with intravenous bisphosphonates, such as zoledronic acid, due to their prolonged skeletal action. For patients treated with denosumab, this period may be slightly shorter but should always be individualized based on clinical evaluation.

Figure 1 illustrates the recommended approach for patients treated with antiresorptive drugs without MRONJ.

PATIENTS WITH MEDICATION-RELATED OSTEONECROSIS OF THE JAW

Therapeutic decisions should be individualized according to disease stage, type of drug, treatment indication, general health status, and, in oncologic contexts, underlying disease prognosis. The creation of multidisciplinary reference units comprising clinicians experienced in MRONJ management is desirable to ensure standardized and coordinated care.

Therapeutic goals focus on pain control, infection resolution, and prevention of disease progression or new complications. Depending on severity, treatment may be medical or surgical. Conservative management, indicated for early stages, includes local antiseptic measures, systemic antibiotics, and symptomatic control (22). However, recent evidence suggests that even in early stages (1 and 2), early surgical intervention may be beneficial when healing fails after several months of conservative therapy. In such cases, platelet-rich plasma used as an adjunct after curettage has shown favorable outcomes (38).

Surgical treatment, ranging from debridement of necrotic areas to bone resection with primary closure, should be tailored to clinical evolution and individual response. Figure 2 summarizes the therapeutic strategy for patients with established MRONJ.

In stage 1 MRONJ, treatment aims to control local symptoms, prevent progression, and maintain oral health. Antiresorptive therapy should be continued, as discontinuation has not demonstrated clinical benefit.

In advanced MRONJ (stages 3 and 4), temporary suspension of antiresorptive therapy may favor faster lesion resolution. However, treatment reintroduction should occur only after complete mucosal healing, absence of inflammatory or infectious signs, and multidisciplinary risk-benefit assessment (1). When therapeutic benefit outweighs recurrence risk, reinitiation should be performed in agreement with the patient under dental and maxillofacial supervision.

When reintroduction is not advisable, therapeutic alternatives should be individualized according to the previously used drug. In patients treated with bisphosphonates, parathyroid hormone (PTH) analogs such as teriparatide may be considered as effective anabolic options for managing osteoporosis and recovering bone mass (22). In contrast, for patients previously treated with denosumab, no equivalent alternatives exist to replace its potent antiresorptive effect. In such cases, close clinical monitoring is advised due to the risk of "rebound effect." If this phenomenon occurs, controlled reintroduction of denosumab may be considered to prevent skeletal complications and maintain bone stability.

IMPACT ON THERAPEUTIC ADHERENCE

Fear of developing MRONJ affects adherence to antiresorptive treatment. This concern, often linked to

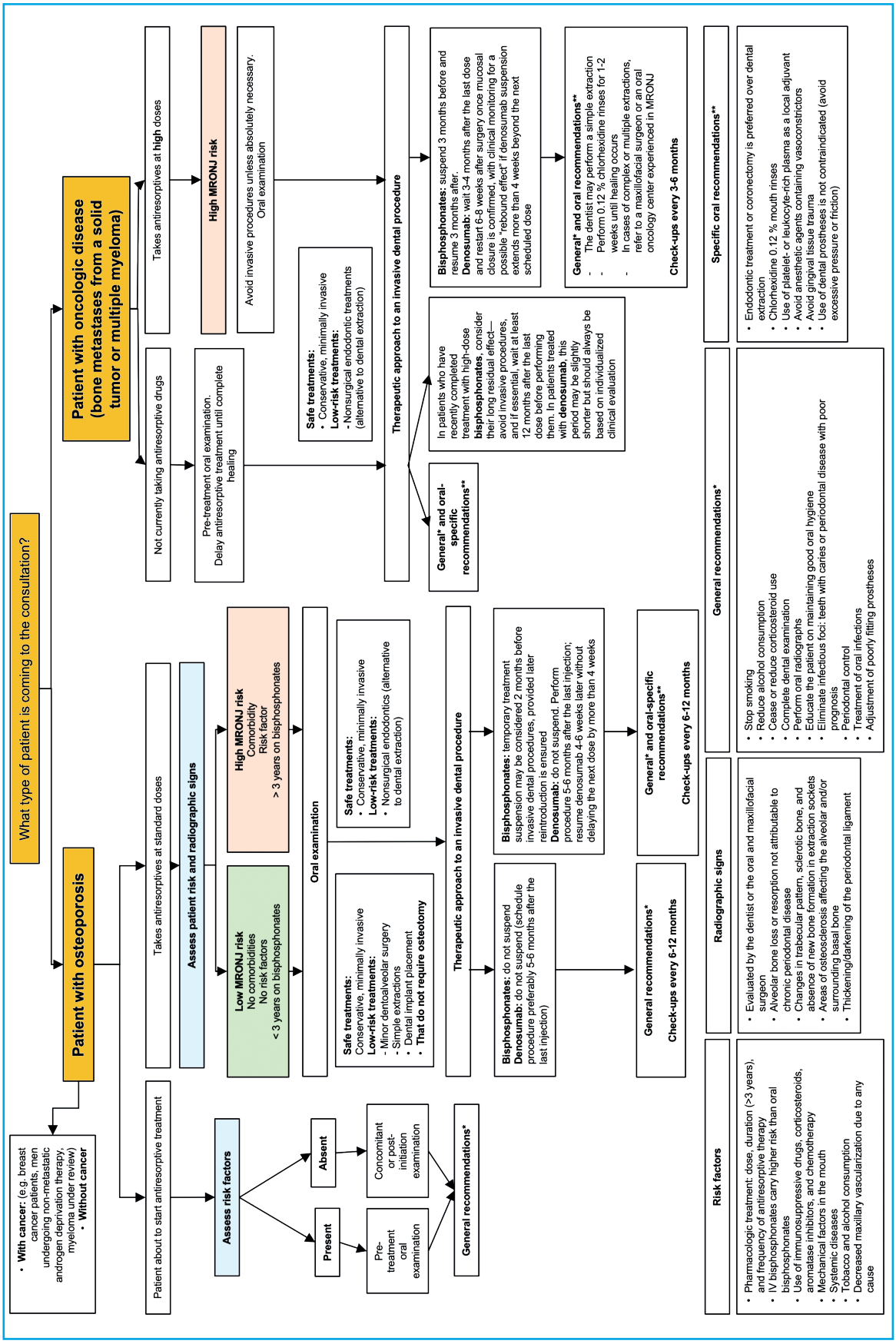

Figure 1. Algorithm for managing patients treated with antiresorptive bone drugs who do not have MRONJ. Specific recommendations are presented according to patient type (osteoporotic or oncologic) and current or planned use of antiresorptive bone drugs, differentiating standard versus high doses. MRONJ risk stratification is established, and therapeutic actions are detailed by low or high risk, including general and oral-specific recommendations, as well as the frequency of dental follow-up. Relevant risk factors and radiographic signs for clinical decision-making are also included..

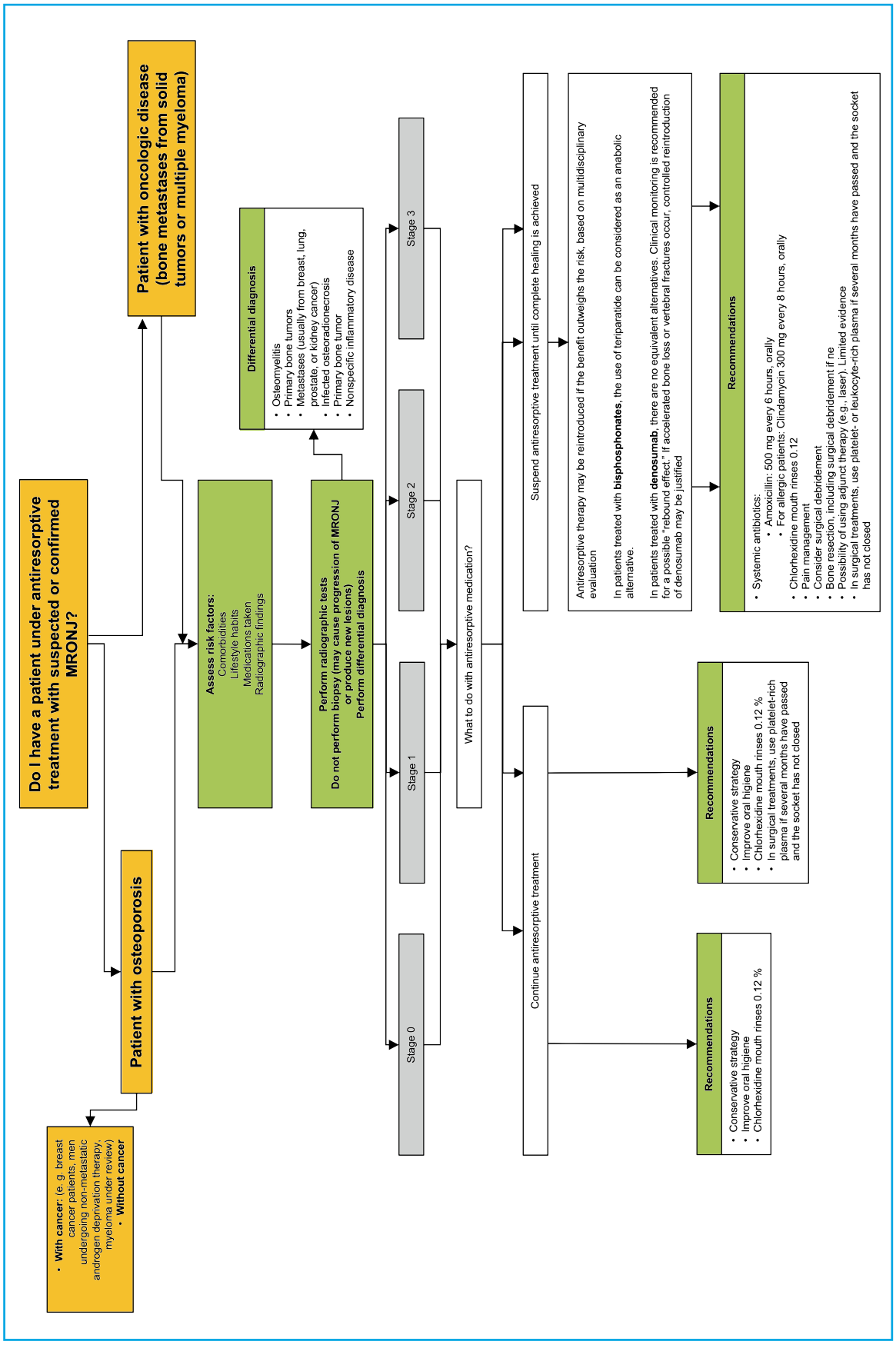

Figure 2. Algorithm for managing patients treated with antiresorptive bone drugs who do have MRONJ. The diagram guides diagnostic and therapeutic approaches according to the clinical stage (0 to 3) of MRONJ in patients with osteoporosis or oncologic disease. It includes risk assessment, exclusion of differential diagnoses, and decision-making regarding continuation or suspension of antiresorptive bone therapy. Recommended interventions are detailed by severity, with emphasis on conservative management, oral hygiene, mouth rinses, antibiotics, pain control, and surgical debridement.

an exaggerated perception of risk, may lead to premature treatment discontinuation, missed doses, or therapy abandonment, thereby increasing the risk of osteoporotic fractures or progression of metastatic bone disease (39).

In the absence of universal management protocols, individualized assessment and shared clinical judgment among specialists are essential to balance treatment risks and benefits. Based on current scientific evidence and the expert group's clinical experience, a set of practical recommendations—summarized in figure 3—is proposed to improve therapeutic continuity and minimize associated complications.

DISCUSSION

This document proposes a structured strategy for the prevention and management of MRONJ, founded on multidisciplinary coordination among dentists, oral and maxillofacial surgeons, and prescribing physicians.

In line with the most recent consensuses, routine suspension of antiresorptive therapy in patients with osteoporosis is discouraged—even for low-risk dental or implant procedures—while emphasizing individualized management according to drug type, treatment duration, and local and systemic risk factors (1,22,23).

Figure 3. Ten-point patient recommendations based on scientific evidence and the experts' clinical experience. This document is part of an educational strategy aimed at preventing MRONJ in patients treated with antiresorptives. The recommendations were agreed upon by specialists in Oral and Maxillofacial Surgery, Dentistry, Rheumatology, Endocrinology, Hematology, and Medical Oncology, and are intended to reinforce therapeutic adherence and oral health through interventions based on the best available evidence.

From a diagnostic standpoint, inclusion of stage 0 in the AAOMS classification (1) represents a meaningful advance, enabling early recognition of incipient forms based on radiographic findings or nonspecific symptoms. Recent studies (12,19,20,40) support the use of cone-beam computed tomography (CBCT) as a complementary tool for early diagnosis, although the need to avoid overdiagnosis remains—particularly in the presence of nonspecific orofacial symptoms attributable to common dental conditions.

Prevention continues to be the most effective tool (33-35). Likewise, fear of developing MRONJ can undermine treatment adherence. Clear communication with health care professionals, multidisciplinary coordination, and patient education are therefore essential.

As a distinctive contribution, this consensus organizes international recommendations into a practical algorithm tailored to different clinical profiles—patients with osteoporosis and those with oncologic disease (bone metastasis or multiple myeloma)—integrating current scientific evidence, clinical experience, and real-world applicability criteria, with the goal of offering clear guidance that is readily implementable in clinical practice. Other recent documents have addressed this clinical problem. Notably, the recommendations by Anastasilakis et al. (22), on behalf of the European Calcified Tissue Society (ECTS), are based on a critical (and nonsystematic) literature review owing to the limited quality of available evidence. As in the present consensus, their conclusions state that recommendations to prevent MRONJ should be adapted to the underlying skeletal condition, individual risk factors, and the type of antiresorptive therapy received. However, our work expands this approach by integrating specific dental recommendations, pharmacologic management guidelines differentiated by clinical profile (osteoporotic vs oncologic) and by antiresorptive agent, providing a more practical and comprehensive view of the multidisciplinary approach that should be followed in these patients.

Separately, the working group led by de Ali et al. (23) conducted a systematic review and meta-analysis to determine whether antiresorptive use in patients with osteoporosis increases MRONJ risk in those undergoing dental implant placement, as well as its potential impact on implant survival. They concluded that the effect of antiresorptive therapy on implant failure remains uncertain and that the available—and very low-quality—evidence does not support routine suspension of antiresorptives prior to the procedure. However, that study does not offer specific management recommendations for different clinical scenarios—an aspect that this multidisciplinary document addresses in detail.

CONCLUSIONS

MRONJ is a serious complication with major clinical implications and marked heterogeneity in its diagnosis, prevention, and treatment. This position paper proposes unifying criteria based on scientific evidence and expert opinion to optimize prevention and treatment strategies. Pre-treatment dental evaluation, risk-factor control, and patient education are essential to minimize incidence. Management—both in oncologic and non-cancer patients—should be individualized, prioritizing conservative strategies and supporting therapeutic continuity. The paucity of prospective studies limits current evidence and underscores the need for further research to standardize clinical protocols.

ARTIFICIAL INTELLIGENCE

The authors declare that no artificial intelligence (AI) or AI-based tools were used in the writing of this article.

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AUTHORS´ CONTRIBUTION

The study conceptualization was carried out by Marta Zafra Poves, María Pilar Aguado Acín, Luis Miguel Redondo González, and Guillermo Martínez Díaz-Guerra. Funding acquisition, methodology, project administration, supervision, validation, and visualization were performed by Guillermo Martínez Díaz-Guerra. The original draft was written by Marta Zafra Poves, Carmen García Insausti, María Casanova Espinosa, María Pilar Aguado Acín, José Nart, and Pedro Infante-Cossio. Review and editing of the manuscript were performed by Marta Zafra Poves, Guillermo Martínez Díaz-Guerra, Carmen García Insausti, José Bagán, María Casanova Espinosa, Luis Miguel Redondo González, María Pilar Aguado Acín, José Nart, José Luis Cebrián Carretero, and Pedro Infante-Cossio.

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CONFLICTS OF INTEREST

Marta Zafra Poves, Carmen García Insausti, María Casanova Espinosa, José Bagán, Luis Miguel Redondo González, Pedro Infante-Cossio, José Luis Cebrián Carretero, and José Nart declare no conflicts of interest related to the content of this manuscript. Guillermo Martínez Díaz-Guerra declares having participated in conferences and scientific meetings organized by Amgen, UCB, Kyowa Kirin, Theramex, and Gedeon Richter; having served as a speaker for Kyowa Kirin, Italfármaco, FAES, and Gedeon Richter; and having been a member of advisory boards for Alexion, Takeda, Italfármaco, Ascendis, Amgen, UCB, and Kyowa Kirin. María Pilar Aguado Acín declares having participated as a speaker in educational activities sponsored by Alexion, Amgen, FAES, Gedeon Richter Ibérica, GP Pharm, Kyowa Kirin, Lilly, MSD, Rubió, Stada, and Theramex; and having participated in occasional advisory roles for Alexion, Amgen, AstraZeneca, Kyowa Kirin, and Theramex.

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