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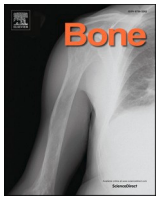

Full Length Article

Maintenance of bone resorption markers in the low premenopausal range during the year following denosumab discontinuation is associated to bone density preservation. The ReoLaus study

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ABSTRACT

Purpose: Denosumab discontinuation (DD) is associated with serum C-terminal X-linked telopeptides (sCTX) increase, bone mineral density (BMD) loss and vertebral fractures (VFs) risk increase. We compared clinical characteristics of women losing or not lumbar spine (LS) BMD one-year after DD, and their sCTX values at different time-points.

Methods: We included women from the ReoLaus cohort having received ≥2 denosumab 60 mg injections, with three BMD measurements on the same device (before (DXA1), at the end of denosumab treatment (DXA2), and one-year after (DXA3)) and sCTX measured at different time-points. *Losers* (LS DXA3-DXA2 > 2.8 %) and *stable* groups were compared.

Results: 63 postmenopausal women were included (mean age 64.2 ± 9.1 years, 7.9 ± 2.7 denosumab injections). 19 months after last denosumab injection, 65 % had lost LS BMD. *Losers* were younger, had lower BMD and higher sCTX before denosumab, received more injections and gained more BMD under denosumab, and had higher sCTX after DD. Same proportion of patients received bisphosphonates in both groups, but 11 (all in *losers* group) received ≥1 zoledronate infusion. Three women developed VFs in the *losers* group (none in the *stable*). Mean sCTX at 10 and 19 months were 590 ± 372 versus 221 ± 101, and 598 ± 324 versus 293 ± 157 ng/l, respectively (premenopausal range < 573 ng/l, $p < 0.01$ for both). LS BMD loss and sCTX levels measured at 10 and 19 months were correlated ($r^2 = 0.29$, $p = 0.01$, and $r^2 = 0.16$, $p < 0.005$).

Conclusion: Maintenance of BMD gained with denosumab is associated with sCTX in the low premenopausal range after DD. Whether this could be achieved by regular sCTX monitoring and adjustment of bisphosphonates doses or frequency administration needs to be confirmed by further studies.

1. Introduction

Denosumab discontinuation induces an increase in osteoclasts activity [1,2], leading to an elevation of bone turnover markers (BTMs) to levels higher than those observed before treatment. BTMs rapidly increase six months after the last denosumab injection, peak approximately 12 months later and return to pre-treatment values after 24 months (18 and 30 months after the last injection, respectively) [3]. If no further antiresorptive therapy is given, bone mineral density (BMD) returns to pre-treatment values as early as 18 months after the last denosumab injection [3–5]. This rebound effect, combining increased BTMs and BMD loss, is associated with an increased risk of multiple and spontaneous vertebral fractures (VFs) [3–9]. This risk seems to be particularly high in people with prevalent VFs, those who did not receive a bisphosphonate (BP) before [7] or at the time of denosumab discontinuation [7,8,10,11], and/or those with longer denosumab treatment [6,8,12–14]. Younger age, lower BMI, lack of prior BP treatment, greater BMD loss after denosumab discontinuation and greater BMD gain during denosumab treatment are other risk factors associated with BMD loss after denosumab discontinuation [10,11,15,16]. Upon discontinuation of denosumab, a potent BP is recommended to prevent the risk of VF and BMD loss, with zoledronate being the most widely used. [16–24]. The optimal BP regimen to limit these adverse effects is still not known. Indeed, the different strategies used to date only partially achieve these

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objectives [4,15–18,20,21,23,25].

After denosumab discontinuation, both the loss of BMD and the occurrence of VFs are associated with high BTMs [1,3,7,9,12,15,26], suggesting an increase in osteoclastogenesis and osteoclast activity. This is supported by the description of a significant increase in RANKL mRNA followed by an increase in cathepsin K mRNA in women with VFs after denosumab discontinuation, compared with treatment-naive osteoporotic women with clinical VFs [1]. In a randomized clinical trial, an infusion of zoledronate given at denosumab discontinuation only partially limited the increase in BTMs and loss of BMD during the first year. However, BMD was maintained during the second year while BTMs remained within the reference range [23]. These results may suggest that controlling BTMs during the first year could prevent BMD loss. Regardless of the risk factors associated with VFs or BMD loss upon denosumab discontinuation, follow-up of BTMs could allow to assess these risks at the individual level. Some authors propose regular monitoring of BTMs to adapt/repeat BP treatment to keep them below a certain threshold. This strategy would avoid VFs and maintain the acquired BMD [21,24]. However, this approach is still empirical. It is therefore essential to define the best strategy to control BTMs after denosumab discontinuation, in order to maintain the BMD gained and to avoid VFs.

We created the Rebound Effect Observatory study in Lausanne (ReoLaus), an observational prospective study of patients discontinuing denosumab and followed at the Interdisciplinary Centre for Bone Diseases of the Lausanne University Hospital, with the objective of providing some answers to these questions. The aim of the actual study was: 1) to compare the clinical characteristics of women who lose BMD at lumbar spine (LS) with those who remain stable during the first year following denosumab discontinuation; and 2) to verify whether BTMs levels below a certain threshold are associated to the maintenance of BMD at LS.

2. Material and methods

2.1. Recruitment and inclusion criteria

All women and men who stopped denosumab since January 2014 and who were assessed at least once in the Interdisciplinary Centre for Bone Diseases, Lausanne University Hospital, Switzerland, are included in the ReoLaus cohort. No specific protocol for bisphosphonates treatment after denosumab discontinuation was applied, as they may have been followed by their medical doctor, or in our Centre. Thus, management was not standardized, with different monitoring intensities, and was progressively modified according to the growing knowledge on the rebound effect at denosumab discontinuation. In the last years, we have tried to homogenize the management of denosumab discontinuation in our Centre: regular BTMs measurement, BMD once a year [21].

We included for the present analysis postmenopausal women treated with denosumab for osteoporosis or for the prevention of aromatase-inhibitor (AI) induced bone loss, and evaluated for denosumab discontinuation between 01.06.2014 to 31.12.2020 in our Centre. To be included, these women needed to have: 1) at least two denosumab 60 mg injections given strictly every 6 months (± 2 weeks); 2) at least three BMD measurements on the same Dual X-rays absorptiometry (DXA) machine: the first before denosumab initiation (DXA1), the second at the end of denosumab treatment (DXA2), and the third one year after the end of denosumab efficacy (DXA3); and 3) serum C-terminal X-linked telopeptides of type 1 collagen (sCTX) measured at the central clinical routine laboratory of the Lausanne University Hospital.

2.2. Data collected

Anthropometric and clinical data were collected from routine clinical records, and included age, BMI, treatments with glucocorticoid or AI, antiosteoporotic treatments, and non-traumatic fractures before denosumab initiation, during denosumab treatment, and after denosumab discontinuation. The exact start and end dates (day/month/year) were recorded for osteoporosis treatments. The treatment with denosumab was described by the dates (day/month/year) of each injection from the beginning to the end, and the number of injections.

BMD was measured by DXA at lumbar spine (LS; vertebra were included by application of ISCD guidelines) and the non-dominant hip (total hip – TH, and femoral neck – FN). All BMD measurements were performed on the same machine for each individual patient (Discovery A System, Hologic, 123 Waltham, MA, USA; or Lunar iDXA, GE Healthcare, Madison, WI, USA). DXA1 was performed during the 12 months before or the first three months after the first denosumab injection. DXA2 was performed during the 3 months before or the six months after the last denosumab injection. DXA3 was done around 18 months after the last denosumab injection (Supplementary Fig. 1). All patients had a vertebral fracture assessment at each DXA examination to screen for asymptomatic radiologic fractures.

sCTX values (premenopausal women normal ranges: 25–573 ng/l) were measured in early morning fasting blood samples at the central clinical routine laboratory of the Lausanne University Hospital. They were recorded for each of the three periods: 1) before denosumab initiation; 2) during denosumab treatment; and 3) one or several times after the last denosumab injection. We collected sCTX values measured after denosumab discontinuation: 1) 7 months ±30 days after last denosumab injection; 2) 10 months ±30 days after the last denosumab injection; and 3) 19 months ±30 days after the last denosumab injection, at closest of DXA3.

2.3. Definition of the losers and stable groups

Patients were classified as *losers* if BMD decreased over the least significant change (LSC) after denosumab discontinuation (DXA3 compared to DXA2), and *stable* in all other cases. We used the least significant changes (LSC) thresholds for each BMD measurement, expressed in gram/cm^2, calculated for the Lausanne University Hospital DXA devices as proposed in the ISCD DXA reporting guidelines [27]: 2.8 % at lumbar spine (LS), 3.0 % at total hip (TH) and 4.3 % at femoral neck (FN). We compared *losers* and *stable* at LS because: 1) most patients lose BMD at this site; 2) LS is more affected than hip sites at denosumab discontinuation; and 3) this site is the most reproducible at follow-up.

2.4. Primary and secondary outcomes

The primary outcome was to identify clinical and/or biological differences between the *losers* and the *stable* groups. The secondary outcome was exploratory, to determine if there was a cut-off value for sCTX associated with no loss of LS BMD after denosumab discontinuation.

2.5. Statistical analysis

Statistical analyses were conducted using Stata ICv14 © (StataCorp, College Station, Texas, USA) for Windows©. Results are expressed as number (percentage) or average ± standard deviation (SD). Association between studied parameters was determined using Student's *t*-test for continuous variables and Chi-squared test for categorical variables. Statistical significance was considered for a bivariate test with a p-value < 0.05. For the sCTX values measured at 10 and 19 months after denosumab discontinuation, that are significantly different between *losers* and *stable* groups: a) we investigated the association between sCTX values (dependent variable) and change in LS BMD after denosumab discontinuation (DXA3-DXA2; independent variable) using linear regression in a model adjusted by the number of denosumab injections (independent variable); b) we defined the receiver operating characteristic (ROC) curves, calculating the area under the curve (AUC), sensitivity, specificity and Youden's index (= sensitivity + specificity –

1).

2.6. Ethical statement

Informed consent was obtained from all participants included in the study. All procedures performed were in accordance with the ethical standards of the institutional research committee (CER-VD 2017-02135) and with the 1964 Helsinki declaration and its later amendments.

3. Results

3.1. Clinical characteristics of participants

Of the 170 patients included in the ReoLaus study, 63 postmenopausal women met the inclusion criteria. The reasons for exclusion are summarized in Fig. 1. Their characteristics are described in Supplementary table 1. Mean age was 64.2 ± 9.1 years at denosumab initiation and they received 7.9 ± 2.7 denosumab doses. After denosumab discontinuation, 54 (85.7 %) received a bisphosphonate (BP) in a delay of 8.1 ± 2.1 months after the last denosumab injection. The BP mean duration exposure after the last injection denosumab until DXA3, was 11.1 ± 2.8 months. The BPs given after the last denosumab injection were oral alendronate, zoledronate or a sequence of both treatments (oral BPs, and then zoledronate). The individualized treatment plan for each patient is detailed in Supplementary Fig. 2.

3.2. Bone mineral density

Results of DXA1 (before denosumab initiation), DXA2 (in mean 2.4 ± 3.9 months after the last denosumab injection), and DXA3 (in mean 19.0 ± 3.4 months after the last denosumab injection) are given in Table 1. Mean BMD increased significantly at all sites between DXA1 and DXA2 and decreased significantly at all sites between DXA2 and DXA3. Fifteen women lost BMD only at LS, and 26 at LS and the hip sites; in total 41 women loosing BMD at LS (75 %) were classified as *losers* (Table 1). Table 2 gives the characteristics of the *losers* and *stable* (n = 22) groups. Women in the *losers* group were younger (61.6 ± 8.2 vs. 69.0 ± 8.7 years), had lower LS T-score value and higher sCTX at denosumab initiation, and received more denosumab injections (8.5 ± 2.6 vs. 6.8 ± 2.4) than women in the *stable* group. LS BMD gain during denosumab treatment at LS and TH was higher in the *losers* group than in the *stable* group, and loss after denosumab discontinuation was correlated with BMD gain during denosumab treatment in the whole cohort ($r^2 = 0.18$, $p = 0.0012$). Although the same proportion of patients received BP treatment in both groups, more patients were treated with alendronate alone or a sole zoledronate infusion (alone, before or after an alendronate treatment) in the *stable* group, while eleven patients in the *losers* group received more than one infusion of zoledronate (Table 2).

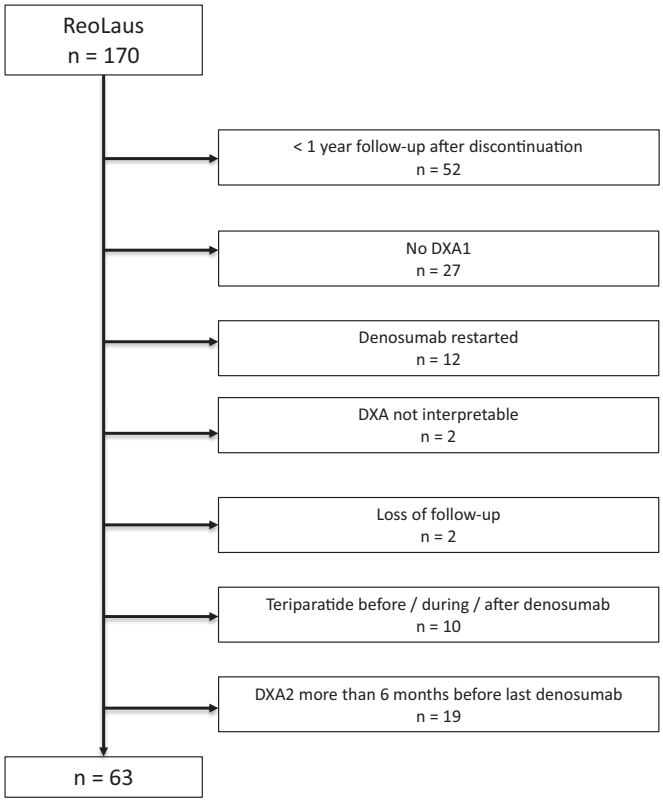


Fig. 1. Patients' exclusion criteria flow chart.
DXA: Dual-X ray Absorptiometry. DXA1 was performed during the 12 months before or the first three months after the first denosumab injection. DXA2 was performed during the 3 months before or the six months after the last denosumab injection.

3.3. Serum sCTX

Respectively, 43 (*losers* 29, *stable* 14), 21 (*losers* 15, *stable* 6), and 49 (*losers* 32, *stable* 17) women had sCTX measures at 7, 10 and 19 months after denosumab discontinuation. sCTX measured 7 months (±30 days) after the last denosumab injection were similar in both groups (Table 2), despite higher proportion of BP treated patients in the *losers* group (4/29; 13.8 %) than in the *stable* group (1/14; 7.2 %). sCTX measured at 10 months (±30 days) and 19 months (±30 days) after the last denosumab injection were significantly higher in *losers* (mean values at the superior limit of the premenopausal range) than in *stable* group (mean values in the middle of the premenopausal range; 590 ± 372 vs. 221 ± 101 ng/l; and 598 ± 324 versus 293 ± 157 ng/l, respectively; $p < 0.001$). There was no significant difference ($p = 0.30$) in the proportion of non-treated patients at the 10 months' time-point in the *losers* group (6/15, 40 %), vs the *stable* group (1/6, 17 %). Mean sCTX values of the whole cohort were in the premenopausal range (Supplementary table 1), but only individual values of *stable* remained within the premenopausal baseline range throughout follow-up (Table 2). The LS BMD loss 19 months after

Table 1
Dual-X ray absorptiometry results at different time-points.

	n (%)	Lumbar spine	Total hip	Femoral neck
a. T-score values ± SD	63 (100)			
DXA1		−2.69 ± 1.11	−1.71 ± 0.70	−1.98 ± 0.71
DXA2		−1.81 ± 1.17*	−1.34 ± 0.77*	−1.63 ± 0.79*
DXA3		−2.14 ± 1.31**	−1.53 ± 0.79**	−1.79 ± 0.77**
b. BMD loss (DXA3 - DXA2; g/cm^2)				
1. only LS	15 (23.8)	−7.2 % ± 4.0 %	−0.5 % ± 1.4 %	0.6 % ± 3.0 %
2. LS and TH or FN	26 (41.3)	−8.6 % ± 4.4 %	−6.3 % ± 3.4 %	−6.1 % ± 5.5 %
3. TH or FN	6 (9.5)	−0.3 % ± 2.0 %	−3.9 % ± 1.2 %	−5.2 % ± 4.3 %
4. none	16 (25.4)	1.1 % ± 3.7 %	1.3 % ± 3.0 %	−0.3 % ± 2.5 %

SD: Standard Deviations; DXA: Dual-X ray Absorptiometry; BMD: bone mineral density; LS: lumbar spine; TH: total hip; FN: femoral neck; n = number of patients. Results are expressed as number (%) or mean ± standard deviation.
* $p < 0.001$ between values at the end of denosumab treatment (DXA2), and before denosumab treatment (DXA1).
** $p < 0.001$ between values 19 months after last denosumab injection (DXA3) and the end of denosumab treatment (DXA2).

Table 2
Characteristics of *Losers* and *Stable* patients.

	Losers n = 41	*Stable* n = 22	p-Value
Age at denosumab initiation (years)	61.6 ± 8.2 (40, 81)	69.0 ± 8.7 (55, 85)	0.003
BMI at denosumab initiation (kg/m^2)	23.2 ± 3.1 (16.5, 30.2)	24.5 ± 3.9 (18.7, 35.6)	0.21
BP before denosumab initiation	6 (14.6 %)	6 (27.3 %)	0.22
Number of denosumab injections	8.5 ± 2.6 (3, 14)	6.8 ± 2.4 (2,10)	0.014
T-score values at LS (SD)			
DXA 1	−3.03 ± 0.98 (−4.7, 0.5)	−2.08 ± 1.10 (−3.8, 0.4)	0.002
DXA 2	−2.07 ± 1.14 (−4.0, 2.0)	−1.32 ± 1.14 (−2.9, 1.4)	0.017
DXA 3	−2.61 ± 1.13 (−4.5, 1.7)	−1.26 ± 1.20 (−3.0, 1.5)	< 0.001
%BMD change (DXA2-DXA1)			
LS	15.76 ± 8.58 (1.40, 40.60)	9.84 ± 5.04 (2.78, 21.24)	0.003
TH	7.58 ± 4.39 (−0.98, 19.43)	4.61 ± 4.85 (−5.02, 21.17)	0.037
FN	7.96 ± 10.62 (−8.5, 51.94)	6.68 ± 7.05 (−3.38, 33.21)	0.609
% BMD change (DXA3-DXA2)			
LS	−8.1 ± 4.3 (−21.8, −3.1)	0.8 ± 3.4 (−2.8, 13.0)	
TH	−4.2 ± 4.0 (−17.3, 2.6)	−0.1 ± 3.5 (−5.8, 7.4)	< 0.001
FN	−3.6 ± 5.7 (−27.3, 8.7)	−1.6 ± 3.8 (−9.0, 4.8)	0.10
sCTX (ng/l)			
Before denosumab initiation	615 ± 159 (291, 987)	446 ± 269 (16, 1060)	0.037
During denosumab treatment	41 ± 21 (10,120)	78 ± 89 (17, 412)	0.13
7 months (±30d) after last denosumab	290 ± 280 (44, 1031)	264 ± 170 (50, 701)	0.72
10 months (±30d) after last denosumab	590 ± 372 (100, 1352)	221 ± 101 (129, 401)	0.007
19 months(±30d) after last denosumab	598 ± 324 (109, 1749)	293 ± 157 (100, 582)	< 0.001
BP after denosumab discontinuation number of treated patients	36 (87.8 %)	18 (81.8 %)	0.52
Type of treatment			0.001
No treatment	5 (12.2 %)	4 (18.2 %)	
Alendronate only treated patients	4 (9.8 %)	6 (27.3 %)	
Zoledronate treated patients[a]	32 (78.0 %)	12 (54.5 %)	
1 infusion	21 (51.2 %)	12 (54.5 %)	
>1 infusion	11 (26.8 %)	0 (0.0 %)	
Delay after last denosumab (months)	8.0 ± 1.9 (5.0, 16.0)	8.2 ± 2.3 (5.5, 13.6)	0.78
Duration of BP treatment until DXA3 (months)	11.4 ± 2.6 (6.2, 16.7)	10.3 ± 3.0 (4.4, 15.5)	0.20
Fractured patients; n (%)			
Before denosumab initiation	21 (51.2)	11 (50.0)	0.93
During denosumab treatment	3 (7.3)	2 (9.1)	0.80
After denosumab discontinuation (VF)	3 (7.3)	0 (0)	0.08
n of VF (3 patients)	5 (12.1)	0 (0)	0.13

Losers are defined by a bone mineral density (BMD) decrease at lumbar spine (LS) over the least significant change (LSC) after denosumab discontinuation (DXA3 compared to DXA2); all other patients are defined as *Stable*. DXA1 was performed during the 12 months before or the first three months after the first denosumab injection. DXA2 was performed during the 3 months before or the six months after the last denosumab injection. DXA3 was performed around 18 months after the last denosumab injection.
BMI: body mass index; BP: bisphosphonates; SD: standard deviation; DXA: Dual X-ray Absorptiometry; TH: total hip; FN: femoral neck; sCTX: serum CrossLaps; d: days; n = number; VF: vertebral fractures. BP stands for oral alendronate or intravenous zoledronate.

[a] Zoledronate alone, or in a sequence with oral alendronate. Values are expressed in number (%) or mean ± standard deviation (minimum, maximum).

the last denosumab injection was correlated to the level of sCTX measured at 10 months ($r^2 = 0.29$, p = 0.01, Fig. 2a), and at 19 months ($r^2 = 0.16$, p = 0.004, Fig. 2b). As denosumab treatment duration influences the intensity of the rebound, we evaluated whether the association between the sCTX measures at 10 and 19 months after last denosumab injections and the BMD changes after denosumab discontinuation (DXA3-DXA2) was related to the length of denosumab treatment. The linear regression analysis found that 1 % decrease in BMD between DXA2 and DXA3 was significantly associated with a 54 ng/l increase in sCTX at 10 months (beta: −0.54, p-value = 0.012), and a 40 ng/l increase in sCTX at 19 months (beta: −0.40, p-value = 0.004). These associations remained significant after adjusting for the number of denosumab injections that the individual had received (beta: −0.50 and − 0.39 respectively; p-value = 0.017 and 0.006 respectively).

We performed a ROC-curve analysis to determine a cut-off value at each time-point that discriminates between patients in the *losers* and *stable* groups (Supplementary Fig. 3). The analysis of sCTX values at 10 months as a marker for BMD loss at LS after denosumab discontinuation (DXA3-DXA2) resulted in an AUC of 0.78. According to Youden's index (0.60), the optimal cut-off value for identifying patients that will lose BMD at LS is 401 ng/ml (70 % of the upper normal range for premenopausal women, 573 ng/ml), with a sensitivity of 0.60 and a specificity of 1.00. The analysis of sCTX values at 19 months resulted in an AUC of 0.82. According to Youden's index (0.61), the optimal cut-off value for identifying patients that will lose BMD at LS is 369 ng/ml (64 % of the upper normal range), with a sensitivity of 0.84 and a specificity of 0.76.

3.4. Subgroup analyses

Including patients with DXA2 performed up to 3 months before the last denosumab injection may have biased the results, as they may have still gained density up to 6 months after last denosumab injection. We thus did a sensitivity analysis of the 23 patients who benefited of DXA2 strictly at 6 months ±30 days of last denosumab (16 *losers*, 7 *stable*). LS T-scores at different time-points were similar to those of the whole cohort, although not anymore statistically different except for DXA3 (*losers* − 2.72 ± 0.75; *stable* − 1.24 ± 1.09 p = 0.015). Main % BMD change after denosumab discontinuation was slightly higher than in the whole cohort as expected (−9.94 % ± 5.03 in *losers*, vs. 0.92 ± 5.03 in *stable*). sCTX values at each time-point were comparable to those of the whole cohort, although the difference between *losers* and *stable* was statistically significant only for the 19-month time point (592.5 ± 272.9 vs. 279.5 ± 177.7; p = 0.016), probably due to the low number of patients as values ranges were similar.

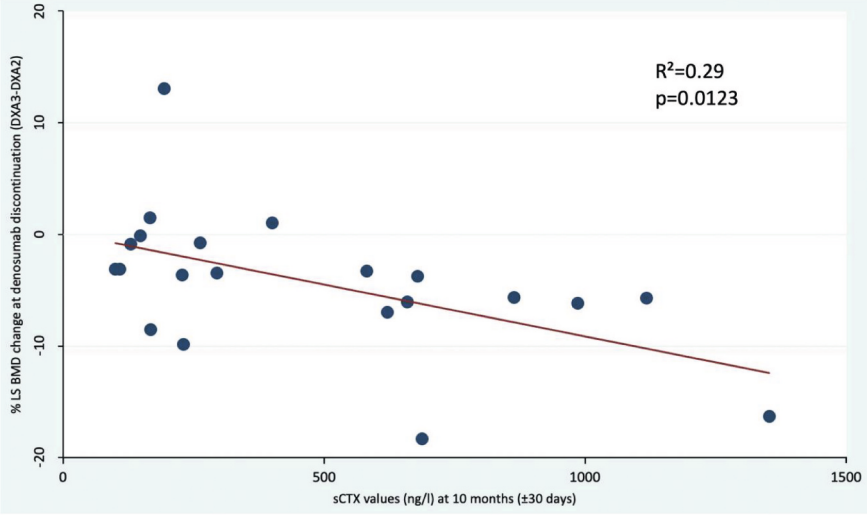
We performed a sensitivity analysis including only patients treated with denosumab for AI-related bone loss (12 patients, evenly distributed between *losers* and *stable* groups), as they may have different characteristics. DXA and sCTX mean values were comparable to those of the whole cohort in both *losers* and *stable* groups (results not shown), although none were statistically different between the groups probably due to the low number of patients as values ranges were also similar.

We did a sensitivity analysis including in the *losers* group the 6 patients who lose BMD at the total hip and/or the femoral neck but not at the LS. Results were almost identical to those of the whole cohort analysis (results not shown), with the exception of the BMD gain during denosumab treatment that became significant at the femoral neck (8.48 ± 10.62 in the *losers* vs. 4.46 ± 3.13 in the *stable*, p = 0.04), and the lack of significant difference in sCTX before denosumab (577.6 ± 184.8 vs. 474.8 ± 291.9, p = 0.29).

3.5. Fractures

Five spontaneous VFs occurred in three women after the end of

a) Correlation between serum crosslaps (sCTX) values (ng/l) 10 months (±30 days) after last denosumab, and % LS BMD change after denosumab discontinuation (DXA3-DXA2)



b) Correlation between serum crosslaps (sCTX) values (ng/l) 19 months (±30 days) after last denosumab, and % LS BMD change after denosumab discontinuation (DXA3-DXA2)

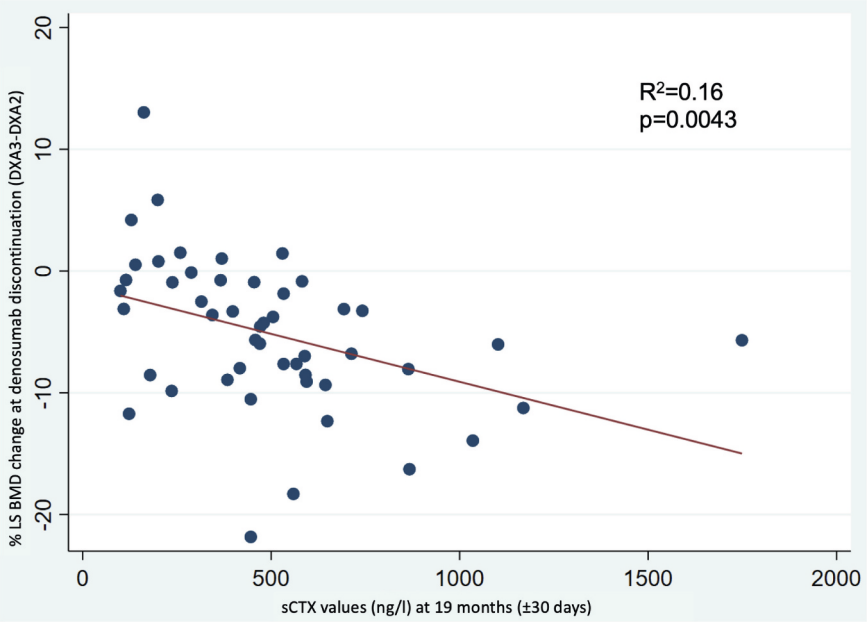


Fig. 2. Correlation between serum crosslaps and BMD change at denosumab discontinuation.
a) Correlation between serum crosslaps (sCTX) values (ng/l) 10 months (±30 days) after last denosumab, and % LS BMD change after denosumab discontinuation (DXA3-DXA2).
b) Correlation between serum crosslaps (sCTX) values (ng/l) 19 months (±30 days) after last denosumab, and % LS BMD change after denosumab discontinuation (DXA3-DXA2).

denosumab efficacy (last injection +6 months): 3/41 (7.3 %) in the *losers* group and none in the *stable* group. VFs occurred 14.5 ± 2.7 months after the last denosumab injection (Table 2): 1) one VF at 12.7 months in a woman who did not receive BP (sCTX not measured at that time); 2) one VF at 18.3 months in a patient who received zoledronate 11.9 months after the last denosumab injection (sCTX = 580 ng/l at the time of VF), 3) three VFs at 12.5 months in a patient who received alendronate 10 months after the last denosumab injection (sCTX =1229 ng/l at the time of VFs). In these 3 patients, one year after denosumab discontinuation LS BMD had decreased by 3.8 %, 6.8 % and 7.0 %, respectively, and the LS T-score was −0.7, −3.4 and −1.9 SD, respectively. Their characteristics were not different from other women in the *losers* group, including prevalent VFs, magnitude of BMD gain or loss. Moreover, a patient from the *losers* group developed a costal fracture at 6.1 months after las denosumab injection (sCTX = 277 ng/l at the time of the fracture). No radiologic asymptomatic vertebral fracture was diagnosed.

4. Discussion

In the ReoLaus cohort, loss of lumbar spine BMD after denosumab withdrawal was associated with younger age, lower BMD and higher sCTX at denosumab initiation, longer denosumab treatment, more BMD gained during treatment, and higher level of sCTX during the year after denosumab discontinuation. Mean sCTX values in the middle of the reference range for premenopausal women after denosumab discontinuation were associated with BMD stability over one year, while they were in the upper normal range in patients losing BMD at LS.

In the ReoLaus cohort, 65 % of the women lost LS BMD 19 months after the last denosumab injection, despite 87.8 % of them receiving

bisphosphonates after denosumab discontinuation. The efficacy of antiresorptive treatments on avoiding BMD loss at denosumab discontinuation varies between studies. Most observational and randomized studies have been conducted with zoledronate, and report a partial loss of the BMD gained, with variable results [7,15,18,22,28], from the maintenance of the BMD gained [17], to a 100 % loss at the total hip [25]. In the only study with weekly alendronate at denosumab discontinuation, the DAPS study, 15.9 % and 21.7 % of the women lost part of the BMD gained at the LS and FN, respectively [20]. The observed differences may be due, among others, to denosumab treatment duration, the timing of BP administration, and the definition used for BMD loss. BMD gain is more likely to be maintained with a BP if denosumab treatment lasted ≤3 years [15,16,24]. In our study, the mean denosumab treatment duration was about 4 years, with shorter treatment in the *stable* group, and we chose the LSC used in clinical practice at our center to define the BMD loss (2.8 % at LS).

4.1. Risk factors for BMD loss before denosumab initiation

Women who lost BMD after denosumab discontinuation were younger and had lower BMD values at denosumab initiation as previously described [11]. They also had higher sCTX before denosumab initiation. One hypothesis is that younger patients have higher BTMs because they are closer to the perimenopause [29], as this BTMs increase is associated with an average bone loss of 10 % in the first 5 years after menopause [30]. They may also have higher constitutive bone turnover and thus have been diagnosed with osteoporosis at an earlier age. The difference in BTMs values prior denosumab initiation was not explained by previous treatments received (same proportion of patients in both groups had received BP); they also had similar prevalent fragility fractures and AI treatments exposure.

4.2. Risk factors for BMD loss during denosumab treatment

As other authors, we found an association between the BMD gained during treatment and the BMD lost after denosumab discontinuation [5,11]. Also, women who lost BMD after denosumab discontinuation had received more denosumab injections as previously suggested in patients treated with bisphosphonates [15,16] or without further treatment after denosumab discontinuation [31]. However, some studies did not show this relationship [4,5]. The intensity of the rebound effect, which can be assessed by BTMs measure, may be an explanation. It has been shown that after 6 years of denosumab treatment BTMs levels increase more between two injections than after the first year of therapy [32]. In addition, denosumab treatment duration seems to be associated with a higher risk of multiple VFs after its discontinuation [6,8,12]. A linear regression analysis performed to evaluate this hypothesis found that denosumab treatment duration did not affect the association between sCTX values at 10 or at 19 months, and BMD changes at denosumab discontinuation. However, the difference in the mean number of denosumab injections between the *losers* and the *stable* group is small (8.5 ± 2.6 vs. 6.8 ± 2.4), and our results do not preclude that longer treatments influence this relationship.

4.3. Risk factors for BMD loss after denosumab treatment

A higher level of sCTX after denosumab discontinuation was associated with BMD loss in our study: the mean sCTX value in the *stable* group was in the middle of the normal range for premenopausal women (221 ± 101 and 293 ± 157 ng/l at 10 and 19 months after the last denosumab injection, respectively; normal range: 25–573 ng/l), but in the upper limit of the normal range in the *losers* group (590 ± 372 and 598 ± 324 ng/l, respectively), a highly significant result (both $p < 0.01$). Moreover, none of the women in the *stable* group experienced new VFs. Other authors have previously suggested that higher BTMs after denosumab discontinuation may be related to the level of BMD loss whether [25] or not [5] the women received zoledronate, but no difference was observed in BTMs values measured after denosumab discontinuation between women who lost and who did not lose BMD under zoledronate or alendronate off-treatment therapy [17,20]. However, exposition to denosumab was short (2.2 and 1 year, respectively), and the threshold defining LS BMD loss was high [17]. An association was found between higher BTMs levels after denosumab discontinuation and TH and FN BMD loss in a large observational study [11].

4.4. Maintaining sCTX at a low level to avoid BMD loss: effect of bisphosphonates

Our results and those of published studies suggest a relationship between the intensity of the rebound, reflected by sCTX levels, and the BMD loss. Thus, we can hypothesize that maintaining sCTX low, i.e., in the lower reference values for premenopausal women (as in the case of the *stable* group in our study) by adapting BP before and/or after denosumab treatment, could allow to preserve the acquired BMD and avoid the risk of VFs.

Patients previously treated with BP had an attenuated rebound effect, as measured by sCTX, after denosumab discontinuation in one study [33]. In one large observational study [11] antiresorptive treatment in the two years before denosumab introduction was related to LS BMD loss in univariate analysis, but disappeared after adjustment. Moreover, in a large retrospective study including 797 women who stopped denosumab, BPs given before denosumab decreased the risk of VFs after stopping denosumab in patients not treated afterwards [7]; this was not the case in another study with low number of events [34]. In our study, one quarter of the patients were previously treated with a BP, and >80 % afterwards, without differences between groups, but the low number of cases prevents drawing conclusions. Also, outcomes could be different depending on the BP used prior to denosumab, and/or the number of years of BP treatment, and/or the time interval between the end of BP treatment and the start of denosumab; no study to date analyzed these aspects to our knowledge. To note, in the study from Burckhardt et al. [7], BPs given after denosumab were more efficacious than BPs given before it in reducing the risk of VFs, and there was no greater benefit of combining both strategies.

There are still few data on how BP given upon denosumab discontinuation affect sCTX. In one study (n = 120) in which mean BMD loss was between 33 % (LS) and 51 % (TH) of the BMD gained during denosumab treatment, sCTX were within the upper normal range one to four years after the last denosumab injection in 23 women treated by a single infusion of 5 mg zoledronate administered six months (±3 weeks) after the last dose of a 2- to 5-year denosumab treatment [18], suggesting that a single zoledronate infusion did not sufficiently control bone resorption. In a smaller study of 27 women similarly treated after the last denosumab injection (mean treatment duration 2.2 years), sCTX levels were higher than the reference range, but BMD loss was prevented for at least 2 years independently of the rate of bone turnover. However, the BMD threshold to define a loss was high (>5 %) [17], and one patient suffered multiple VFs despite the zoledronate infusion, suggesting insufficient control of the rebound. In a randomized controlled trial including 61 women, zoledronate was administrated 6 or 9 months after the last denosumab injection, or when BTMs increased [22]. sCTX decreased below baseline 1 month after zoledronate infusion, but increased at months 3, 6 (peak; above the reference range for premenopausal women) and 12; 65 % of the patients from all groups lost BMD at LS beyond the LSC (> 3 %) one year after zoledronate administration [22]. In this and another large observational study [15], a second or third zoledronate infusion was given to patients with increased sCTX, without better BMD results at two years than in women who received a single infusion [15,23]. However, sCTX threshold for repeating the zoledronate infusion was very high (> 50 % up the normal range of postmenopausal women for the randomized trial, or after a ≥ 2-fold increase of CTX and/or P1NP in the observational study) [15,23]. It is

not excluded that with a lower sCTX threshold, the 2-year BMD results would have been better.

We found, using ROC curves, similar cut-off values of sCTX discriminating women in the *losers* vs. the *stable* group at 10 months (70 % of the upper normal value for premenopausal women) and 19 months (64 %), with high sensitivity and specificity. Because of the high variability of the sCTX measures, and the variability of the normal ranges depending on the test used, we may consider a cut-off around two-thirds of the upper normal value for premenopausal women. Our results support the only position statements published by the European Calcified Tissue Society. An adequate response for the management of denosumab discontinuation is defined as "a level of sCTX below the mean found in healthy premenopausal women, with a threshold below 280 ng/l" [24]. Higher doses or higher frequency of BP administration than those used in the treatment of osteoporosis could be required. Recommending a standardized BP treatment would be ideal, but it may be necessary to adjust the dose and/or frequency of BP administration once the sCTX exceeds the indicated threshold. In our study, the percentage of women treated with a BP, the time of initiation and the duration of treatment with a BP were the same in the *losers* and *stable* groups. However, only patients in the *losers* group received more than one zoledronate infusion during the follow-up, probably as a result of an increase in sCTX, as we progressively implemented the intensification of BP treatment as proposed [21]. We can hypothesize that without repeated treatment, the BMD loss would have been more marked. Although various risk factors influence the rebound effect, it is not possible to predict its intensity. Regular monitoring of BTMs after denosumab discontinuation, in order to adjust BP therapy to keep them within a given target, is probably the best strategy to propose at this time.

4.5. Limitations

Several limitations should be considered. First, this is a real-life, single-center observational study with patients exposed to varying durations of denosumab treatment and different follow-ups after its discontinuation. Thus, treatments were not standardized, nor were the times at which blood and densitometry monitoring was performed. Second, the rebound effect after denosumab discontinuation lasts for two years. We report here the results of the first year; the evolution of BMD and BTMs in the second year, and their relationship, may be different. Third, we used a threshold of 2.8 % to define LS BMD loss. This is a stringent threshold, but it is the one used in clinical routine. It is also imaginable to use a different threshold, depending on the clinical significance of the BMD loss. Our study also has strengths. For each patient, the different DXA measurements were done on the same machine, and sCTX measurements were all performed in the morning under fasting conditions in the same laboratory. The persistence of the observed results in the different sensitivity and subgroup analysis despite the heterogeneity of the cohort follow-up reinforces our conclusions.

5. Conclusions

The results of the ReoLaus study support the hypothesis that it could be necessary to maintain sCTX in the low reference range of premenopausal women after denosumab discontinuation to preserve the BMD gain achieved. Whether patients with stable values had a less intense rebound, or whether regular monitoring of sCTX and adjustment of doses and/or frequency of administration of bisphosphonates may allow to achieve this target should be analyzed in a dedicated study.

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CRediT authorship contribution statement

Giovanni Liebich: Data acquisition, formal analysis, manuscript drafting and reviewing visualization. Olivier Lamy: Conceptualization, data interpretation, manuscript drafting and reviewing, supervision. Berengere Aubry-Rozier: Formal analysis, manuscript reviewing. Elena Gonzalez-Rodriguez: Formal analysis, data interpretation, manuscript drafting and reviewing.

Declaration of competing interest

All authors state that they have no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2023.116764.

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