Title: Bisphosphonates after Denosumab withdrawal reduce the vertebral fractures incidence

Short title: Bisphosphonates and Denosumab withdrawal

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Abstract

Objective: Several studies showed the occurrence of vertebral fracture (VFx) in patients discontinuing denosumab (Dmab), suggesting the need of bisphosphonate (BPs) therapy to mitigate this VFx risk increase. However, the morphometric VFx (morphoVFx) incidence after Dmab discontinuation and the BPs effect on VFx risk in this setting are still a matter of debate.

Design: Retrospective, monocentric study**.**

Methods: In 120 patients (111 females) discontinuing Dmab, 19 have not been treated (Not-treated Group, 16 females, age 63.5±15.0 years) and 101 patients have been treated (Treated Group, 95 females, age 70.0±10.6 years) with BPs (28 alendronate, ALN; 73 zoledronate, ZOL, single infusion), respectively. We evaluated the incidence of both clinical VFx and morphoVFx in Treated Group and Non-treated Group.

Results: Patients in Treated Group showed a 5.5% VFx incidence (n=6, 3 clinical, 3 morpho VFx), which was anyway lower than Not-treated Group patients (n=4, 21.1%, 4 clinical, 3 multiple, p=0.029), despite a comparable FRAX score at the time of Dmab initiation. The logistic regression analysis showed that the VFx incidence was independently associated with the lack of BPs treatment (odds ratio 13.9, 95% confidence interval 1.7-111.1, p=0.014), but not with the number of Dmab injections, age, duration of BPs before Dmab initiation, the BMD at Dmab withdrawal and the prevalence of VFx at Dmab withdrawal.

Conclusions: The Dmab withdrawal is associated with an increased risk of clinical but not morphometric VFx. Therapy with ALN or with a single ZOL treatment are partially effective in reducing the increased VFx risk after Dmab withdrawal**.**

Introduction

Denosumab (Dmab), a monoclonal antibody against the receptor activator of nuclear factor κB ligand (RANKL), is a potent antiresorptive agent, which profoundly and continuously suppresses bone turnover markers (BTMs), increases bone mineral density (BMD), and reduces fracture risk [1]. Nowadays, a good safety profile is guaranteed for up to 10 year (1).

At variance with bisphosphonates (BPs), Dmab does not incorporate into bone matrix, and, for this reason, its effects are reversible when therapy is discontinued. Indeed, after Dmab discontinuation, BTMs increase rapidly and, at 9 months after last injection, exceed their baseline levels and remain elevated for about 2 years, decreasing slowly to baseline levels approximately 30 months after the last injection. In keeping with the BTMs behavior, after Dmab withdrawal, the BMD gained during treatment is lost, reaching baseline values within 12 and 24 months (2–5). This phenomenon, commonly described as the "rebound phenomenon", has been suggested to be due, among other causes, to the fact that osteoclast precursors, which remained quiescent during the treatment period, retake simultaneously their activity (6). Notably, longer is the Dmab therapy, faster is the BMD decline (7).

This rebound phenomenon seems to be associated with an increased risk of clinical vertebral fractures (VFx). Indeed, since 2015, several case reports and series have been published describing the occurrence of unexpected VFx, including multiple VFx (MVFx) in patients discontinuing Dmab (6, 8). A post hoc analysis of the FREEDOM and FREEDOM Extension studies showed that as compared with the placebo arm, patients discontinuing Dmab had significantly higher frequency of multiple VFx (7). However, many authors believe that the increased VFx incidence after Dmab withdrawal is evident mainly in patients with high-risk of fracture before Dmab therapy and that after Dmab discontinuation this high risk population returns to the pretreatment fracture risk (9).

To prevent this "rebound phenomenon" in patients stopping Dmab, a BPs treatment, such as oral alendronate (ALN) or intravenous zoledronate (ZOL) has been advocated, irrespective of the attained BMD at the time of the transition between treatments. Although, several scientific societies have issued position statements on this topic, the optimal BPs regimen to mitigate bone loss and the subsequent fracture risk is still a matter of ongoing research (9–11).

The available studies show that ALN and ZOL therapy after Dmab discontinuation only attenuate the rebound-related bone loss (12–15). Indeed, recent data suggest that a not negligible number of patients discontinuing Dmab show a BMD loss after a single ZOL irrespective of the duration of Dmab therapy and of the timing of ZOL administration (9,12,13). Up to now, data about the BPs effect on VFx in this setting are not conclusive, although some data suggest that BPs may have a protective effect on MVFx after Dmab withdrawal (14). Finally, data on morphometric VFx (morphoVFx) incidence after Dmab discontinuation have been reported in only one study (Burckhardt)

This retrospective real-life monocentric study was designed to evaluate in a sample of consecutive patients who discontinued Dmab the incidence of both clinical VFx and morphoVFx and the effect of post Dmab BPs treatment on VFx risk, and possibly the factors associated with VFx risk in treated patients.

Patients and Methods

Patients

In this observational study, we retrospectively examined the available data at September 2020 of all patients (n=415), referred to our outpatient clinic for Metabolic Bone Diseases, who had been treated since May 2011 with Dmab (60 mg subcutaneously every 6 months) for at least 12 months (2 injections), on the basis of the Italian prescription rules (http://www.agenziafarmaco.gov.it/sites/default/files/Determinazione_446-2017_agg_nota79.pdf). Among these, we evaluated data of all patients (n=195), who discontinued the Dmab therapy within September 2018.

Among these patients we excluded 75 subjects on the basis of the following criteria: i) administration of less than 2 injections of Dmab (n=6); ii) premenopausal status and/or secondary osteoporosis other than aromatase inhibitors (AI) related osteoporosis (n=39); iii) lack of BMD measurement by dual-energy x-ray absorptiometry (DXA) and/or of thoraco-lumbar spinal radiographs at the time of Dmab discontinuation and/or at the end of follow-up (n=25); vi) treatment after Dmab discontinuation with bone active drugs beyond bisphosphonates (n=5).

Eventually we enrolled 120 patients (9 males and 111 females). According to the Italian prescription rules, all patients had been treated for primary osteoporosis (n=106) or for concomitant therapy with aromatase-inhibitors (AI, n=14). Among the included patients, 19 patients expressly wanted to discontinue Dmab, regardless BMD levels, and did not accept to be treated with BPs (Not-treated Group) due to the fear of jaw osteonecrosis, although they have been exhaustively warned about the high fracture risk related to the rebound phenomenon and about the very low risk of jaw osteonecrosis. The remaining 101 patients were suggested to discontinue therapy (Treated Group) and were subsequently treated with BPs. Within the treated group 11 patients (10.8%) were suggested to discontinue Denosumab due to a suboptimal compliance, regardless the BMD status. During each consultation patient were asked to report the dates of Denosumab injections. A good compliance was defined if patients reported an injection interval $\leq$7 months (16). All patients, defined as non-compliant, showed a moderate adherence (injection delay 1-3 months) but they were considered at risk of possible unplanned further discontinuation. One of these patients experienced a clinical vertebral fracture during Denosumab therapy possibly due to Denosumab delay. All non-compliant patients were treated with a single infusion of ZOL in order to avoid a subsequent lack of compliance. Within the remaining 90 patients, 78 were suggested to discontinue Denosumab in the presence of BMD at lumbar spine (LS) and total hip (TH) above -2.5. This BMD threshold, which until 2017 was considered safe, was evaluated together with other possible risk factors in order

to define the overall patients fracture risk (17) (18). Twelve patients were suggested to discontinue Dmab for AI therapy discontinuation and without other criteria to continue Dmab therapy, regardless BMD (according for Italian prescription rules).

Among patients in Treated Group, 73 and 28 patients were treated with ZOL (single infusion, administered between 30 and 60 days after Dmab discontinuation) and ALN (once a week, immediately after Dmab discontinuation, for the whole follow up), respectively. We planned a single ZOL infusion on the basis of the evidence that the ZOL infusion effect persists well beyond 12 months (19) and in keeping with the ECTS position statement, which suggested to administer a single ZOL infusion (to be given possibly after Dmab discontinuation at the time of bone turnover increase) or to initiate oral bisphosphonates after Dmab withdrawal (20). During each consultation, patients treated with ALN were asked about their compliance (referred compliance ≥80% in al patients) and they were encouraged to contact our center in case of poor drug tolerability to evaluate ZOL infusion. All patients discontinued Dmab and AI therapy simultaneously. All patients were taking with vitamin D and calcium supplements. Among Treated Group and Not-Treated Group 71 and 8 patients respectively had been treated with BPs prior to Dmab therapy (27 and 1, respectively discontinued BPs ≤12 months before Dmab therapy). The study plan is depicted in figure 1.

Methods

We report data of all patients at Dmab withdrawal (t_1) and at the end of the follow-up period (t_2), which lasted 24 months. From all the enrolled patients, we collected information on body mass index (BMI), smoking habits, family history of osteoporosis and hip fractures.

Moreover, information on prevalent and incident clinical fragility fractures at Dmab initiation (t_0, t_1 and t_2) were obtained from all subjects at consultation. Fracture was considered prevalent and due to bone fragility if occurred before Dmab therapy and without any evident trauma or after a low-energy trauma (e.g., a fall from a standing height), respectively. The information given by patients were confirmed by reviewing the medical records. Traumatic fractures were not considered in the analysis. Information on incident clinical fragility fractures were obtained from all subjects at regular clinical visits and confirmed by reviewing the medical records. We also encouraged patients to contact us in case of ascertained VFx and/or symptoms suggesting VFx. All data were confirmed by reviewing the medical records. We reported as prevalent at t_1 all fractures occurred before Dmab withdrawal. Fractures at t_2 were considered incident only if occurred after Dmab discontinuation (t_1) and if due to bone fragility.

For all patients we calculated, at baseline (before starting Dmab therapy) the 10 years probability of a major fracture with FRAX (21). For all patients we reported serum creatinine and 25hydroxy-vitamin D (25OHD) before starting Dmab, at Dmab discontinuation and at the end of follow up.

In all patients BMD was measured by DXA (Hologic Discovery, software version 13.3:3, Bedford, MA) at the lumbar (L1-L4) spine (LS, Z-LS, in vivo precision 1.0%), femoral neck (FN, Z-FN, in vivo precision 1.8%), and total hip (TH, Z-TH, in vivo precision 1.7%) at t_0, t_1 (~6 months after the last Dmab injection) and t_2 (~30 months after last Dmab injection). We calculated the LS-, FN and TH-BMD changes between t_1 and t_0 (Δt_1-t_0) to evaluate BMD variation during Dmab therapy, between t_2 and t_1 (Δt_2-t_1) to evaluate BMD variation after Dmab discontinuation, and between t_2 and t_0 (Δt_2-t_0) to evaluate BMD variation between the end of the follow-up and the Dmab initiation. The BMD variation was considered significant if above or below the LSC (LS 2.8%, FN 5.9%, TH 4.8%). Patients were classified as "improved" or "worsened" if their BMD were significantly increased or reduced, respectively.

At t_0, t_1 and t_2, a conventional spinal radiograph in lateral and anteroposterior projection (T4–L4) was obtained in all subjects using a standardized technique. Morphometric VFx (morphoVFx) were diagnosed using the semiquantitative visual assessment (SQ) (22). Fractures were defined as reductions of >20% in anterior, middle, or posterior vertebral height. From lateral spine radiographs, 13 vertebrae from T4 to L4 were assessed visually as intact (SQ grade 0) or as having approximately mild (20% to 25% height reduction), moderate (25% to 40% height reduction), or severe (>40% height reduction) deformity (SQ grades 1, 2, and 3, respectively. In all patient we calculated Spinal Deformity Index (SDI) by summing the fracture grades of all vertebrae (T4 to L4) that is considered an tool for assessing future VFx risk (23). Two radiologists, who were blinded to BMD data, independently reviewed the radiographs. The questionable cases were collectively discussed to agree on a diagnosis.

Statistical analysis

Statistical analysis was performed by SPSS version 26.0 statistical package (IBM, Chicago, IL).

The results were expressed as mean±SD. The normality of distribution was tested by Kolmogorov–Smirnov test. The comparison of continuous variables was performed using Student's t-test or Mann–Whitney U test as appropriate. Categorical variables were compared by $\chi 2$ test or Fisher Exact test, as appropriate.

The logistic regression analysis assessed the association between the BPs therapy after Dmab withdrawal and the subsequent occurrence of VFx after adjusting for the variables that resulted to be different between Treated Group and Non-treated Group at the time of Dmab withdrawal and for the factors commonly associated with the risk of VFx after Dmab withdrawal, such as age, BMD at LS and prevalent multiple VFx at the time of Dmab initiation.

P-values of less than 0.05 were considered significant.

Results

The clinical and biochemical parameters of all patients (n=120) at the time of Dmab discontinuation and the comparison between BPs treated patients (Treated Group, n=101) and non-treated patients (Not-treated Group, n=19) at

the time of Dmab discontinuation are reported in table 1. Overall,10 patients (8.3%) experienced a VFx, which occurred in 4 patients (21.1%) from Not-treated Group, but, importantly, even in 6 patients (5.9%) from Treated Group.

In Not-treated Group, all VFx (n=4) were clinical, and 3 out of 4 were multiple VFx (2, 4 and 9 VFx, respectively). These 4 patients presented symptoms of clinical VFx 3-4 months after Dmab discontinuation (i.e. 9-10 months after last Dmab injection). At variance, in Treated Group, only 3 patients (2.5% of Treated patients) experienced the occurrence of clinical VFx, while the other 3 fractured patients had only morphoVFx. No treated patients experienced multiple VFx. In ALN group 2 patient experienced a clinical VFx (6.1% of ALN patients), 1 and 3 months after Dmab discontinuation (i.e. 7 e 9 months after last Dmab injection), no patient in ALN presented morpho VFx. In ZOL group, only 1 patient experienced a clinical VFx (1.4% of ZOL patients), 11 months after ZOL infusion (i.e. 18 months after last Dmab Injection), while 3 patients showed a morpho VFx. Incidence of VFX between ALN and ZOL group, both considering all VFx or only clinical VFx, was not significantly different (p=0.752 and p=0.126 respectively).

As compared with patients in Treated Group, those in Not-treated Group were younger and had less Dmab injections and Dmab therapy duration, lower prevalence of multiple VFx and had less frequently been treated with BPs before Dmab and had less frequently withdrawn BPS therapy ≤12 months before Dmab initiation. The gender distribution, BMI, family history of fragility fractures, AI treatment, current smoking, SDI, FRAX score for all fracture, BMD at any site at Dmab discontinuation, and 25OHD and creatinine levels were not different between patients from Not-treated Group and those from Treated Group. As compared with these latter, patients in Not-treated Group, had a higher incidence of VFx, both clinical and multiple, despite a similar, if not lower, overall risk profile for fractures (10 years probability of a major fracture with FRAX 16.8±10.2 and 20.8±13.3, respectively, p=0.224).

Considering BMD change after Dmab withdrawal, we found no statistically significant difference between treated and non-treated group, however non treated patients seems to present a more pronounced worsening of TH and FN BMD (ΔTH t_2-t_1 and ΔFN t_2-t_1, respectively). Among treated patents, we found no difference between ALN and ZOL groups in ΔLS t_2-t_1, ΔTH t_2-t_1 and ΔFN t_2-t_1 (-2.7±6.9 versus -2.7±5.0, p=0.976; -1.5±5.6 versus -1.4±4.8, p=0.923; -3.3±7.6 versus -1.5±6.4, p=0.343 respectively). Bone mineral density changes at LS, TH and FN during Dmab therapy and after Dmab discontinuation in non-treated and ALN and ZOL groups are reported in figure 2.

In keeping, the frequency of patients who experienced a worsening of TH BMD between the beginning of Dmab therapy and the end of follow-up ≥LSC was higher in Not-treated Group than in Treated Group. A similar, though not statistically significant, trend was found even as far as LS BMD was concerned.

Considering only treated subjects no difference was found between ALN and ZOL groups (10.7% versus 13.7%, p=0.488; 10.7% versus 8.2, p=0.478 and 14.2% versus 19.2%. p=0.773, BMD worsening ≥LSC at LS, TH and

FN respectively). Moreover, Dmab therapy duration was similar between patients with and without BMD loss ≥LSC at LS, TH (5.7±2.5 versus 6.8±2.6, p=0.155; 5.4±2.3 versus 6.8±2.6, p=0.146; 7.0±2.4 versus 6.6±2.6, p=0.536, respectively).

The logistic regression analysis showed that the VFx incidence was 15.4-fold higher in patients not treated with BPs after Dmab discontinuation regardless of the number of Dmab injections, age, duration of BPs treatment before Dmab therapy, the LS-BMD at Dmab withdrawal and the prevalence of VFx at Dmab discontinuation (table 2). The same results were obtained even considering, TH-BMD at Dmab withdrawal (odds ratio 12.3, 95% confidence interval 1.6-100.0, p=0.016) and considering only clinical VFx (odds ratio 30.3, 95% confidence interval 2.0-500.0, p=0.013)

The clinical and biochemical parameter of BPs treated patients with and without VFx after Dmab discontinuation are reported in table 3. The age, gender distribution, BMI, AI treatment, type of BPs used (ALN or ZOL), frequency of BPS withdrawal ≤12 months before Dmab initiation, number of Dmab injections and therapy duration, period of time between the last Dmab injection and the beginning of BPs therapy, family history of fragility Fx, current smoking, SDI, FRAX score, Dmab compliance, BMD at Dmab discontinuation, BMD changes between Dmab initiation and discontinuation and between Dmab discontinuation and end of follow-up, 25OHD and creatinine levels were not different between fractured and not-fractured BPs treated patients.

Discussion

The present study confirms that the risk of both clinical and multiple VFx is increased in patients who discontinued Dmab therapy in the absence of a BPs treatment. After Dmab discontinuation, BPs treatment has a protective effect on the VFx risk. Indeed, the lack of a BPs treatment is associated with a 13.9-fold increased VFx risk independent of age, the duration of Dmab therapy, BMD, previous BPs treatment and prevalent VFx. Finally, the present data confirm that the rebound VFx, when present, are clinical and often multiple as already reported (7).

Up to date, the optimal regimen to prevent the risk of rebound fragility VFx in patients stopping Dmab is yet to be clarified and the BPs treatments effectiveness in preventing VFx is still debated (9). Indeed, some studies suggested a possible beneficial effects of BPs (mainly ZOL) on the VFx risk (9,14) (Burkhardt). However, the reduced sample size and/or the absence of a control group of not-treated subjects render these data still preliminary. On the other hand, a large study showed a lower incidence of clinical VFx and the absence of multiple VFx in a group of patients treated with ZOL after Dmab withdrawal, but the authors did not adjust for confounding factors possibly influencing their findings (14)..

,The present data show that the BPs therapy is effective in reducing the VFx risk regardless of several potential factors known to influence the "rebound phenomenon". The finding that no patient in Treated Group experienced multiple VFx, in keeping with the previous study of Evert-Graber and co-authors (14), is almost reassuring as regards of BPs therapy effectiveness, after Dmab discontinuation, in preventing this dangerous event that often imply several irreversible effects. However, it should be noted that the percentage of patients who experienced the worsening of BMD between the beginning of Dmab therapy and the end of follow-up (LS 12.9%, TH 8.9%, FN 17.8%) was meaningful even in Treated Group, with no difference between ALN and ZOL group. This finding is in keeping with the recent RCT of Sölling and co-authors (13) and points out the not fully satisfactory effectiveness of BPs treatment to prevent bone loss in patients treated with Dmab for more than 2.5 years. Up to now, the available studies, evaluated only the protective effect of a single ZOL infusion, in keeping with data showing that the effect of ZOL persist well beyond 12 months (19). Our study suggests that a single infusion of ZOL might not be sufficient to preserve BMD over time. This finding confirms the need of optimizing the antiresorptive therapy in patients undergoing Dmab withdrawal in terms of type of drug, administration interval, dose, frequency and duration of treatment (9). In keeping with the data of bone turnover markers reported by Solling and co-authors, it could be hypothesized that a second infusion of ZOL, before 12 months after the first infusion, could be necessary to ensure that bone turnover remain within the lower part of the reference range (13).

Investigating the possible factors associated with the higher VFx risk after Dmab discontinuation was another aim of the present study. The limited sample of Not-treated patients prevented us to deeply investigate this issue. However, the present data give anyway some interesting insights, and it is worth noting that, in future studies, not treated group will probably not be available for ethical reasons. It should be noted that the non-treated Group included patients who were younger, had a considerable shorter period of Dmab treatment and, above all, a less severe osteoporosis with a significantly lower prevalence of multiple VFx. In spite of being at lower risk of fragility fracture as compared with treated patients, not-treated patients had, in fact, a significantly higher number of VFx. This further underscores the validity and importance of BPs use following Dmab discontinuation.

Indeed, some authors suggested that the increased VFx incidence after Dmab withdrawal is evident mainly in patients with high-risk of fracture before Dmab therapy and that after Dmab discontinuation this high risk population returns to the pretreatment fracture risk (9). This seems not to be the case in the present study, as in both Treated and Not-treated Group, fractured patients had an overall fragility fracture risk profile (as mirrored by FRAX score) comparable to that of not-fractured patients. In keeping, the VFx occurrence after Dmab discontinuation was independent of prevalent multiple VFx and of spinal BMD at the time of Dmab withdrawal. Furthermore, ZOL has been suggested to be more effective in maintaining BMD when Dmab treatment did not exceed 2.5 years (5 injections) (9),

but data on VFx as outcome are still lacking. In the present study, patients from the Treated Group showed a beneficial BPs effect in terms of clinical and multiple VFx risk despite a mean Dmab duration above 2.5 years (6.6±2.6 injections). In addition, we did not find an independent association between the VFx occurrence and the Dmab therapy duration (table 2) and, finally, among treated subjects the Dmab therapy duration was not significantly associated with the VFx occurrence (table 3). Therefore, the present data seem to suggest that the BPs is effective in reducing the rebound VFx regardless of the Dmab therapy duration.

We found that the prevalence of patients treated with BPs within the year before Dmab therapy was higher in the Treated Group. This could have played a confounding role on the effect of BPs treatment after Dmab withdrawal. Indeed, the effect of BPs administration before Dmab treatment on the rebound phenomenon is still debated (9) (aggiungere Burkhardt). However, in the present study, the VFx risk after Dmab withdrawal was associated with the lack of BPs therapy regardless of BPs therapy before Dmab administration. Therefore, at least from the present data, the previous BPs therapy seems to play a minor role in protecting from rebound VFx after Dmab discontinuation,as suggested even by a recently published study (Burkhardt).

The small number of subjects in Not-treated Group did not consent to deeply explore the factors associated with rebound VFx after Dmab discontinuation. The large confidence interval (1.8-142.8) of the association between the lack of BPs and the occurrence of VFx after Dmab discontinuation is in keeping with the small sample size. However, the present results are in line with literature data showing the importance of a BPs therapy in preventing the rebound VFx related to Dmab withdrawal (9,12–15) (Burkahrdt). Beside the small sample size of patients in Not-Treated Group, our study has other limitations. First, the lack of a randomized design suggests that these finding should be taken cautiously. Second, the use of the of BTMs could have been more informative on the possible use of a different schedule of BPs administration. Indeed, adapting the ZOL schedule to the BTM changes could importantly increase the ZOL efficacy in reducing the rebound related VFx risk. Finally, the relatively small sample of patients treated with ALN prevents us to reliably compare the effect of ZOL and ALN on the VFx risk after Dmab discontinuation, even though our findings did not reveal a meaningful difference of effects between the two BPs.

Notwithstanding these limitations, this study deserves clinical attention as it shows that BPs therapy at the time of Dmab withdrawal can reduce the VFx risk independently of the Dmab duration and the fragility fracture risk profile at the beginning of Dmab therapy.

Further studies are needed to investigate which antiresorptive drug and with which schedule should be administered after Dmab discontinuation, to personalize the drug therapy for osteoporosis with a view of establishing the correct sequential therapy for each patient affected with this severe condition.

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Informed consent: Informed consent was obtained from all individual participants included in the study.

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356 **Figure 2**. Percent changes (mean±SE) during Denosumab treatment(t_0-t_1) and after Denosumab discontinuation (t_1-t_2) in

357 Lumbar Spine (LS), Total Hip (TH), Femoral Neck (FN) BMD in patients not treated or treated with weekly

358 alendronate (ALN) or with a single zoledronate infusion (ZOL) after Denosumab discontinuation.