# The relationship between length of denosumab treatment for postmenopausal osteoporosis and serum TRAcP5b measured six months after the last injection

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SHORT COMMUNICATION

# The relationship between length of denosumab treatment for postmenopausal osteoporosis and serum TRAcP5b measured six months after the last injection

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## Abstract

**Summary** To test the hypothesis that during treatment with denosumab osteomorphs and precursors recycle to higher number of osteoclasts with time, we measured TRAcP5b in serum taken 6 months after the last injection in postmenopausal women treated for 1–10 years. Serum TRAcP5b values were not related to time of exposure to denosumab.

**Purpose** In women with postmenopausal osteoporosis the aetiology of the observed inverse relationship between duration of denosumab (Dmab) therapy and bone loss after its discontinuation is currently unknown. In studies in mice inhibition of RANKL is associated with an increase in osteomorphs and osteoclast precursors that recycle into osteoclasts and may accumulate with time. We hypothesized that longer inhibition of RANKL by Dmab will be followed by the synchronous formation of a larger number of osteoclasts after stopping treatment. To test this hypothesis, we measured serum TRAcP5b, a marker of osteoclast numbers, in postmenopausal women treated with Dmab for different periods of time up to 10 years.

**Methods** TRAcP5b, C-terminal telopeptide of type 1 collagen (CTX) and procollagen type 1 N-terminal propeptide (P1NP) were measured at 6.0 months ± 15 days after last Dmab injection in 59 women who had received Dmab for 4.0 ± 2.3 years (range 1–10 years). Of these, 38 were treatment naïve (group 1) and 21 had received other treatments prior Dmab (group 2).

**Results** Duration of Dmab treatment was not related to serum TRAcP5b values or to TRAcP5b/CTX ratio either in the whole cohort or in each of the two groups separately. In contrast, serum TRAcP5b values were significantly correlated with serum CTX values ($r_s = 0.619$; $p < 0.001$), but not with serum P1NP values or BMD at all skeletal sites.

**Conclusion** Our observations indicate that serum TRAcP5b, measured at 6 months after a Dmab injection, is not a useful early marker for time-dependent increased accumulation of osteoclasts in humans and for identification of patients at risk for a higher rebound increase in bone resorption.

**Keywords** Bone turnover markers · Denosumab · Denosumab discontinuation · Postmenopausal osteoporosis · TRAcP5b

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## Introduction

In women with postmenopausal osteoporosis the well-established rapid bone loss that follows the cessation of denosumab (Dmab) therapy can be mitigated or even prevented by a single intravenous infusion of zoledronate given 6 months after the last Dmab injection [1, 2]. The efficacy, however, of zoledronate to maintain the bone mineral density (BMD) gains achieved with treatment is significantly affected by the duration of Dmab treatment before its discontinuation [3, 4]. We recently showed that among osteoporotic women, who became osteopenic with Dmab therapy for up to 3 years, an infusion of zoledronate maintained the BMD gains for 1 year. In contrast, women treated with Dmab for periods longer than 3 years experienced significant BMD losses. Moreover, a highly significant inverse relationship between the time on Dmab therapy and loss of spine BMD was demonstrated [3]. The pathogenesis of these responses is largely unknown and may involve osteoclast recycling [5, 6], a mechanism recently proposed to contribute to the rapid decrease of BMD following discontinuation of Dmab treatment [7]. In studies in mice, it was shown that osteoclasts live longer after completing their function on bone resorption and are capable of undergoing fission into daughter non-resorbing cells called osteomorphs [8]. These cells, that are transcriptionally distinct from osteoclasts, can subsequently fuse with neighbouring osteoclasts and with each other and recycle back to osteoclasts in the process of osteoclast recycling. Inhibition of RANKL blocks cellular recycling resulting in accumulation of osteoclast precursors and osteomorphs. We hypothesized that inhibition of RANKL for longer periods of time will be followed by higher accumulation of osteomorphs and osteoclast precursors in the bone microenvironment that will recycle and will form a larger pool of osteoclasts after stopping treatment and will subsequently lead to more bone resorption. To test this hypothesis in the clinical setting, we measured the active isoform 5b of tartrate-resistant acid phosphatase (TRAcP5b), a serum marker believed to reflect osteoclast cell numbers [9] and the marker of bone resorption CTX in postmenopausal women treated with Dmab for different periods of time up to 10 years. Evidence in support of our hypothesis was recently presented as abstract from the group that described the concept of osteoclast recycling [10]. The authors examined changes of biochemical markers of bone turnover following discontinuation of RANKL inhibition in mice and reported that a significant overshoot in serum TRAcP5b occurs prior to the overshoot in serum CTX and P1NP and bone loss.

## Patients and methods

### Study design

This was an open-label, multicenter, cross-sectional study of women with osteoporosis treated with Dmab for different periods of time. The primary objective of the study was the examination of the relationship between the number of osteoclasts measured by serum TRAcP5b and the duration of treatment with Dmab. A secondary objective was the examination of the relationship between the ratio of serum TRAcP5b to serum CTX and the duration of Dmab treatment; an exploratory objective was the potential use of serum TRAcP5b values in the identification of patients with osteoporosis at risk for a higher rebound increase in bone resorption after stopping Dmab therapy.

### Patients

Included in the study were ambulatory women $\geq 50$ years with postmenopausal osteoporosis receiving treatment with 6-monthly s.c. injections of Dmab, cholecalciferol 800 IU/day and calcium carbonate 500 mg b.i.d.; all had normal serum 25-hydroxyvitamin D ($\geq 50$ nmol/l) and calcium concentrations Exclusion criteria were low-energy clinical fracture(s) during the last 6 months, use of medications affecting bone metabolism (other than antiosteoporotic agents) during the last 3 years, bone disease(s) other than postmenopausal osteoporosis, creatinine clearance $< 60$ mL/min/1.73m$^2$, any type of cancer, uncontrolled endocrine diseases, and abnormal liver function. The study was approved by the local Medical Ethical Committees and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. All patients provided written informed consent for participating in the study.

### Methods

Morning fasting blood samples were obtained at 6.0 months $\pm$ 15 days after the last Dmab injection for the measurement of TRAcP5b, C-terminal telopeptide of type 1 collagen (CTX) and procollagen type 1 N-terminal propeptide (P1NP); the samples were stored at -30 °C until assayed in a single batch. TRAcP5b and CTX were measured by Human Immunoassay ELISA, Immunodiagnostic Systems Holdings Ltd (http://www.idspl.com), with intra- and inter-assay CVs $\leq 8.9\%$; P1NP was measured by P1NP ELISA kit, Abbexa Ltd. (http://www.abbexa.com), with intra- and inter-assay CVs $< 10\%$. Upper limit of normal range for postmenopausal women: P1NP 76 ng/mL, CTX 1.0 ng/mL,

TRAcP5b 4.04 U/L; and for premenopausal women: 56 ng/mL, 0.57 ng/mL, and 3.37 U/L, respectively. Areal BMD was measured by dual energy X-ray absorptiometry (DXA) at the lumbar spine (LS; L1-L4), total hip (TH), and femoral neck (FN) (Hologic Inc., USA) and lateral radiographs of the spine were obtained in all patients.

### Statistical analysis

Continuous data are presented as mean ± standard deviation (SD). Categorical data are presented as numbers or proportions. Kolmogorov–Smirnov test was used to check the normality of distributions of continuous variables. Independent samples T-test or Mann–Whitney test were used for between group comparisons. Chi-square or Fisher's exact test was used to compare categorical variables. Spearman's coefficient was used for bivariate correlations. Linear regression analysis was also used for investigating independent associates with TRAcP5b or TRAcP5b/CTX ratio. For the need of this analysis, variables with skewed distribution were logarithmically transformed. In all tests, $p < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 27 for Macintosh (IBM Corp., Armonk, NY).

## Results

Between February and November 2022, 60 consecutive postmenopausal women fulfilling the inclusion criteria were invited to participate in the study of whom one did not accept. The 59 women studied had received Dmab for a mean period of 4.0 ± 2.3 years (range 1–10 years). Of these, 38 had received no treatment for osteoporosis other than denosumab (group 1) and 21 had also received other treatments before denosumab (group 2). The duration of prior treatments in group 2 was 5.4 ± 3.8 years and consisted of bisphosphonates either alone (n = 14) or with other therapies (e.g., teriparatide, raloxifene, strontium ranelate, calcitonin; n = 7). The characteristics of the total cohort and those of the two groups separately are summarized in Table 1. Except for serum P1NP values which were higher in group 1, the two groups had similar values of all variables examined.

Serum TRAcP5b values were higher than the upper limit of normal premenopausal range in 6.8% of patients and 2 of them had values higher than the upper limit of normal postmenopausal range. TRAcP5b values were not related to the duration of Dmab treatment either in the whole cohort ($r_s = 0.149$; $p = 0.259$; Fig. 1A) or in each of the two groups separately, [group 1 ($r_s = 0.108$; $p = 0.517$),

**Table 1** Characteristics of the total cohort and of the two groups of women studied

| | All | Group 1 *(no prior treatment)* | Group 2 *(prior treatment)* | *p*-value * |
|---|---|---|---|---|
| Patients | 59 | 38 | 21 | – |
| Age (years) | 65.0 ± 7.6 | 64.1 ± 7.9 | 66.6 ± 6.7 | 0.218 |
| Age at menopause (years) | 48.8 ± 4.2 | 48.2 ± 4.5 | 50.1 ± 3.6 | 0.105 |
| BMI (kg/m$^2$) | 25.4 ± 4.4 | 25.2 ± 4.4 | 25.9 ± 4.3 | 0.577 |
| Smoking (N; %) | 15 (25.4) | 11 (28.9) | 4 (19.0) | 0.403 |
| Vertebral fractures (N; %) | 5 (8.5) | 3 (7.9) | 2 (9.5) | 0.830 |
| Non-vertebral fractures (N; %) | 9 (15.3) | 4 (10.5) | 5 (23.8) | 0.258 |
| Hip fractures (N; %) | 0 (0.0) | 0 (0.0) | 0 (0.0) | – |
| Parent hip fracture | 12 (20.3) | 7 (18.9) | 5 (23.8) | 0.741 |
| Dmab duration (years) | 4.0 ± 2.3 | 3.7 ± 2.1 | 4.4 ± 2.7 | 0.386 |
| LS BMD (g/cm$^2$) | 0.876 ± 0.073 | 0.877 ± 0.078 | 0.874 ± 0.067 | 0.897 |
| FN BMD (g/cm$^2$) | 0.678 ± 0.124 | 0.687 ± 0.150 | 0.663 ± 0.058 | 0.932 |
| TH BMD (g/cm$^2$) | 0.815 ± 0.074 | 0.807 ± 0.082 | 0.827 ± 0.059 | 0.378 |
| 25-OHD (ng/mL) | 32.5 ± 10.7 | 32.2 ± 11.4 | 32.7 ± 10.3 | 0.882 |
| CTX (ng/mL) | 0.329 ± 0.289 | 0.336 ± 0.317 | 0.317 ± 0.238 | 0.812 |
| PINP (ng/mL) | 26.0 ± 13.0 | 30.1 ± 14.2 | 18.8 ± 5.5 | < 0.001 |
| TRAcP5b (U/L) | 1.97 ± 0.92 | 1.87 ± 1.04 | 2.14 ± 0.63 | 0.569 |
| TRAcP5b/CTX ratio | 7.9 ± 4.2 | 7.3 ± 4.0 | 9.1 ± 4.3 | 0.141 |

Data are presented as in mean ± standard deviation (SD) or numbers

* Between groups comparisons: Mann–Whitney test for continuous and Chi-square or Fisher's exact test for categorical variables

Abbreviations: *BMI* body mass index; *CTX* C-terminal cross-linked telopeptide; *Dmab* denosumab; *FN* femoral neck; *LS* lumbar spine; *25-OHD* 25-hydroxy vitamin D PINP, procollagen type I propeptide; *TH* total hip; *TRAcP5b* tartrate-resistant acid phosphatase activity type 5

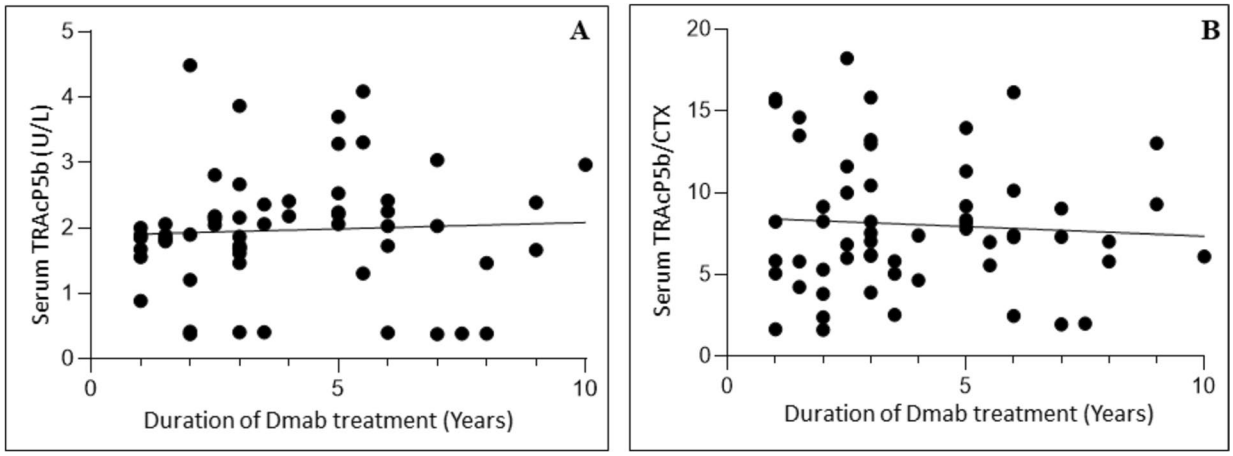


**Fig. 1** Relationship between serum TRAcP5b values and duration of Dmab treatment (panel A); relationship between serum TRAcP5b/CTX ratio and duration of Dmab treatment (panel B)

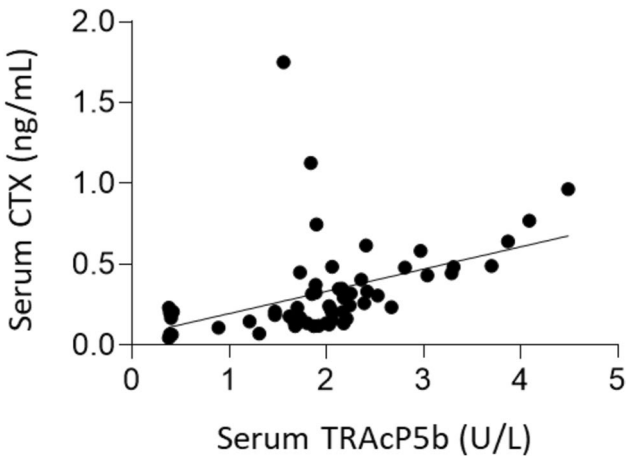


**Fig. 2** Relationship between serum TRAcP5b and CTX values at 6 months after the last denosumab injection

group 2 ($r_s = 0.108$; $p = 0.642$)]. Similarly, duration of Dmab treatment was not associated with the TRAcP5b/CTX ratio either in the whole cohort ($r_s = -0.033$; $p = 0.805$; Fig. 1B) or in any of the two groups of patients [group 1 ($r_s = -0.113$; $p = 0.501$), group 2 ($r_s = -0.005$; $p = 0.984$)].

Serum CTX values were higher than the upper limit of normal premenopausal range in 13.2% of patients and 2 patients had values higher than the upper limit of normal postmenopausal range; in these two patients serum TRAcP5b values were within the low normal range. Serum TRAcP5b values were significantly correlated with serum CTX values ($r_s = 0.619$; $p < 0.001$; Fig. 2), but not with serum P1NP values or BMD (LS, FN or TH). The relationship between serum TRAcP5b and CTX values remained significant also after adjusting for age, BMI and duration of Dmab treatment (Beta = 0.404; $p < 0.001$; 95% CI: 0.182 – 0.625) as well as after adjusting for age, BMI and previous antiosteoporotic treatment (Beta = 0.398; $p < 0.001$; 95% CI: 0.184 – 0.613).

## Discussion

We show here that in postmenopausal women with osteoporosis treated with Dmab for 1 to 10 years there was no correlation between serum TRAcP5b values, obtained 6 months after the last injection, and duration of treatment. The lack of such relationship was not confined to the total cohort of the studied women but also to each of the two groups of women who received or not other antiosteoporotic treatment before Dmab.

The kinetics of the rebound increase in bone turnover after discontinuation of Dmab therapy are adequately described in prospective randomized studies by sequential measurements of bone turnover markers in serum; values do not generally change at 6 months but increase thereafter above pretreatment levels reaching a peak 9 to 12 months after the last denosumab injection and remain elevated for at least 18 months in the majority of patients [11]. While the mechanism underlying this response still needs to be determined, increased osteoclastogenesis has been documented in blood and bone samples obtained at 8 months or longer after stopping Dmab in human studies [12, 13]. Formation and activity of osteoclasts following cessation of Dmab therapy is, however, a dynamic process that starts with the uninhibited, synchronous, rapid but transient release of RANKL. The magnitude of these changes as well as factors that may modify them have not been elucidated but some have been recently addressed in two types of studies. Firstly, a study of women with postmenopausal osteoporosis that showed a highly significant correlation between the time of exposure to Dmab and the loss of LS-BMD following an infusion

of zoledronate [3]. Secondly, a study in mice that led to the formulation of the concept of osteoclast recycling [8]. These studies combined suggested that part at least of the magnitude of the responses of patients exposed to Dmab for different periods of time may be due to different total numbers of osteoclast precursors and osteomorphs that will accumulate and fuse to form new actively resorbing osteoclasts. Confirming this hypothesis in humans would not only support the concept of osteoclast recycling so far reported only in preclinical studies but may also help in the appropriate management of individual patients who stop Dmab treatment. As measurements of serum CTX values at 6 months cannot capture differences in early responses, we examined the relationship between length of Dmab therapy and osteoclast numbers as these can be assessed in humans in vivo with measurement of TRAcP5b in serum.

Our results did not support this hypothesis. Moreover, to capture a potential imbalance in the levels of TRAcP5b and lack of an early effect on bone resorption, as measured by serum CTX, we also examined the relationship between duration of Dmab treatment and the TRAcP5b/CTX ratio. Again, we found no relationship between the two. We also found no relationship between TRAP5b and P1NP values. We believe that a correlation between TRAcP5b and P1NP would be demonstrated at a later time point due to the coupling of bone resorption to bone formation, as already shown by others.

It may be argued that examining the kinetics of serum TRAcP5b by measuring it in samples taken at additional time points, earlier or later than 6 months after the last injection of Dmab might have provided a better assessment of its value as marker of osteoclast number in humans discontinuing treatment; lack of such measurements could, therefore, be considered a limitation of our study. The rationale of our choice of sampling time was as follows. In previous studies, including our earlier reported prospective RCT, a rapid increase in serum CTX between 6 and 9 months following Dmab discontinuation has been documented [14–16]. In addition, rebound-associated vertebral fractures have been reported already at 8 months after the last Dmab injection [12]. Importantly, in the study of Anastasilakis et al. [14] 17.2% of the patients treated with Dmab had already serum CTX values higher than the upper limit of the premenopausal normal range at 6 months after the last injection, a result confirmed in the present study. These findings, implying that serum TRAcP5b values should have been already increased at 6 months at least in patients with the longest treatment and theoretically the largest number of osteoclasts, were considered and helped to determine the time of blood sampling. Examining TRAcP5b values later than 6 months would have, therefore, been meaningless for the aim of our study because such increase would have occurred almost concurrently with the increase in serum CTX. Moreover, a delay in the administration of the next Dmab dose or of bisphosphonate beyond 6 months would have been unethical particularly in patients exposed to Dmab for longer periods of time.

Apart from these considerations and the failure of our study to meet its objectives, the significant correlation between serum TRAcP5b and CTX levels confirm the value of this biomarker in the evaluation of bone resorption in general and following Dmab use in particular. Solling et al. recently reported also a significant correlation between serum TRAcP5b and CTX 6 months after the last injection of Dmab [16].

Our observations indicate that serum TRAcP5b is not a useful early marker for identification of patients at risk for a higher rebound increase in bone resorption due to time-dependent increased accumulation of osteoclasts. These results need to be confirmed in future studies that might also include measurements of serum TRAcP5b one month later (e.g. 7 months) or earlier than 6 months after the injection of Dmab. Direct measurement of osteoclast precursors and/or osteomorphs in humans is, however, essential to definitely refute or support the hypothesis.

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**Data availability** Data will be available upon reasonable request.

## Declarations

**Ethical approval** All procedures performed in the study were in accordance with the ethical standards of the institutional research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

**Conflict of interest in respect to the article** Polyzois Makras reports fees for lectures/advisory boards and research grants from Amgen and Galenica and fees for lectures/advisory boards from UCB, Elpen, Bianex, Eli-Lilly, ITF, Unipharma, and Rapharm; Maria P. Yavropoulou reports fees for lectures/advisory boards and research grants from Galenica; Socrates E. Papapoulos reports consulting/speaking fees from Amgen, Entera Bio, Qualix Dot, Radius Health and UCB; Athanasios D. Anastasilakis reports lecture fees from Amgen, Bianex, Eli-Lilly, Galenica, ITF, Unifarma, and UCB; Stergios A. Polyzos, Danai Georgakopoulou and Athanasios Papatheodorou have nothing to declare.

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