Zinc improves Denosumab and eldecalcitol efficacy for bone mineral density in patients with hypozincemia

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Abstract

Purpose: We aimed to investigate the effects of zinc deficiency and zinc medication in osteoporosis patients undergoing denosumab (DMAb).

Methods: This retrospective study was conducted at a single hospital. The participants were female osteoporosis patients visiting between April 2019 and April 2020. All patients were treated with DMAb and eldecalcitol and recommended zinc-rich food. Based on zinc medication and serum zinc levels at the 12th month of dietary guidance, patients were categorized into the following four groups: hypozincemia with zinc medication, latent zinc deficiency with zinc medication, without zinc medication, and control without zinc medication. Longitudinal serum zinc concentrations, bone mineral density (BMD), and occurrence of fractures were measured. We investigated the factors influencing no response to DMAb and eldecalcitol treatment.

Results: Among the 145 patients followed up for 24 months, dietary guidance did not change the serum zinc concentration; however, zinc medication significantly increased these levels. The hypozincemia group did not show a significant BMD increase in the lumbar spine and femoral neck after DMAb and eldecalcitol treatment during dietary guidance; however, zinc medication increased these to the same levels as the other groups. In multivariate analyses, hypozincemia and thyroid disease were identified as the factors affecting no response. While 28.2% of patients with latent zinc deficiency without zinc medication suffered fractures, no fractures occurred in hypozincemia patients with zinc medication.

Conclusion: Hypozincemia may reduce the efficacy of DMAb and eldecalcitol in increasing BMD and fracture prevention.

41 **Keywords:** bone mineral density; denosumab; hypozincemia; osteoporosis; zinc

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Introduction

The importance of trace elements in the body has been emphasized. Zinc is essential for human and animal growth and is related to insulin-like growth factor 1 [1,2]. Its concentrations tend to decrease with age and chronic diseases such as cirrhosis, diabetes, chronic inflammatory bowel disease, and chronic kidney disease [3-5]. An estimated 17.3% of the global population is at risk of inadequate Zn intake [6]. In Japan, the Practical Guideline proposed hypozincemia as a serum zinc concentration below 60 μg/dL, with latent zinc deficiency as 60–80 μg/dL [7].

Bone has one of the highest zinc concentrations, and bone growth retardation is a common finding in zinc deficiency [8]. The first report on the association between zinc and bones was by Miller et al. in 1962, for which zinc deficiency caused the stunting of long bones in cattle [9]. Several studies on the relationships between zinc and bone growth, strength, and bone mineral density (BMD) in rats and chickens have been conducted since the 1970s [10-12]. The relationship between zinc deficiency and osteoporosis has recently been reported [13-17]. Suppression of osteoclast activity by zinc has been thought to be a factor in osteoporosis [18]. In an in vitro study, Moonga et al. reported that osteoclasts were exquisitely sensitive to zinc, with a significant decrease in bone resorption occurring even at low concentrations, and a highly potent and selective inhibitor of osteoclastic bone resorption [19]. In vivo study, Hadley et al. showed that in growing rats, the up-regulation of extracellular matrix modeling indexes and concomitant decrease in bone resorption activities as dietary zinc increased provide evidence of one or more physiological roles for zinc in modulating the balance between bone formation and resorption [20]. In this way, animal experiments have reported that zinc has the dual effect of promoting bone formation

and inhibiting bone resorption. However, limited information is available on the association between the efficacy of osteoporosis therapy and Zn deficiency in clinical trials.

Denosumab (DMAb), an anti-bone resorptive drug, is a human monoclonal antibody that targets the osteoclast differentiation factor/receptor activator of the NF-kB ligand (RANKL) [21]. Several studies have shown that it causes a greater increase in BMD and a reduction in bone resorption than bisphosphonates [22-24]. On the other hand, some populations of patients receiving DMAb did not show significant increases in BMD. Although identifying the factors for poor reactions might be very important for DMAb treatment, only one study has examined it [25]. We hypothesized that zinc deficiency affects the efficacy of DMAb and zinc supplementation enhances its efficacy. To address these hypotheses, the objective of this study was to investigate the effects of zinc deficiency and zinc medication in patients with osteoporosis with zinc deficiency treated with DMAb.

Materials and methods

Study design and participants

This retrospective study was conducted at a single hospital. This study was approved by the local ethics committee of Hakodate Central Hospital (2022-33). All patients provided informed consent before enrolment. The participants were female patients who visited the outpatient clinic of the same physician for osteoporosis treatment with DMAb and active vitamin D3 analog, “eldecalcitol” (0.75 μg) between April 2019 and April 2020. All patients were recommended zinc-rich foods, such as meat and seafood, to prevent zinc deficiency. According to serum zinc concentration at 12 months, hypozincemia (< 60 μg/dL) and latent deficiency (60–80 μg/dL) patients provided informed consent for taking zinc medication. The patients who agreed to receive the medication were administered 25

mg zinc (83.92 mg zinc acetate dihydrate) (NOBELZIN®, Nobelpharma, Tokyo, Japan) twice a day, and only zinc-rich food intake was recommended for the other patients who objected to providing consent. The zinc measurement and zinc administration were part of routine medical care. An additional 12 months of follow-up was conducted after grouping, and the medication of DMAb and eldecalcitol was continued during the entire period.

The patient demographic data included age, sex, body mass index (BMI), comorbidities, such as thyroid disease, parathyroid disease, malignant tumor, rheumatoid arthritis (RA), diabetes, chronic kidney disease (CKD), and chronic obstructive pulmonary disease (COPD), history of vertebral fractures, and glucocorticoid use. Blood samples, including serum calcium, phosphorus, and zinc concentrations, and estimated Glomerular Filtration Rate (eGFR) were collected from all patients every 6 months. Blood samples were collected at the same time in the morning without a fasting period. The serum calcium concentrations were corrected for the serum albumin concentrations. BMD was automatically calculated using dual-energy X-ray absorptiometry (DXA (PRODIGY Fuga-C, GE Healthcare, Madison, WI, USA)) every 6 months. The number of months of DMAb use was also investigated for all patients before enrollment. Various clinical parameters of the groups were compared at 12 months. The baseline was set to 0 or 12 months, and longitudinal changes in the serum zinc concentration and lumbar and femoral neck BMD were determined. Additionally, the occurrence of fragility fractures (proximal femoral fractures and vertebral fractures) and other fractures were examined as secondary outcomes.

We defined a non-responder to DMAb and eldecalcitol treatment as a patient who had increased lumbar spine or femoral neck BMD of less than 0 g/cm^2 by 12 months from

baseline (BMD loss). The factors influencing no response based on the background factors at 12 months were examined.

Statistical analysis

One-way ANOVAs with post-hoc Tukey-Kramer tests were used to investigate the differences between the groups. Repeated ANOVAs with post-hoc Tukey–Kramer tests were used to investigate differences in BMD at each time point compared to BMD at baseline. We used the chi-square test to compare the occurrence of fractures. We examined the factors influencing no response using single logistic regression models, and then created multivariate logistic regression models based on the results of the single logistic regression models and one-way ANOVA; the odds ratios (ORs) of the regression coefficients were calculated. We conducted power analysis for ANOVA (with an α of 0.05 (2-tailed), power of 80%), and a sample size of 124 patients would be required. These analyses were performed using JMP Pro version 16.2 (SAS Institute, Inc., Cary, (NC, USA), with statistical significance set at $p < 0.05$.

Results

Participants and their characteristics at 12 months

A total of 173 female patients visited the same outpatient clinic for osteoporosis treatment with DMAb and eldecalcitol prescribed by the same physician and were recommended zinc-rich foods for 12 months. Zinc deficiency was managed in 137 patients (79.2%, [137/173]) with dietary guidance only, and they were recommended zinc medication. The patients were divided into the following four groups according to serum zinc concentrations at the 12th month of dietary guidance and zinc medication:

hypozincemia with zinc medication group (Hypo-Zn), latent zinc deficiency with zinc medication (Latent-Zn), latent zinc deficiency without zinc medication (Latent), and normal zinc concentration (> 80 μg/dL) without zinc medication (Control). Twenty-five patients could not be followed up for more than 24 months and three patients requested discontinuation because of nausea. Finally, 33, 18, 55, and 39 patients could be followed up for more than 24 months in the Control, Hypo-Zn, Latent-Zn, and Latent groups, respectively (Fig. 1). Hypozincemia without zinc medication group was excluded from the analysis of this study because there was only one case.

The clinical characteristics at baseline of the 145 patients who were followed up for > 24 months are shown in Table 1 and Supplementary Table 1. The mean age was 72.33, 78.33, 74.87, and 72.62 years, and the mean BMI was 22.83, 22.01, 21.82, and 22.16 kg/m^2 for the Control, Hypo-Zn, Latent-Zn, and Latent groups, respectively. The mean serum concentrations of calcium and phosphorus at 12 months were not significantly different among the four groups (9.81 vs 9.84 vs 9.75 vs 9.62 mg/dL and 3.61 vs 3.53 vs 3.60 vs 3.49 mg/dL, respectively). The mean eGFR at 12 months differed significantly among the four groups (65.92 vs 55.07 vs 59.79 vs 66.17 ml/min/1.73m^2, $P = 0.040$). The mean serum concentration of zinc at 12 months was significantly different for the groups (92.45 vs 54.11 vs 66.91 vs 71.56 μg/dL, $P < 0.001$), and there were significant differences between any of the two groups. The latent-Zn group had also significantly lower serum zinc concentrations than the latent group ($P = 0.039$). There was a significant difference among the groups in the proportion of the patients with RA ($P = 0.026$), but not in other comorbidities. The hypo-Zn group had significantly higher RA morbidity than the control group and the latent-Zn group ($P = 0.037$ and 0.021). History of vertebral fractures ($P = 0.007$) differed significantly among groups. The hypo-Zn group showed a more prominent

history of vertebral fractures than the control group ($P = 0.007$). There were no significant differences in glucocorticoid use and the BMD of the lumbar spine and femoral neck between the groups. All patients had been treated with DMAb and eldecalcitol from > 24 months before this study, and there were no significant differences during the periods of pre-treatment among these groups (33.90 vs 33.13 vs 41.61 vs 34.48, P = 0.290).

Longitudinal changes in the serum concentration of zinc and BMD

The longitudinal changes in Zn are shown in Fig. 2. The serum concentrations of zinc for the hypo-Zn and latent-Zn groups from baseline to 12 months were significantly lower than those for the latent and control groups ($P < 0.001$). The serum concentrations of Zinc for the hypo-Zn group were significantly lower at baseline and 12 months than those for the latent-Zn group ($P = 0.049, < 0.001$). The serum concentrations of zinc for the hypo-Zn and latent-Zn groups at 24 months were significantly higher than those for the latent and control groups ($P < 0.001$, respectively).

The control, latent, and latent-Zn groups showed a significant increase in the BMD of the lumbar spine at 12 months after dietary guidance compared with baseline ($P < 0.001$, respectively); however, the hypo-Zn group did not show a significant increase ($P = 0.104$) (Fig. 3). All groups showed a significant increase in the BMD of the lumbar spine at 18 and 24 months after dietary guidance relative to baseline ($P < 0.001$, respectively). While only the control group showed a significant increase in BMD of the femoral neck at 6 and 12 months relative to the baseline ($P < 0.001$), all groups showed a significant increase in the BMD of the femoral neck at 24 months compared with the baseline ($P = 0.002, < 0.001, = 0.001, < 0.001$, respectively).

The % change of BMD in the latent-Zn and hypo-Zn groups was compared between the first 12 months of dietary guidance alone and the subsequent 12 months of zinc medication. In both groups, the % change of BMD of both the femoral neck and the lumbar spine showed greater rates of increase in the subsequent 12 months but the differences were not significant.

Logistic regression analyses for non-responders to DMAb and eldecalcitol treatment

As shown in Table 2, we examined the results of simple logistic regression analyses in non-responders to DMAb and eldecalcitol treatment. We identified the three factors, namely thyroid disease, diabetes, and hypozincemia. Specifically, 77.8% (7/9) of patients with thyroid disease were non-responders (OR, 4.568; 95% confidence interval (CI), 0.915–22.798, $P = 0.041$); 17.6% (3/17) of patients with diabetes were non-responders (OR, 0.328; 95% CI, 0.101–1.059, $P = 0.046$); 72.2% (13/18) of patients with hypozincemia were non-responders (OR, 5.980; 95% CI, 1.678–21.311, $P = 0.006$), and 45.7% (43/94) of patients with latent zinc deficiency were non-responders (OR, 1.939; 95% CI, 0.832–4.519, $P = 0.125$).

We identified two factors, namely thyroid disease and hypozincemia from multivariate logistic regression analyses (Table 3). In model A using all identified factors in single logistic regression analyses, thyroid disease and hypozincemia significantly influenced no response (OR 4.545 and 6.354, 95% CI 0.893–23.124 and 1.742-23.173, $P = 0.046$ and 0.005, respectively). In model B, with all factors that showed significant differences in one-way ANOVA, only hypozincemia influenced no response (OR, 6.009; 95% CI, 1.604–22.504, $P = 0.008$). In model C by combining factors of models A and B, thyroid disease and hypozincemia were found to be significantly associated with non-

responders (OR 4.532 and 6.542, 95% CI 0.885–23.206 and 1.704–25.124, $P = 0.047$ and 0.006, respectively).

The occurrence of fragility fractures and other fractures

The fragility fractures (proximal femoral fractures and vertebral fractures) and other fractures that occurred in each group during the 24-month observation period were examined. Proximal femoral fractures occurred in 1 case (3.0%) in the control group, 1 case (2.6%) in the latent group, and 3 cases (5.5%) in the latent-Zn group, but not in the hypo-Zn group ($P = 0.708$). Vertebral fractures occurred in 1 case (3.0%) in the control group, 7 cases (17.9%) in the latent group, 1 case (1.8%) in the latent-Zn group, but not in the hypo-Zn group ($P = 0.004$). Other fractures occurred in 2 cases (6.1%) in the control group, 3 cases (7.7%) in the latent group, and 2 cases (3.6%) in the latent-Zn group, but not in the hypo-Zn group ($P = 0.617$). No patients showed atypical femoral fractures (Table 4). In each two group analysis, the latent group had significantly higher overall fracture rates than the hypo-Zn group and the latent-Zn group ($P = 0.012$ and 0.032). Moreover, the latent group had significantly higher vertebral fracture rates than the latent-Zn group ($P = 0.006$).

We also performed three-group analyses (the hypo-Zn and the latent-Zn combined group (the Zn group) vs. the latent group vs. the control group). The overall trend was similar to that of the four-group analyses. In each two group analyses, the latent group had significantly higher fracture rates and vertebral fracture rates than the Zn group ($P = 0.005$ and 0.001).

Discussion

This retrospective study investigated the effects of zinc deficiency and zinc medication in patients with osteoporosis with zinc deficiency treated with DMAb and eldecalcitol, to identify the factors for poor reactions for DMAb and eldecalcitol must be very important for DMAb and eldecalcitol treatment. While the control, latent, and latent-Zn groups showed a significant increase in lumbar BMD at 12 months relative to the baseline following dietary guidance (approximately 4%), the hypo-Zn did not show a significant increase (approximately 2%). The BMD of the femoral neck showed a similar trend. In particular, the hypo-Zn group showed a slight increase in the femoral neck BMD at 12 months. Additionally, hypozincemia and thyroid disease were identified as factors influencing no response to DMAb and eldecalcitol treatment. The hypo-Zn group showed the same level of increase in BMD of the lumbar from 12 to 24 months, after adding zinc medication, relative to the other groups, and a significant increase in the BMD of the femoral neck at 24 months. However, the % change of BMD was not significantly different between the first 12 months of dietary guidance alone and the subsequent 12 months of zinc medication. Therefore, while hypozincemia may reduce the efficacy of DMAb and eldecalcitol in increasing BMD, the results were inconclusive regarding the superiority of dietary guidance versus zinc medication. No patients suffered fractures in the observation period in the hypo-Zn group, and the latent-Zn group had significantly lower all fracture rates and vertebral fracture rates than the latent group. Therefore, zinc medication may be effective in fracture prevention in patients with hypozincemia treated with DMAb and eldecalcitol.

This study showed that 80.1% of female patients with osteoporosis remained zinc deficient despite a year of dietary guidance to promote zinc sufficiency. There have been several previous reports of lower serum zinc concentrations in postmenopausal women with

248 osteoporosis than in healthy controls [26,27]. Despite the limitation of not measuring zinc
249 intake in the current study, the finding of longitudinal serum zinc levels from baseline to 12
250 months suggests that dietary guidance alone is not sufficient to prevent zinc deficiency.
251 Consistent with a previous report [28], this study showed that oral zinc medication
252 significantly improved serum zinc concentrations in postmenopausal women with
253 osteoporosis. Therefore, it may be necessary to consider introducing oral zinc medication,
254 as well as dietary therapy, to improve zinc deficiency.

255 Although the Japanese standard of serum zinc concentration was 65−130 μg/dL
256 previously, it was revised in 2016 due to the frequent occurrence of zinc deficiency
257 symptoms (such as taste disorder) for patients with zinc levels in the 65−79 μg/dL range. In
258 this study, patients with latent zinc deficiency showed similar changes to those with normal
259 zinc concentration regardless of zinc medication. Additionally, latent zinc deficiency was
260 not associated with non-responders to DMAb and eldecalcitol treatment. Our results
261 suggested that latent zinc deficiency may have no effect on the efficacy of osteoporosis
262 treatment by DMAb and eldecalcitol.

263 Numerous animal studies have confirmed the biphasic influence of zinc on bone
264 metabolism. Initially, zinc has been demonstrated to promote osteoblast differentiation,
265 significantly enhancing alkaline phosphatase activity and Runx2 expression [29]. Notably,
266 Yamaguchi et al. observed that the rise in alkaline phosphatase activity was further
267 augmented by the combined administration of vitamin D3 and zinc, compared to vitamin
268 D3 alone [12]. Therefore, the findings of this study may reflect the synergistic effects of
269 vitamin D and zinc. Moreover, zinc is known to exert inhibitory effects on RANKL-
270 induced osteoclastogenesis [30]. It was also reported that zinc treatment led to increased
271 expression of osteoprotegerin (OPG), a decoy receptor for RANKL, in osteoblastic cells

[31]. Corroborating these findings, Suzuki et al. reported that in zinc-deficient rats, markers of bone formation were diminished, while markers of bone resorption increased, aligning with the previously mentioned effects [17]. DMAb is a neutralizing antibody of the RANKL, and suppress strongly in osteoclast differentiation [32]. Because DMAb fundamentally suppressed RANKL-induced osteoclastogenesis, we inferred that bone resorption activity was strongly suppressed regardless of the degree of zinc sufficiency. Therefore, the poor response to DMAb of hypozincemia patients in this current study might be due to decreased osteoblast activity. The significance of changes in bone metabolic markers under conditions of bone metabolism suppression after an average of 33 months of DMAb administration is unclear, but future studies should investigate bone metabolic markers to elucidate the mechanism.

In the group of patients with hypozincemia who received zinc medication, all fractures, including fragility fractures, did not occur during the observation period. Specifically, 28.2% of patients with latent zinc deficiency without zinc medication suffered fractures. Patients with latent zinc deficiency without zinc medication had significantly higher all fracture risk and vertebral fracture risk than those on zinc medication. While there was no significant difference in the effect of increasing bone density, there was a significant difference in terms of fracture prevention. Therefore, the latent zinc deficiency range might be a useful criterion in considering fracture risk. As far as we know, there have been no reports about the effect of zinc medication on fracture prevention. Increased zinc intake has been reported to improve bone quality and strength in several animal studies [33-35]. Wang et al. reported that ducks with higher zinc concentrations diet exhibited higher tibia ash, strength, and trabecular volume [36]. Our results suggest that zinc may influence the improvement of bone quality and strength and fracture prevention in humans. Some

296 reports of zinc supplementation promoting muscle development in infants and children
297 have been documented but no reports on older the elderly have been published, such as
298 those with osteoporosis [37,38]. The fracture prevention effect in this study might be
299 related to the reduction of fall risk by muscle development with zinc medication. It would
300 be useful to perform a body composition examination or motor function test to compare
301 muscle mass and function.

302 This study has several limitations. First, we did not assess any bone metabolic
303 markers or 25-hydroxyergocalciferol (25(OH)D) concentrations. The patients in this study
304 had already been treated with DMAb and an eldecalcitol for more than one year and were
305 expected to have a stable low bone metabolic turnover. However, future studies should
306 examine the changes in bone metabolic markers and 25(OH)D concentrations following
307 zinc administration to clarify its efficacy. In addition, we did not investigate the urinary
308 zinc concentration, which has been reported to be elevated in postmenopausal patients [39-
309 41]. Second, serum zinc concentration may have been influenced by dietary factors. Fasting
310 is considered necessary for more accurate measurements of serum zinc concentrations;
311 therefore, future studies should be conducted in hospitalized patients on controlled diets.
312 Third, we did not measure the serum concentrations of copper or iron. Since zinc acetate
313 hydrate was originally used to treat Wilson's disease [42], copper and iron deficiencies are
314 severe side effects. It is very important to examine the frequency of side effects to confirm
315 their safety. Fourth, secondary causes of bone fragility other than menopause have not been
316 ruled out in consideration of comorbidities and other factors. Some of the patients in this
317 study did not have pure postmenopausal osteoporosis. This is a retrospective study and the
318 results may have a weak statistical power owing to the small number of cases. Future large

prospective studies with matched backgrounds should be conducted to confirm these findings.

In conclusion, hypozincemia may reduce the efficacy of DMAb and eldecalcitol in increasing BMD and fracture prevention.

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Conflicts of interest/Competing interests

All authors have no conflicts of interest.

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Figure legends

Fig. 1 Participant selection flow chart for this study.

Fig. 2 Longitudinal change in the serum concentration of zinc. White circle: latent zinc deficiency group (Latent), black circle: latent zinc deficiency with zinc treatment group (Latent-Zn), black triangle: hypozincemia with zinc treatment group (Hypo-Zn), black inverted triangle: normal zinc concentration group (Control). *P < 0.05 vs latent zinc deficiency group. †P < 0.05 vs latent zinc deficiency with the zinc treatment group. BL: baseline, DMAb: denosumab.

Fig. 3 Longitudinal % changes in lumbar spine and femoral neck BMD relative to baseline (dietary guidance). White circle: latent zinc deficiency group (Latent), black circle: latent zinc deficiency with zinc treatment group (Latent-Zn), black triangle: hypozincemia with zinc treatment group (Hypo-Zn), black inverted triangle: normal zinc concentration group (Control). *P < 0.05 vs baseline. BL: baseline, DMAb: denosumab.

Table 1. Comparison of the clinical characteristics of female patients in each group at 12 months

	Control group N=33	Hypo-Zn group N=18	Latent-Zn group N=55	Latent group N=39	P-value
Mean age, years	72.33 (11.81)	78.33 (9.07)	74.87 (9.95)	72.62 (8.81)	0.149
BMI, kg/m^2	22.83 (4.00)	22.01 (2.98)	21.82 (2.70)	22.16 (3.04)	0.540
Serum concentration					
Calcium (mg/dL)	9.81 (0.48)	9.84 (0.64)	9.75 (0.50)	9.62 (0.37)	0.282
Phosphorus (mg/dL)	3.61 (0.51)	3.53 (0.39)	3.60 (0.57)	3.49 (0.61)	0.737
eGFR ($mL/min/1.73m^2$)	65.92 (17.60)	55.07 (19.15)	59.79 (14.96)	66.17 (16.11)	0.040*
Zinc (µg/dL)	92.45 (14.22)	54.11 (5.53)	66.91 (5.28)	71.56 (5.38)	<0.001*
Comorbidities, cases					

Thyroid disease	1 (3.0%)	0 (0%)	3 (5.5%)	5 (12.8%)	0.194
Parathyroid disease	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)	0.441
Malignant tumor	8 (24.2%)	2 (11.1%)	6 (10.9%)	6 (15.4%)	0.380
RA	0 (0%)	2 (11.1%)	0 (0%)	1 (2.6%)	0.026*
Diabetes	5 (15.2%)	2 (11.1%)	6 (10.9%)	4 (10.3%)	0.922
CKD	2 (6.1%)	0 (0%)	0 (0%)	2 (5.1%)	0.234
COPD	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
History of vertebral fractures, cases	8 (24.2%)	5 (27.8%)	24 (43.6%)	24 (61.5%)	0.007*
Glucocorticoid use, cases	0 (0%)	0 (0%)	1 (1.8%)	0 (0%)	0.656
Lumbar BMD, g/cm^2	0.852 (0.140)	0.857 (0.149)	0.819 (0.164)	0.810 (0.133)	0.452

Femoral neck BMD, g/cm^2	0.729 (0.108)	0.671 (0.126)	0.702 (0.093)	0.712 (0.077)	0.123
DMAb use period, years	34.48 (24-70)	33.90 (24-60)	33.13 (24-70)	41.61 (24-70)	0.290
Non responder, cases	10 (30.3%)	13 (72.2%)	23 (41.8%)	20 (51.3%)	0.027*

Data are presented as mean (standard deviation of the mean, percentage, or range). BMI: Body Mass Index, eGFR: estimated Glomerular Filtration Rate, RA: Rheumatoid Arthritis, CKD: Chronic Kidney Disease, COPD: chronic obstructive pulmonary disease, BMD: Bone Mineral Density, DMAb: Denosumab. *p < 0.05: Significant differences among the four groups were determined using ANOVA

Table 2. Odds ratios for predicting non responder to DMAb and eldecalcitol treatment using simple logistic analysis

Variables	OR	95%CI	*P*-value
Age (1 year increase)	1.018	0.985–1.052	0.278
BMI (one unit increase)	0.914	0.820–1.018	0.096
Serum concentration (one unit increase)			
Calcium	0.878	0.445–1.731	0.706
Phosphorus	0.738	0.402–1.354	0.323
eGFR	0.996	0.976-1.015	0.671
Comorbidities			
Thyroid disease	4.568	0.915–22.798	0.041*
Malignant tumor	0.506	0.193–1.328	0.156
RA	2.438	0.216–27.497	0.456
Diabetes	0.328	0.101–1.059	0.046*
CKD	1.188	0.163–8.670	0.866
History of vertebral fractures	1.151	0.594–2.231	0.677
DMAb use period (1 year increase)	1.130	0.896–1.426	0.301
Hypozincemia	5.980	1.678–21.311	0.006*
Latent zinc deficiency	1.939	0.832–4.519	0.125

DMAb: Denosumab; OR, odds ratio; CI, confidence interval; BMI: Body Mass Index, eGFR: estimated Glomerular Filtration Rate, RA: Rheumatoid Arthritis, CKD: Chronic Kidney Disease. *$p<0.05$: This parameter significantly affected no response to DMAb and eldecalcitol treatment

Table 3. Odds ratios for predicting a non responder to DMAb and eldecalcitol treatment using multivariate logistic analysis

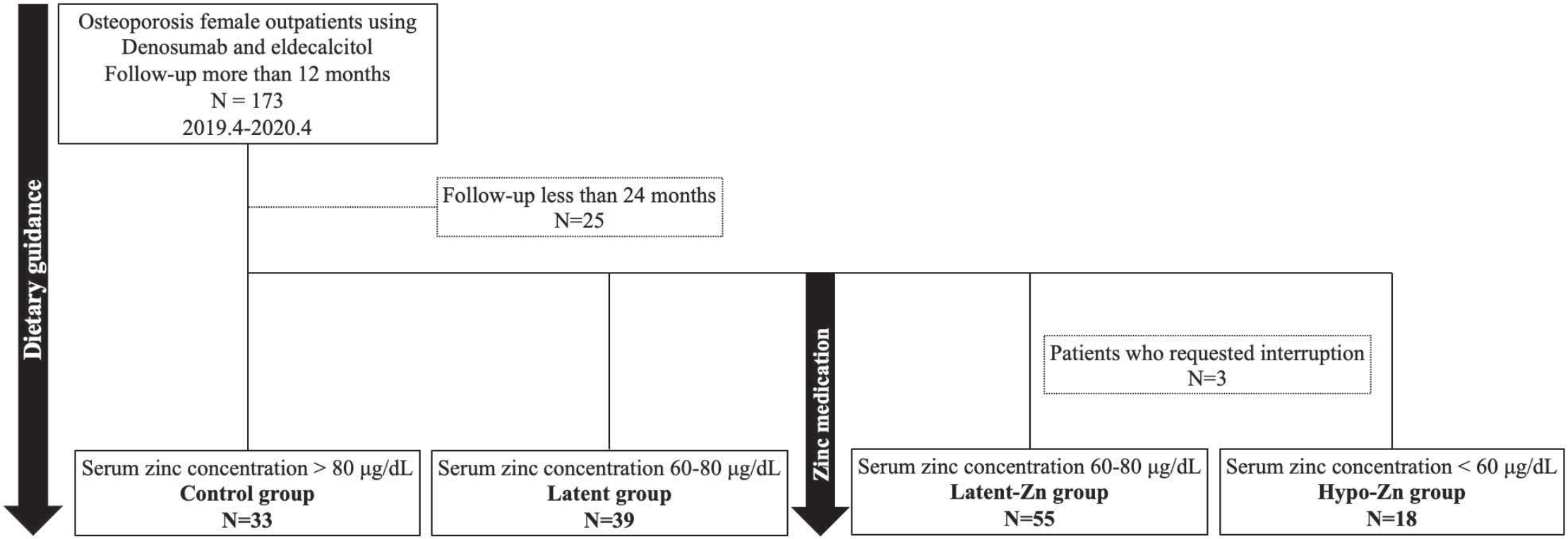
Variables	OR	95%CI	*P*-value
Model A			
Thyroid disease	4.545	0.893–23.124	0.046*
Diabetes	0.354	0.104–1.197	0.076
Hypozincemia	6.354	1.742–23.173	0.005*
Latent zinc deficiency	1.768	0.743–4.207	0.198
Model B			
eGFR	1.001	0.981–1.022	0.889
RA	1.084	0.079–14.836	0.952
History of vertebral fractures	1.137	0.556–2.324	0.725
Hypozincemia	6.009	1.604–22.504	0.008*
Latent zinc deficiency	1.883	0.790–4.486	0.153
Model C			
eGFR	1.002	0.981–1.024	0.831
Thyroid disease	4.532	0.885–23.206	0.047*
RA	0.954	0.067–13.589	0.973
Diabetes	0.342	0.100–1.176	0.071
History of vertebral fractures	1.216	0.582–2.542	0.603
Hypozincemia	6.542	1.704–25.124	0.006*
Latent zinc deficiency	1.698	0.698–4.133	0.243

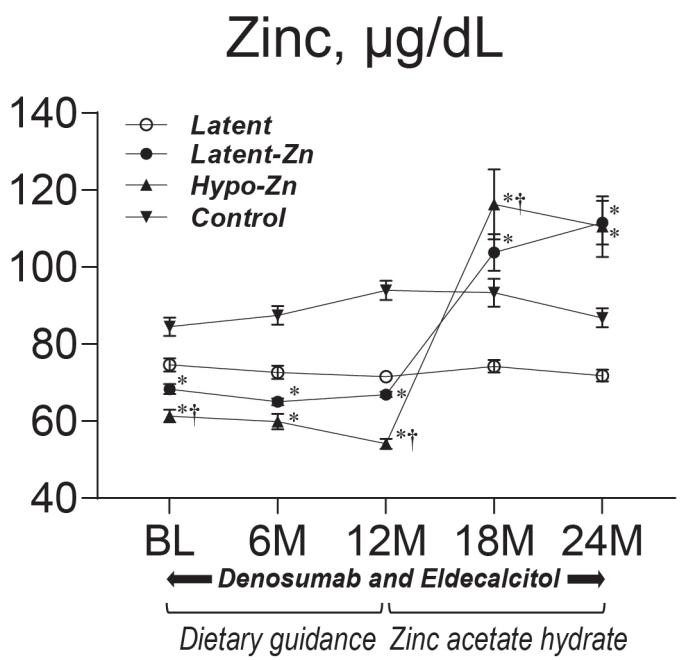
DMAb: Denosumab; OR, odds ratio; CI, confidence interval; eGFR: estimated Glomerular Filtration Rate, RA: Rheumatoid Arthritis. *$p<0.05$: This parameter significantly affected no response to DMAb and eldecalcitol treatment

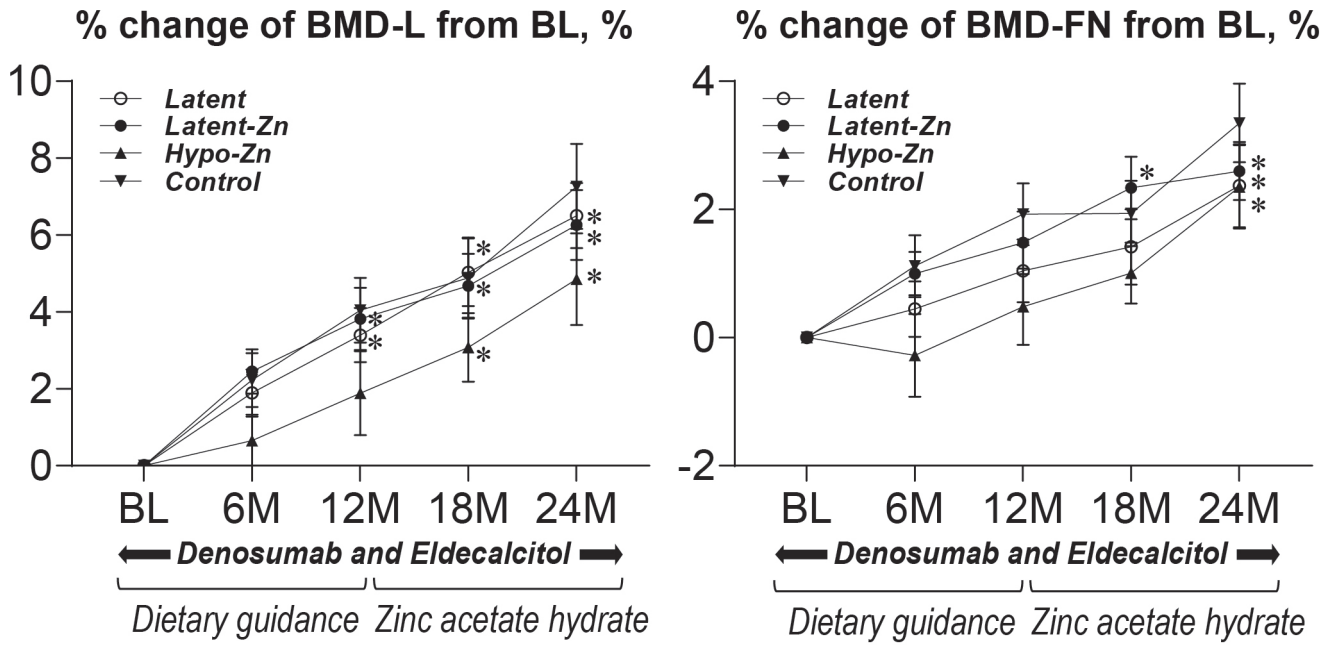
Table 4. Fractures in each group that occurred during the observation period

	Control group, N=33	Hypo-Zn group, N=18	Latent-Zn group, N=55	Latent group, N=39	*P-value*
All fractures, cases	4 (12.1%)	0 (0%)	6 (10.9%)	11 (28.2%)	0.021*
Proximal femoral fractures, cases	1 (3.0%)	0 (0%)	3 (5.5%)	1 (2.6%)	0.702
Vertebral fractures, cases	1 (3.0%)	0 (0%)	1 (1.8%)	7 (17.9%)	0.005*
Other fractures, cases	2 (6.1%)	0 (0%)	2 (3.6%)	3 (7.7%)	0.596

Data are presented as mean (percentage). *$p < 0.05$: Significant differences among the four groups were determined using chi-square tests.


Dietary guidance
Osteoporosis female outpatients using
Denosumab and eldecalcitol
Follow-up more than 12 months
N = 173
2019.4-2020.4
Follow-up less than 24 months
N=25
Zinc medication
Patients who requested interruption
N=3
Serum zinc concentration > 80 μg/dL
Control group
N=33
Serum zinc concentration 60-80 μg/dL
Latent group
N=39
Serum zinc concentration 60-80 μg/dL
Latent-Zn group
N=55
Serum zinc concentration < 60 μg/dL
Hypo-Zn group
N=18


Zinc, μg/dL
140
120
100
80
60
40
Latent
Latent-Zn
Hypo-Zn
Control
BL
6M
12M
18M
24M
Denosumab and Eldecalcitol
Dietary guidance
Zinc acetate hydrate


% change of BMD-L from BL, %
10
8
6
4
2
0
Latent
Latent-Zn
Hypo-Zn
Control
BL
6M
12M
18M
24M
Denosumab and Eldecalcitol
Dietary guidance
Zinc acetate hydrate
% change of BMD-FN from BL, %
4
2
0
-2
Latent
Latent-Zn
Hypo-Zn
Control
BL
6M
12M
18M
24M
Denosumab and Eldecalcitol
Dietary guidance
Zinc acetate hydrate