

Effects of 12-months treatment with zoledronate or teriparatide on intima-media thickness of carotid artery in women with postmenopausal osteoporosis: A pilot study

International Journal of
Immunopathology and Pharmacology
Volume 33: 1–7

© The Author(s) 2019



Article reuse guidelines:

sagepub.com/journals-permissions

DOI: 10.1177/2058738418822439

journals.sagepub.com/home/iji



Elena Passeri¹, Daniela Mazzaccaro², Veronica Sansoni³,
Silvia Perego³, Giovanni Nano^{2,4}, Chiara Verdelli⁵,
Giovanni Lombardi³  and Sabrina Corbetta^{1,4} 

Abstract

Atherosclerosis and osteoporosis are interrelated entities and share similar pathogenic mechanisms. Recent studies showed that key proteins of bone metabolism, such as osteoprotegerin (OPG) and osteopontin (OPN), are also involved in vascular atherosclerosis and calcifications. The carotid intima-media thickness (CA-IMT) is an early quantitative marker of generalized atherosclerosis. Aim of study was to investigate whether 12-months treatment with zoledronate (ZLN) or teriparatide (TPT) affects CA-IMT and circulating OPG and OPN levels. In this study, 11 postmenopausal osteoporotic women (aged 73, 70.5–74.5 years; median, range interquartile) treated with 5 mg/year iv ZLN; 9 postmenopausal osteoporotic women (aged 70, 62.5–73.5 years) treated with 20 µg/day sc TPT; and 10 aged-, body mass index (BMI)-, glycemic, and lipid profiles-matched, free from anti-osteoporotic and hypocholesterolemic drugs, controls were prospectively investigated at baseline and after 12 months. At baseline, median CA-IMT was similar in the three groups and increased after 12 months. CA-IMT increased significantly in TPT-treated patients (1.0, 0.8–1.2 vs 1.1, 0.9–1.5 mm, $P=0.04$), though the change was minimal. After 12 months of treatment, CA-IMT positively correlated with alkaline phosphatase (ALP) levels ($r=0.767$, $P=0.008$) and negatively with high-density lipoprotein (HDL) cholesterol levels ($r=-0.65$, $P=0.03$), suggesting interplay between active bone remodeling and lipid profile. At baseline and after 12 months, median serum OPG and OPN levels did not differ among the groups and did not correlate with changes in CA-IMT. In conclusion, ZLN and TPT treatments are safe on carotid walls in osteoporotic women with subclinical atherosclerosis; circulating OPG and OPN are not affected by long-term anti-osteoporotic treatments and do not correlate with CA-IMT.

Keywords

carotid atherosclerosis, intima-media thickness, osteopontin, osteoprotegerin, teriparatide, zoledronate

Date received: 5 July 2018; accepted: 6 December 2018

¹Endocrinology and Diabetology Service, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

²First Operative Unit of Vascular Surgery, IRCCS Policlinico San Donato, Milan, Italy

³Laboratory of Experimental Biochemistry and Molecular Biology, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

⁴Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

⁵Laboratory of Experimental Endocrinology, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

Corresponding author:

Sabrina Corbetta, Endocrinology and Diabetology Service, Department of Biomedical sciences for Health, University of Milan, IRCCS Istituto Ortopedico Galeazzi, Via R. Galeazzi 4, 20161 Milan, Italy.
Email: sabrina.corbetta@unimi.it



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons

Attribution-NonCommercial 4.0 License (<http://www.creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

Introduction

Atherosclerosis and osteoporosis globally account for high morbidity and mortality. In the general population, atherosclerosis and cardiovascular calcifications are associated with low bone mineral density (BMD).¹ Moreover, key molecules of bone metabolism, such as osteoprotegerin (OPG) and osteopontin (OPN), are also involved in vascular atherosclerosis and calcifications.²

Although the interaction between osteoporosis and cardiovascular disorders is emerging, the clinical effects of osteoporosis treatment on atherosclerosis are poorly investigated. Bisphosphonates, a major class of antiresorptive drugs, inhibit atherosclerosis in animal models and in small-scale clinical trials,³ though the underlying mechanism has not yet been clarified. The parathyroid hormone (PTH) amino-terminal fragment (1–34), teriparatide (TPT), is an osteoanabolic agent, which may play a role in the atherosclerosis development. PTH targets the endothelial and smooth muscle cells of the vascular wall causing endothelial dysfunction. Clinical evidence support the effect of PTH on vascular atherosclerosis: in postmenopausal normocalcemic women, circulating PTH levels positively correlated with carotid intima-media thickness (CA-IMT),⁴ and patients with primary hyperparathyroidism, a metabolic disorder with concomitant hypercalcemia and elevated serum PTH levels, had increased CA-IMT.⁵ Besides, in low-density lipoprotein (LDL) receptor-deficient diabetic mice, daily subcutaneous administration of TPT greatly reduced the extent of both aortic and cardiac valve calcifications.⁶

This study aimed to investigate whether 12-months treatment with intravenous zoledronate (ZLN) or subcutaneous TPT affects the CA-IMT, a widely used surrogate marker for carotid atherosclerosis, and the circulating levels of the biochemical markers of bone metabolism and vascular calcifications, OPG, and OPN.

Patients and methods

Study population

This prospective observational pilot study investigated 11 postmenopausal osteoporotic females (aged 73, 70.5–74.5 years, median, interquartile (IQ) range) treated with 5 mg/year iv ZLN and 9 postmenopausal osteoporotic females (aged 70, 62.5–73.5 years) treated with 20 mg/day sc TPT. All patients were well compliant to the TPT treatment.

Control group consisted in 10 age-, body mass index (BMI)-, glycemic and lipid profiles-matched, non-fractured, free from anti-osteoporotic drugs and free from statins postmenopausal women.

Exclusion criteria were as follows: active smoke, overt carotid disease, statin treatment, poorly controlled hypertension, obesity (BMI >30 kg/cm²), liver and heart failures (NYHA class II, III, or IV), chronic kidney diseases, diabetes mellitus, corticosteroid therapy and endogenous hypercortisolism, malignances, hypercalcemia and primary hyperparathyroidism, connective autoimmune diseases, and anti-estrogenic drugs. Causes of secondary osteoporosis were excluded through extensive diagnostic workout, including serum protein electrophoresis and urine Bence Jones protein determination.

Osteoporotic patients with vitamin D insufficiency were corrected individually according to the serum 25-OH vitamin D levels during the diagnostic workout. Both patients and controls at time of enrollment started monthly supplementation with 100,000 U of oral colecalciferol. Hypercalciuria was detected in two osteoporotic patients and corrected with thiazide diuretics.

All procedures performed were in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained from all individual participants included in the study.

Clinical and metabolic assessment

All metabolic and diagnostic evaluations were performed baseline and after 12 months. Medical history was collected from all participants. Anthropometric measurements, including height, weight, waist, and hip circumferences, were obtained. Weight was measured on a standard balance beam scale; height was measured using a calibrated, wall-mounted Harpenden Stadiometer. BMI was calculated.

BMD and T score were determined by dual-energy X-ray absorptiometry (DXA; Hologic Discovery scanner, Zaventem, Belgium) at lumbar spine and femoral levels in all osteoporotic women; thoracic and lumbar spine X-ray images for the diagnosis of morphometric fractures according to Genant's semi-quantitative criteria were also obtained.

Bone metabolism was investigated in osteoporotic patients and controls as follows: overnight fasting venous blood samples were collected for determination of serum calcium, phosphate, creatinine, and

alkaline phosphatase (ALP) according to routine laboratory assays. Albumin-corrected calcium levels were calculated as follows: albumin-corrected calcium = $(0.8 \times \text{normal albumin} - \text{patient's albumin}) + \text{serum calcium levels}$. The normal albumin level was defaulted to 4.0 g/dL. Serum PTH levels were determined by ElectroChemiLuminescence on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). Serum 25-OH vitamin D (25-OHD) was measured by a chemiluminescent assay (LIAISON® test; DiaSorin Inc., Stillwater, MN, USA). Circulating OPG and OPN were measured by enzyme-linked immunosorbent assay (ELISA) kits (BioVendor—Laboratorní Medicina a.s., Brno, CZ, EU, and IBL Co. Ltd, Fujioka, Japan, respectively). The lower limit of detection was 0.03 pmol/L for OPG and 3.33 ng/mL for OPN. The intra-assay and inter-assay variation were 2.5%–4.9% and 1.7%–9.0% for OPG and 8.0%–11.0% and 4.4%–10.1% for OPN, respectively.

Metabolic profile was investigated in osteoporotic patients and controls as follows: overnight fasting serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, and insulin were measured by routinely used laboratory kits. LDL cholesterol was calculated with the Friedewald *formula*. The insulin resistance index homeostasis model assessment (HOMA) was calculated.

Carotid ecocolor Doppler assessment

Carotid duplex ultrasound assessment was performed with a linear-array, 3- to 11-MHz transducer (Esaote MyLab™ 25 Gold; Esaote S.p.A., Genoa, Italy). Each subject was evaluated at rest, in a comfortable supine position on a bed with the neck fully exposed. The subjects were asked to extend their head and turn it about 30° toward the contralateral side. The common carotid artery of the right side was examined within 1.5 cm proximal to the carotid bifurcation on a two-dimensional ultrasound image. A single sonographer, that was blind to the assignment group of each patient, manually measured the CA-IMT on the frozen frame of a suitable longitudinal view. CA-IMT was evaluated in the posterior wall of the common carotid artery, as the distance from the leading edge of the first echogenic line (lumen-intima interface) to the leading edge of the second line (media-adventitia interface). CA-IMT was measured at its maximal site, as well as 10 mm distal and proximal of the maximal site, respectively. The average of these three measurements was taken as the CA-IMT value.⁷

Statistical analysis

Data failed to pass the normality test; therefore, they were presented as median, and IQ range and comparison of continuous variables between patient and control groups were performed by Mann–Whitney U test. Categorical data were presented as percentages and compared by Fischer's exact test. Comparison was performed using Wilcoxon test matched-pairs for paired data. Bivariate associations between the biochemical parameters were tested by Spearman correlation. A *P* value lower than 0.05 was considered significant. Statistical analysis was performed using PRISM 6.1 (GraphPad Software, La Jolla, CA, USA).

Results

Effects of 12-months ZLN or TPT treatment on clinical and biochemical parameters

Baseline clinical characteristics of patients treated with 5 mg/year iv ZLN (ZLN group) and patients treated with 20 µg/day sc teriparatide (TPT group) and controls are reported in Table 1. At 12 months, anthropometric measurements, including weight, BMI, and waist/hip circumferences, did not vary in response to ZLN or TPT treatment.

ZLN administration was not associated with significant changes in serum albumin-corrected calcium, ALP activity, or PTH levels (Table 1); conversely, as expected, TPT treatment induced a mild decrease in median serum PTH levels (39.0 (28.0–50.5) vs 21.0 (12.2–23.7) pg/mL, *P* < 0.01; baseline vs 12 months) and an increase in median ALP activity levels ((56.5 (55.2–76.2) vs 83.5 (73.2–101.5) U/L, *P* < 0.01; baseline vs 12 months; Table 1).

Regarding the metabolic profile, serum glucose and insulin levels as well as HOMA-IR were unaffected in both patient and control groups (Table 1). Similarly, lipid profile, namely, serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, did not show significant variations in response to ZLN or TPT therapy, as well as in controls (Table 1).

Effects of 12-months ZLN or TPT treatment on CA-IMT

At baseline, median CA-IMT values were similar in the ZLN and TPT groups as well as in controls. Subclinical atherosclerosis, defined as CA-IMT > 0.9 mm,⁷ was diagnosed in about

Table 1. Clinical and Biochemical Characteristics of ZLN and TPT Patients and Effects of 12-months ZLN or TPT Treatment.

Fractures	ZLN patients (n = 11)		TPT patients (n = 9)		Controls (n = 10)	
	Basal	12 months	Basal	12 months	Basal	12 months
Femoral neck T score	10 vertebral, 2 hip, 5 others #		9 vertebral, 5 others #		-	
	-2.2 (-3.4, -1.9)		-3.0 (-3.4, -2.0)		-	
Waist (cm)	83 (72-92)	79 (76-90)	84 (71-91)	87 (78-90)	83 (73-93)	85 (79-92)
BMI (kg/m ²)	27.9 (23.5-29.9)	28.2 (23.9-30.2)	27.1 (23.3-29.3)	25.4 (22.7-27.8)	24.8 (22.4-28.8)	23.8 (22.1-28.3)
Glucose (mg/dL)	89.0 (80.0-99.0)	91.5 (84.0-96.0)	82.5 (72.3-92.7)	87.0 (80.0-94.5)	85.5 (79.3-96.5)	96.5 (86.1-107.2)
Insulin (mU/L)	6.6 (3.6-13.4)	6.6 (5.6-12.7)	5.5 (3.8-7.9)	5.9 (3.8-9.2)	8.0 (4.7-10.7)	8.0 (7.1-12.0)
HOMA-IR	1.51 (0.72-3.03)	1.57 (1.17-3.03)	1.28 (0.97-1.95)	1.43 (0.82-1.99)	1.65 (0.93-2.57)	1.81 (1.43-3.19)
Total CHO (mg/dL)	208 (192-234)	209 (184-244)	235 (229-266)	236 (220-258)	206 (188-252)	218 (190-244)
HDL CHO (mg/dL)	73 (64-77)	71 (65-80)	74 (63-92)	66 (62-111)	66 (47-71)	65 (49-76)
LDL CHO (mg/dL)	124 (84-139)	125 (87-146)	155 (113-171)	152 (116-170)	121 (105-157)	121 (105-157)
Triglycerides (mg/dL)	91 (84-146)	85 (71-140)	97 (72-116)	107 (64-122)	119 (62-182)	109 (76-165)
Hypertension	5/11 (45%)	5/11 (45%)	6/9 (67%)	6/9 (67%)	5/10 (50%)	5/10 (50%)
Calcium (mg/dL)	9.1 (8.8-9.5)	9.2 (9.0-9.4)	9.3 (9.0-9.6)	9.7 (9.5-9.7)	9.2 (9.0-9.4)	9.1 (8.9-9.4)
Phosphate (mg/dL)	3.6 (3.3-3.8)	3.4 (2.8-3.8)	3.6 (3.5-3.7)	3.5 (3.3-3.8)	3.4 (3.2-3.7)	3.4 (3.1-4.0)
PTH (pg/mL)	47.0 (41.0-79.0)	45.0 (33.0-67.0)	39.0 (28.0-50.5)	21.0* (12.2-23.7)	49.5 (41.7-68.5)	54.0 (42.0-62.0)
25-OHD (ng/mL)	32.6 (27.9-33.4)	46.9 (36.4-60.4)	28.1 (23.1-38.3)	43.2 (15.1-48.3)	16.6 (14.6-23.2)	32.9* (25.7-44.4)
ALP (U/L)	62.0 (57.0-84.0)	61.0 (46.0-78.0)	56.5 (55.2-76.2)	83.5* (73.2-101.5)	74.0 (62.2-80.7)	80.0 (65.2-89.0)
CA-IMT (mm)	1.2 (0.95-1.5)	1.2 (1.0-1.8)	1.0 (0.8-1.2)	1.1* (0.9-1.5)	1.35 (0.9-1.75)	1.7 (0.97-2.45)

ZLN: zoledronate; TPT: teriparatide; # wrist: ribs or metatarsal fractures; BMI: body mass index; HOMA-IR: homeostasis model assessment insulin resistance; CHO: cholesterol; calcium: serum albumin-corrected calcium; PTH: serum intact parathyroid hormone; 25-OHD: serum 25 hydroxyvitamin D; ALP: serum alkaline phosphatase activity levels; CA-IMT: carotid artery intima-media thickness.

Data are presented as medians and interquartile ranges.

*P < 0.05 versus basal levels.

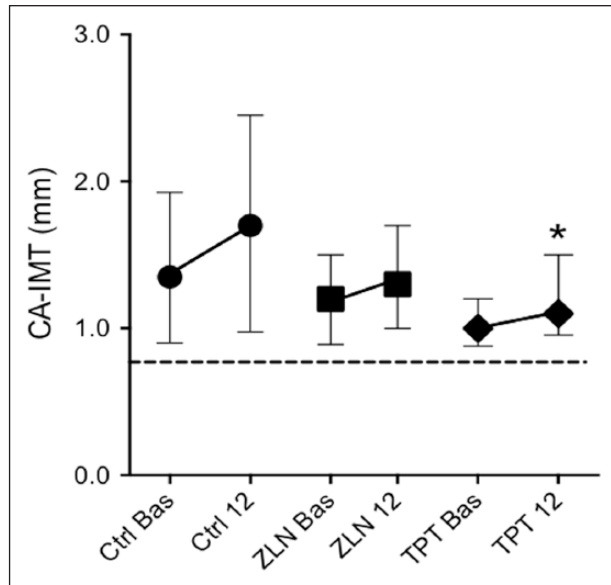


Figure 1. Effect of 12-months treatments on CA-IMT in osteoporotic women compared with 12-months observation. Circles: healthy controls; squares: patients treated with 5 mg iv ZLN; diamonds: patients treated with 20 µg/day TPT sc. Dashed line indicates subclinical atherosclerosis, defined as CA-IMT >0.9 mm.⁷ Data are presented as median and interquartile range; * $P < 0.05$ (Wilcoxon matched-pairs signed rank test). Ctrl, controls; ZLN, patients treated with zoledronate; TPT, patients treated with teriparatide; Bas, measurements at the enrollment; 12, measurements after 12 months of observation or treatment.

two-thirds of subjects (63% of TPT patients, 72% of ZLN patients, and 70% of controls), in agreement with data reported in age-matched Italian population.⁷

Median CA-IMT showed an increase in trend after 12 months in all groups, though the increase was statistically significant only in TPT-treated patients (1.0, 0.8–1.2 vs 1.1, 0.9–1.5 mm, $P = 0.039$); indeed, changes were limited to 0.1 mm (Figure 1).

Correlations between CA-IMT and biochemical parameters at baseline and after 12 months of treatment

Basal CA-IMT positively correlated with age ($r = 0.40$, $P = 0.01$) and triglyceride levels ($r = 0.44$, $P = 0.007$) in all groups. Any further correlation with metabolic parameters could be detected in the ZLN-treated group, while in the TPT group, CA-IMT at 12 months positively correlated with ALP activity levels ($r = 0.767$, $P = 0.008$) and negatively with HDL cholesterol levels ($r = -0.65$, $P = 0.03$), suggesting a potential effect of TPT

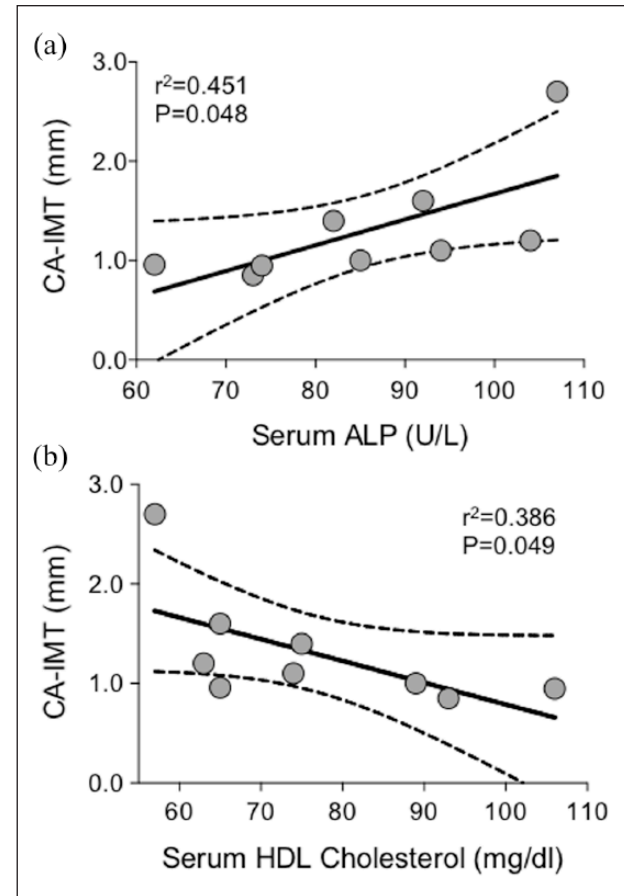


Figure 2. Correlation between (a) serum ALP activity and CA-IMT after 12 months of TPT treatment and (b) serum HDL cholesterol at basal and CA-IMT after 12 months of treatment with TPT. Dashed lines indicate 95% confidence interval.

treatment in association with an adverse lipid profile (Figure 2).

Effects of 12-months ZLN or TPT treatment on serum OPG and OPN levels

At baseline, median serum OPG and OPN levels did not differ among ZLN and TPT groups and controls. At 12 months, any significant variation of OPG and OPN levels was detected in TPT and ZLN groups (Figure 3). In particular, serum OPG and OPN concentrations did not correlate with both basal and 12-months CA-IMT levels.

Discussion

The present pilot study showed in a small but well-defined series of osteoporotic women with subclinical atherosclerosis that 12-months ZLN or TPT treatments are safe from a vascular point of view.

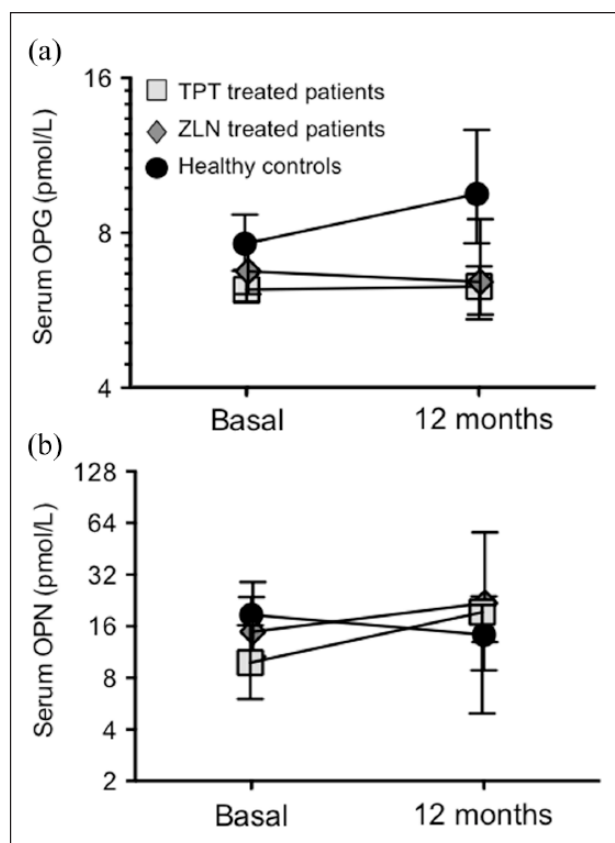


Figure 3. (a) Effect of 12 months of treatments on serum OPG levels in osteoporotic women compared with 12 months of observation in controls. (b) Effect of 12 months of treatments on serum OPN levels in osteoporotic women compared with 12 months of observation in controls. Light gray squares indicate patients treated with TPT; dark gray diamonds, patients treated with ZLN; black circles, healthy controls. Values on Y-axis are expressed as Log₂; data are presented as median and interquartile range.

ZLN did not affect CA-IMT, reinforcing the emerging outcomes that bisphosphonates do not have beneficial or harmful effects on atherosclerotic cardiovascular events,⁸ at variance with a previous study reporting a small CA-IMT reduction after 1-year ZLN therapy.⁹ CA-IMT showed a trend to increase over the 1-year period of follow-up in both treated groups and controls, achieving the significance in TPT-treated patients; admittedly, due to the limited size of the samples, interpretation of such a statistical significant change needs caution. The effect of TPT on CA-IMT has been previously investigated in 28 Asian osteoporotic women, where a decrease in CA-IMT measurements after 12 months of TPT treatment was detected,¹⁰ at variance with what is reported in this study. Indeed, Yoda et al.¹⁰

included patients treated with statins for their dyslipidemia, a therapy that may affect CA-IMT. Our finding of a negative correlation between CA-IMT and HDL cholesterol levels and of a positive correlation with ALP activity after 12 months of treatment in TPT-treated women suggests a synergy between an adverse lipid profile and the bone remodeling modulation. However, we could not detect any significant effects of TPT treatment on circulating levels of OPG and OPN, two molecules involved in the interaction between bone metabolism and atherosclerosis. In particular, serum OPG levels were not affected by ZLN neither by TPT treatments, in agreement with previous reports.¹¹ As far as OPN is concerned, this is the first human report about the effect of ZLN and TPT on circulating OPN levels: both medical interventions did not exert significant changes on serum OPN levels, at variance with the reduction observed in an animal model in response to TPT treatment.⁶

This study suffered from an important limitation, above all, the small size of the enrolled series and, as a pilot study, extension is mandatory. Nonetheless, it provides some consistent items. First, this is the first study comparing the effects on CA-IMT of two commonly used anti-osteoporotic drugs, ZLN and TPT, with distinct effects on bone remodeling. Second, at variance with previous reports, we provided a control group matched for the most common pro-atherosclerotic risk factors; patients and controls were supplemented with vitamin D, and they were non-active smokers and free from other diseases. Finally, a single-blind examiner performed all CA-IMT measurements.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This paper was supported by the Italian Ministry of Health.

ORCID iDs

Giovanni Lombardi  <https://orcid.org/0000-0002-8365-985X>

Sabrina Corbetta  <https://orcid.org/0000-0001-8140-3175>

References

1. Ye C, Xu M, Wang S, et al. (2016) Decreased bone mineral density is an independent predictor for the development of atherosclerosis: A systematic review and meta-analysis. *PLoS ONE* 11: e0154740.
2. Vassalle C and Mazzone A (2016) Bone loss and vascular calcification: A bi-directional interplay? *Vascular Pharmacology* 86: 77–86.
3. Caffarelli C, Montagnani A, Nuti R, et al. (2017) Bisphosphonates, atherosclerosis and vascular calcification: Update and systematic review of clinical studies. *Clinical Interventions in Aging* 12: 1819–1828.
4. Choi HS, Kim SH, Rhee Y, et al. (2008) Serum parathyroid hormone is associated with carotid intima-media thickness in postmenopausal women. *International Journal of Clinical Practice* 62: 1352–1357.
5. Walker MD, Fleischer J, Rundek T, et al. (2009) Carotid vascular abnormalities in primary hyperparathyroidism. *Journal of Clinical Endocrinology and Metabolism* 94: 3849–3856.
6. Shao JS, Cheng SL, Charlton-Kachigian N, et al. (2003) Teriparatide (human parathyroid hormone (1–34)) inhibits osteogenic vascular calcification in diabetic low density lipoprotein receptor-deficient mice. *Journal of Biological Chemistry* 278: 50195–50202.
7. Ciccone MM, Balbarini A, Porcelli TM, et al. (2011) Carotid artery intima-media thickness: Normal and percentile values in the Italian population (camp study). *European Journal of Cardiovascular Prevention and Rehabilitation* 18: 650–655.
8. Kim DH, Rogers JR, Fulchino LA, et al. (2015) Bisphosphonates and risk of cardiovascular events: A meta-analysis. *PLoS ONE* 10: e0122646.
9. Gonnelli S, Caffarelli C, Tanzilli L, et al. (2014) Effects of intravenous zoledronate and ibandronate on carotid intima-media thickness, lipids and FGF-23 in postmenopausal osteoporotic women. *Bone* 61: 27–32.
10. Yoda M, Imanishi Y, Nagata Y, et al. (2015) Teriparatide therapy reduces phosphate and intima-media thickness at the carotid wall artery in patients with osteoporosis. *Calcified Tissue International* 1: 32–39.
11. Anastasilakis AD, Goulis DG, Polyzos SA, et al. (2008) Serum osteoprotegerin and RANKL are not specifically altered in women with postmenopausal osteoporosis treated with teriparatide or risedronate: A randomized, controlled trial. *Hormone and Metabolic Research* 40: 281–285.