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Original Article

Hylonome

Additive impact of alfacalcidol on bone mineral density and bone strength in alendronate treated postmenopausal women with reduced bone mass

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Abstract

Objectives: Assessment of additive impact of alfacalcidol 1 μg daily (Alfa) on bone mineral density (BMD) and on bone strength in postmenopausal women treated with alendronate 70 mg weekly + 500 mg calcium daily. **Subjects and methods:** In a randomized, double-blind, placebo controlled study, 279 postmenopausal women with osteoporosis or osteopenia participated (intention to treat analysis [ITT]; aged 73.6±4.7 years) and were treated with 70 mg alendronate (ALN) weekly and 500 mg calcium daily for 36 months. In addition, these patients received either 1 μg alfacalcidol (Alfa) or placebo (PLC) daily. BMD was measured with Dual-Energy-X-ray-Absorptiometry (DXA) at the lumbar spine and proximal femur and at forearm and tibia with peripheral quantitative computed tomography (pQCT) at regular intervals for 36 months. **Results:** DXA-BMD of lumbar spine (L1-4) increased after 36 months, by 6.65% ($p<0.0001$) in the Alfa/ALN group versus 4.17% ($p<0.0001$) in the PLC/ALN group. Group difference was significant after 3 years ($p=0.026$). At the end of the study, significant differences were found in favor of the Alfa/ALN group in trabecular density (tibia) ($p=0.002$), cortical density (midshaft tibia) ($p=0.043$), and bone strength ($p=0.001$). The remaining parameters showed no differences between the treatment arms, apart cortical bone density at midshaft radius. **Conclusions:** Alfacalcidol significantly increases the efficacy of alendronate treatment in osteopenic/osteoporotic postmenopausal women on spinal DXA-BMD, cortical and trabecular BMD of the tibia and also bending stiffness of the tibia.

Keywords: Osteopenia/Osteoporosis, Alfacalcidol, Alendronate, Cortical Density, Bone Strength

Introduction

Alfacalcidol (1 alpha-hydroxy Vitamin D_3) is a synthetic Vitamin D analogue which contains a hydroxyl group in position 1 and, therefore, does not need to pass the kidney for hydroxylation. It is hydroxylated fully in the liver and bone at position 25 to 1,25 $(OH)_2D_3$ (Calcitriol, D-Hormone), the effective Vitamin D metabolite and has been used as a therapeutic agent for osteoporosis treatment for more than 20 years, in particular in Japan and Europe[49]. Several clinical studies have shown alfacalcidol to impede accelerated bone turnover in osteoporosis[49], to maintain or increase bone mineral density (BMD) in postmenopausal women[32,47,48], and to prevent osteoporotic fractures in spine[24,30,32] and hip[50]. Two meta-analyses confirmed the effects of hydroxylated Vitamin D analogs

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(alfacalcidol, calcitriol) on bone mass, vertebral and non-vertebral fractures[33,34]. We should carefully differentiate between studies with alfacalcidol (1 alpha-hydroxy vitamin D_3) and calcitriol (1,25 dihydroxy vitamin D_3), the active vitamin D metabolite.

Preclinical studies in rats have shown an advantage of alfacalcidol over vitamin D in the treatment of osteoporosis[45] and postulate that alfacalcidol acts on bone independently from PTH-suppression. Other preclinical studies showed that alfacalcidol causes a dose-dependent suppression of bone resorption, but maintains or even stimulates bone formation and results in an increase of bone mineral density with alfacalcidol improvement of mechanical strength with a more pronounced effect on cortical bone[46].

Some studies suggest that the superiority of the alfacalcidol/calcitriol treatment on bone metabolism compared to plain Vitamin D may be due to the fact that the final kidney activation on vitamin D is regulated by a negative feedback mechanism[30,35]. In patients with Vitamin D insufficiency or patients with impaired kidney function, increased D-Hormone action at the target tissues cannot be achieved. Alfacalcidol is effective in increasing bone mass independent from renal function, benefiting especially elderly patients with impaired kidney function. Increased D-hormone action cannot be achieved with plain vitamin D[36].

The effect of osteoporosis treatment with bisphosphonates (BPs) on DXA-BMD of spine and hip has been frequently documented[8,9,13,23]. Alendronate (ALN) was one of the first BPs which was shown to reduce fracture incidence of vertebral, hip, and forearm fractures in women with postmenopausal osteoporosis[8]. There is paucity of data available to distinguish the different treatment effects on cortical and trabecular bone, as well as on bone strength[19,44,51]. In previous studies Roschger et al[38] analyzed iliac crest biopsies before and after ALN treatment and illustrated that the relative calcium content of osteoporotic bone was significantly lower than that of normal controls and that mineralization was significantly higher and more uniform after ALN treatment. The porosity of cortical bone was reduced significantly by ALN treatment. One may infer from these results that this effect may contribute to the observed reduction in fractures. Possibly, the diminished porosity of cortical bone can explain the increased bone strength. Measurements of DXA-BMD cannot distinguish between cortical and trabecular bone and, thus, some understanding of the treatment effect of medications may not be detected and/or cannot be completely understood.

There have been some efforts over the last years to generate data with DXA measurements to calculate bone strength, particularly at the proximal femur[3-5,10]. Using pQCT method to measure bone mineral density [mass/volume] and bone geometry at the distal forearm and tibia, bone strength can be evaluated[19,20,29]. Boonen et al[11] have investigated these differences between cortical and trabecular bone and illustrated in a cross sectional study of selected community dwelling elderly women with pQCT measurements in the forearm a different bone loss with age in cortical (-0.21 mg/cm^3/year, $p<0.05$) and trabecular bone (-0.14 mg/cm^3/year, n.s.).

In this study, we aimed to assess the potential additional benefit of alfacalcidol + ALN treatment on bone density and bone strength in comparison to ALN alone in osteoporotic and osteopenic postmenopausal women. In addition to standard DXA measurements, we aimed to assess the cortical and trabecular bone mineral density of the distal radius and tibia. We hypothesized that alfacalcidol has an additional effect on trabecular and cortical bone in postmenopausal women above that of alendronate alone.

Subjects and methods

In a randomized, double-blind, placebo controlled, bi-centric study 282 postmenopausal women with a mean age 73.6±4.7 years were recruited. 279 were included in the ITT analysis. Three patients were classified as non eligible as they withdrew consent prior to taking the study medication. All patients included suffered from low bone mass and were treated with 70 mg alendronate (ALN)(Fosamax® 70 mg tablets, MSD) once weekly and 500 mg calcium daily. These patients received, additionally, either alfacalcidol 1 µg (Bondiol® gelatin capsule, TEVA) or a placebo (PLC) daily. Further, they received medication for a continuous 36 month period. The mean baseline DXA-BMD spine (L1-L4) T-score was -2.40±0.87 SD (Alfa/ALN-group) or -2.40±0.91 SD (PLC/ALN group) (p=0.940). Trabecular and cortical density (in mg/cm^3) was measured at distal forearm and distal tibia with pQCT (XCT2000 Stratec, Pforzheim, Germany) at baseline, 3, 6, 9, 12, 18, 24, 30, and 36 months. Trabecular density of distal radius and tibia was measured at standardized regions, at 4% distal ulna/tibia length. Cortical density of the forearm was measured at midshaft (=66% of the ulna length) and of the tibia at 14%, 38% und 66% of the tibia length. Cortical cross sectional area was measured at 66% of radius and tibia length and at 38% of the tibia. Strength-Strain-Index (SSI) was measured at 66% of the radius and 38% and 66% of the tibia length. The method is described elsewhere[6]. DXA-BMD measurements of the spine (L1-L4) and proximal femur (Hologic QDR Delphi and Hologic QDR 1000W, Hologic Inc., Waltham, MA, USA) were performed at screening, 12, 24, and 36 months. 62 patients (21.3%) had been pre-treated with bisphosphonates before inclusion in the study (mean time 2.2 years, min. 2 months, max. 8.1 years). These patients had been randomized in both groups (28 in Alfa/ALN group and 34 in the PLC/ALN group).

Inclusion criteria: Written informed consent signed by subject, postmenopausal women ≥65 years of age, Caucasians, chair rising-test of more than 10sec and/or tandem standing less than 10 sec and/or tandem gait less than 8 steps, BMD (DXA, L-spine or total hip) of ≤-1SD T-Score, minimum of one fall in history of 5 years from date, and willingness/ability to adhere to the protocol.

Exclusion criteria: Patients with dementia, any subjects who were not able to perform the tests, patients, who had had more than one syncopal fall the previous year, neuromuscular diseases with known influence on locomotoric competence, stroke with locomotoric impairments, using 2 crutches/room frame constantly, neoplasm or other severe diseases with life expectancy less than one year or expectation of rapid worsening within one year, chronic inflammatory rheumatoid disease, arthritis with con-

tinuous pain and influence on locomotion, inflammatory or metabolic bone disease, excluding osteoporosis, subjects with anti-osteoporotic medications who were not willing to switch over to alendronate treatment, estrogen treatment can be continued, current treatment of active vitamin D, randomization performed after a four week treatment-free time interval, 25-OH-Vitamin D_3<12 ng/ml (12 ng/ml=30 nmol/L), current treatment with statins and increased serum value of CK (creatinine kinase), systemic corticosteroid treatments of more than one month within previous 12 months, intolerance to alfacalcidol, hypercalcaemia (>2,7 mmol/l), milk alkali syndrome, uncorrected and severe visual impairments, hypermagnesaemia (>1,03 mmol/l), creatinine >2.5 mg/dl (>220 µmol/L) (in case of higher values than defined by the regional laboratory as normal and below given limit, patients can be included but creatinine value has to be controlled during following visits), known alcohol or drug addiction or abuse (excessive alcohol means >100 g alcohol/day), known allergy/intolerance of alfacalcidol or alendronate and/or analogue.

Biochemical measurements

All subjects underwent laboratory blood tests. Calcium measurement occurred every 3 months. Intact parathyroid hormone (PTH) was measured at baseline and every 12 months. $1,25(OH)_2D_3$ was measured at baseline, 6, 12, 24, and 36 months. The remaining parameters were measured at baseline, 3, 6, 12, 18, 24, and 36 months. Serum samples were obtained between 8am and 11am after overnight fasting. The samples were processed immediately and then kept frozen at -80°C. The assays for the outcome biochemical measurements were carried out at the end of the study for all sampling dates to assure consistency of assay charges.

Biochemical parameter measured in serum for safety purposes: Aspartate-Aminotransferase (ASAT), Alanine-Aminotransferase (ALAT), blood count (BC), magnesium, creatinine kinase, creatinine. Ca total (accepted values for inclusion: 2.20-2.65 mmol/l, was measured by an automated clinical chemistry analyzer [Modular Analytics; Roche Diagnostics, Mannheim, Germany]). For monitoring the efficacy of the treatment: intact parathyroid hormone (iPTH, reference 11-43 ng/L; iPTH and 25-OH-Vitamin D measurements were performed by means of an automated electrochemiluminescence immunoassay [ECLIA; Modular Analytics E170, Roche Diagnostics, Penzberg, Germany]), 25 $(OH)D_3$ (reference 20-70 µg/L, see iPTH), 1,25 $(OH)_2D_3$ (reference 35-90 ng/L, measured with coated tube assay (RIA) [DIAsource ImmunoAssays S.A., Nivelles, Belgium]), bone specific alkaline phosphatase (BAP)(3-14 µg/L, measured by means of a paramagnetic particle chemiluminescent immunoassay [Access OSTASE; Access Immunoassay System, Beckman Coulter GmbH, Krefeld, Germany]), N-telopeptide of type I collagen (NTX) (reference values 6.2-19.0 nmol BCE, Osteomark, Wampole Lab, ELISA), insulin like growth factor 1 (IGF-1)(reference values are strongly age related, measured with solid-phase enzyme-labelled chemiluminescent immunoassay [Immulite 2000 IGF-I, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany]).

Statistical analysis

The primary analysis was done with the intent to treat population (ITT) with least observation carried forward. All covariates analysed were defined as secondary endpoints in the study protocol. All covariates were quantitative measurements and normal distribution could be confirmed for all measurements. Thus, for baseline comparisons the t-test for independent samples was applied. For intra-individual comparisons between visits the t-test for dependent samples was applied and for the primary analysis (baseline adjusted group comparisons), analysis of covariance was used. The level of significance was 0.05 (two-sided). With 279 subjects standardized differences of 0.34 (difference of means divided by the standard deviation) could be identified with power of 80%. According to the protocol, 22 secondary endpoints regarding DXA and pQCT measurements were defined. Thus, p-values of less than 0.05/22=0.002 were significant, controlling the experiment, while the error rate was measured with the Bonferroni method. However, due to the high correlation of measurements, this approach is rather conservative. Standardized differences of 0.48 could be detected using the Bonferroni correction. All analyses were done using SPSS for Windows 15.0.

Results

Baseline characteristics

Baseline characteristics are illustrated in Table 1. No significant differences were found between the groups at baseline measurements, apart from cortical density and cross sectional bone area at 38% of the tibia length.

According to the DXA-BMD 171 subjects were classified as osteopenic and 108 as osteoporotic patients.

The dropout rate after 36 months was 22.7% in the Alfa/ALN group and 24.8% in the PLC/ALN group, mainly influenced by the extension of the study after 12 months. Originally, the study was planned for 1 year only but, subsequently, was extended to three years without unblinding the study.

DXA-BMD of spine and proximal femur

Each spine DXA measurement was reviewed visually and compared with the corresponding x-ray of the lumbar spine (anteroposterior and lateral view, at visit 5 [12 months]) by an expert, concerning degenerative changes of the lumbar spine. Cases with spondylophytes with a degree of 3 or more according to the Kellgren classification were excluded from analysis. Consequently, 99 cases were excluded from DXA spine analysis. 89 and 91 spine DXA measurements were eligible in the Alfa/ALN and in the PLC/ALN group, respectively (Figure 1). On the other hand, 273 hip DXA measurements withstood the critical review (Figure 1).

DXA-BMD spine/hip

DXA-BMD of spine, measured every 12 months in L1-L4, increased in both groups, 6.65% ($p<0.0001$) in the Alfa/ALN group compared to baseline and 4.17% ($p<0.0001$) in the PLC/ALN group compared to baseline after 36 months (Figure 2). The dif-

Variable	All	Alfa/ALN	PLC/ALN	p-Value
	Mean±SD, N	Mean±SD, N	Mean±SD, N	
Age (years)	73.67±4.75, 279	73.76±5.01, 140	73.59±4.49, 139	0.749
Years since menopause	25.34±7.70, 279	25.96±8.09, 140	24.73±7.25, 139	0.182
Height (cm)	1.59±0.06, 279	1.59±0.067, 140	1,59±0.06, 139	0.515
Weight (kg)	68.98±11.32, 279	68.76±10.73, 140	69.2±11.91, 139	0.744
BMI (kg/m²)	27.19±4.13, 279	27.21±4.02, 140	27.18±4.25, 139	0.942
pQCT radius				
Trab dens (mg/cm³)	134.9±40.9, 235	134.5±42.6, 121	135.4±39.2, 114	0.875
Cortic dens 66% (mg/cm³)	1078.5±60.7, 233	1071.6±60.2, 112	1085.5±60.7, 111	0.089
CSA cortic radius 66% (cm²)	54.5±11.3, 223	53.4±11.1, 112	55.7±11.5, 111	0.122
SSI radius (66%)	220.0±45.3, 225	215.6±44.3, 113	224.4±45.9, 112	0.146
CSA muscle (cm²)	2424.5±310.1, 223	2433.0±315.2, 113	2415.7±305.9, 110	0.676
pQCT tibia				
Trab dens (mg/cm³)	182.2±36.1, 275	181.0± 7.6, 136	183.4±34.7, 139	0.573
Cortic dens 14% (mg/cm³)	1035.8±58.1, 264	1030.3±56.7, 132	1041.3±58.9, 132	0.124
Cortic dens 38% (mg/cm³)	1134.3±47.1, 270	1128.6±48.5, 134	1139.9±45.2, 136	**0.048**
Cortic dens 66% (mg/cm³)	1079.9±46.4, 269	1074.8±45.3, 135	1085.0±47.1, 134	0.072
SSI tibia (38%)	1347.8±217.2, 273	1326.6±206.2, 136	1368.9±226.3, 137	0.107
SSI tibia (66%)	1904.2±343.6, 273	1873.5±323.7, 138	1935.7±361.4, 135	0.135
CSA cortic tibia 38 % (cm²)	230.4±43.1, 270	225.7±32.6, 134	234.9±35.0, 136	0.027
CSA cortic tibia 66% (cm²)	230.2±42.1, 269	225.6±41.6, 135	234.8±42.3, 134	0.075
CSA muscle 66% calf (cm²)	5831.8±812.4, 190	5779.4±901.4, 99	5888.7±703.5, 91	0.355
DXA				
Spine BMC (L1-L4)(g)*	36.0±11.3, 180	36.3±11.5, 89	35.6±11.1, 91	0.652
Spine BMD (L1-L4)(g/cm²)*	0.79±0.11, 180	0.78±0.10, 89	0.79±0.12, 91	0.445
Spine T-Score	-2.4±0.89, 179	-2.4±0.87, 88	-2.4±0.91, 91	0.940
Femur total BMC (g/cm²)*	28.4±4.5, 273	28.4±4.4, 137	28.5±4.6, 136	0.867
Femur total BMD (g/cm²)*	0.77±0.10, 273	0.77±0.10, 137	0.77±0.11, 136	0.836
Femur T-Score	-1.43±0.9, 273	-1.40±0.85, 137	-1.40±0.90, 136	0.736

**cross calibrated values, CSA = cross sectional area, dens = density, cortic = cortical, trab = trabecular, L = lumbar, SSI = strength/strain –index.*

Table 1. Subjects' characteristics and results of the pQCT and DXA measurement at baseline (ITT-population). The bold numbers document the only statistical differences at baseline (cortical density tibia, CSA cortical tibia).

ference between the groups after three years was significant (p=0.026). In the first year the relative increase was 69.2% higher in the Alfa/ALN compared to PLC/ALN group (p=0.004). The total hip measurement documented after 36 months showed an increase of DXA-BMD in the Alfa/ALN group and PLC/ALN group of 2.33% and 1.59%, respectively. The difference between the groups was not significant (p=0.849).

BMD in trabecular and cortical bone of the radius and tibia was measured with pQCT

Radius measurements

After 36 months, the trabecular density increased in the Alfa/ALN group by 0.26%, in the PLC/ALN the radius trabecular density decreased by -0.13%, both not significant to baseline. After 3 years, the difference between the groups represented a trend (p=0.083).

After 36 months the cortical density in midshaft of the radius increased significantly by 6.0 mg/cm³ (+0.52%; p<0.0001) in the Alfa/ALN group and decreased by -1.84 mg/cm³ (-0.09%; p=0.435) in the PLC/ALN group. The difference between the groups was significant at the end of the study (p=0.010) (Figure 3).

Tibia measurements

Trabecular density at distal tibia significantly increased after 36 months of treatment in the Alfa/ALN group (0.69%; p=0.002) and decreased in the PLC/ALN group (-0.41%; p=0.068). At the end of the study the group difference was strongly significant (p=0.002; Figure 4).

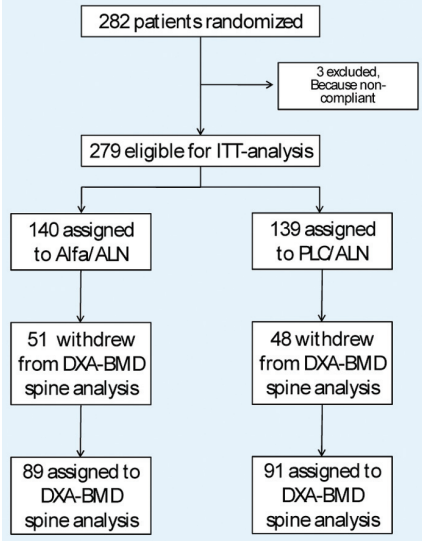

Figure 1. Selection pattern of DXA-BMD assessments of the lumbar spine because of degenerative patterns which influence evaluation of DXA-BMD spine measurements.

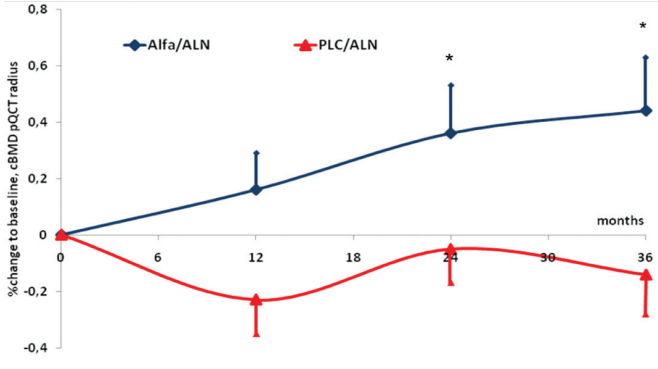

Figure 3. Changes (in %) of cortical BMD (mg/cm^3) in the radius from baseline after 12, 24, and 36 months of treatment with PLC/ALN or Alfa/ALN, measured with pQCT (XCT2000 Stratec, Pforzheim, Germany). At any time point (12, 24, and 36 months) the group differences were significant (p=0.024, 0.036, and 0.01 respectively). In the PLC/ALN group no significant changes to baseline at any time pointAt the end of the study the group differences were significant. In the Alfa/ALN group significant increases of the bone density after 24 and 36 months (*p=0.003 and <0.001 respectively).

Cortical density at 14% of the tibia length increased after 36 months in the Alfa/ALN group (0.27%; p=0.005). The cortical density decreased in the PLC/ALN group (-0.41%; p<0.001). The difference between the groups at the end of the study was significant (p<0.0001; Figure 5). Furthermore, cortical density increased in the Alfa/ALN in all measured regions significantly compared to the PLC/ALN group which mostly lost cortical density (Table 2).

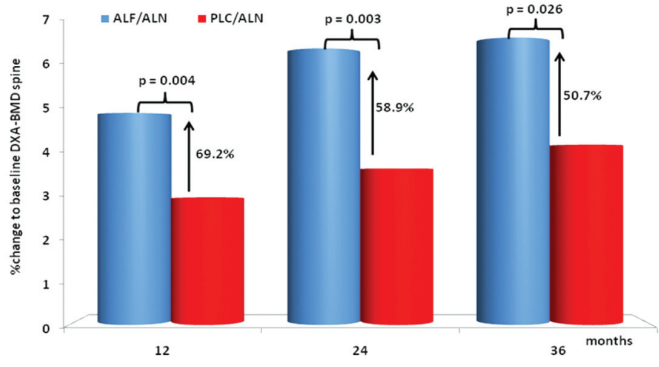

Figure 2. Changes (in %) of DXA-BMD (g/cm^2) in spine from baseline after 12, 24, and 36 months of treatment with PLC/ALN (red) or Alfa/ALN (blue). At any time point (12, 24, and 36 months) the group differences (p=0.004, 0.003, and 0.026 respectively) and differences to baseline were significant (p<0.001).

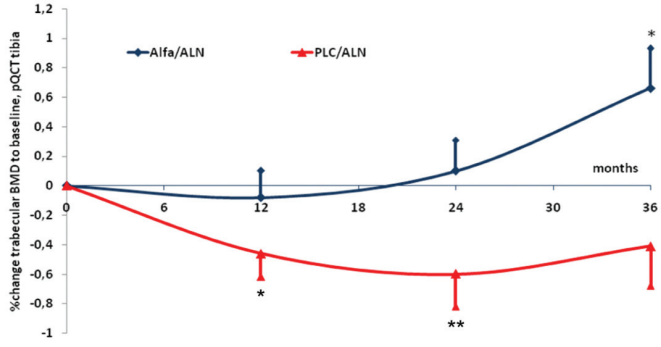

Figure 4. Changes (in %) of trabecular BMD (mg/cm^3) in the distal tibia from baseline after 12, 24, and 36 months of treatment with PLC/ALN or Alfa/ALN, measured with pQCT (Stratec XCT2000 Stratec, Pforzheim, Germany). After 24 and 36 months the group differences were significant (p=0.015 and 0.002 respectively). Significant changes to baseline *(p=0.002), **(p=0.001).

Cortical cross-sectional bone area (cCSA): cCSA was measured at 60% of radius length and 38% and 66% of the length of the tibia. After 36 months no significant changes of the cCSA at radius and no significant difference between the groups (p=0.343) was noted. Significant increase of the cCSA at the tibia at both measurement areas was recorded. cCSA at 38% tibia length increased by 0.17% (p=0.200) in the Alfa/ALN group and decreased by -0.41% (p=0.015) in the PLC/ALN group after 36 months. The group difference at the end of the study was significant (p=0.006). cCSA at 66% tibia length increased by 0.40% (p=0.015) in the Alfa/ALN group and decreased by -0.37% (p=0.060) in the PLC/ALN group after 36 months. The group difference at the end of the study was significant (p=0.022).

ROI pQCT measurement	Alfa/ALN [in mg HA/cm³]	PLC/ALN [in mg HA/cm³]	P value
cBMD radius midshaft	+4.65	-1.59	0.010
cBMD tibia 14% tibia length	+2.46	-4.36	<0.001
cBMD tibia 38% tibia length	+5.10	+1.74	0.017
cBMD tibia 66% tibia length	+4.77	+2.25	0.043
cBMD = cortical bone mineral density, ROI = region of interest, HA = hydroxyapatite.			

Table 2. Changes of cortical BMD in radius and tibia in mg HA/cm³ between baseline and after 3 years of treatment (ITT population, p-values reflect the significance between the groups after 3 years).

	Alfa/ALN (N=140)				PLC/ALN (N=139)			
	baseline	**12 mo**	**24 mo**	**36 mo**	**baseline**	**12 mo**	**24 mo**	**36 mo**
BAP [U/l]	11.85	7.94	8.23	8.35	11.22	9.29	9.50	9.47
±SD	*4.78*	*2.82*	*2.80*	*3.11*	*5.44*	*3.56*	*3.95*	*3.95*
NTX [nmol/l]	15.53	9.92	11.55	10.77	15.11	10.72	12.83	11.32
±SD	*4.11*	*4.13*	*3.84*	*3.25*	*4.98*	*3.84*	*4.75*	*4.12*
25(OH) D[µg/l]	28.03	23.10	21.90	20.51	27.17	22.75	22.47	20.96
±SD	*9.78*	*6.52*	*6.49*	*6.45*	*8.33*	*6.07*	*5.96*	*6.13*
1.25(OH)$_2$D[ng/l]	54.96	55.05	50.82	49.59	54.34	49.33	45.13	44.41
±SD	*15.67*	*14.36*	*12.62*	*13.04*	*16.37*	*14.14*	*12.71*	*13.81*
PTH[ng/l]	43.41	37.39	39.00	41.78	40.15	48.89	53.72	57.59
±SD	*18.33*	*29.54*	*29.79*	*30.50*	*15.81*	*18.98*	*22.80*	*25.56*
IGF 1[µg/l]	96.92	100.81	101.17	96.75	93.63	95.90	97.88	93.40
±SD	*30.42*	*32.37*	*32.73*	*28.53*	*35.14*	*30.71*	*31.48*	*33.67*
Ca[mmol/l]	2.41	2.47	2.47	2.40	2.40	2.40	2.39	2.34
±SD	*.10*	*.13*	*.13*	*.15*	*.11*	*.09*	*.09*	*.10*
mo = months, SD = standard deviation.								

Table 3. Biochemical parameter at baseline, and after 12, 24 and 36 months. Assessed in postmenopausal women with osteopenia/osteoporosis. All group differences have been significant after 3 years compared to baseline, except NTX, 25(OH) D_3, and IGF.

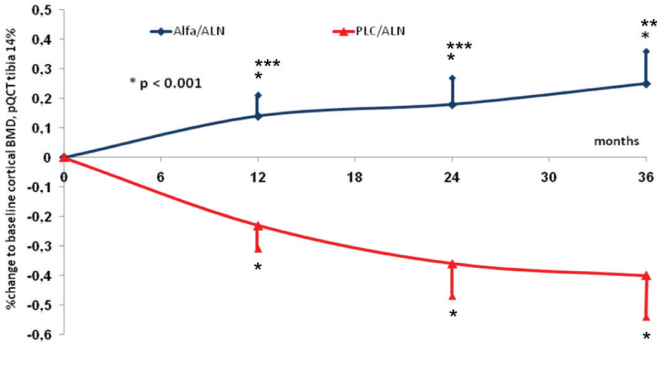

Figure 5. Changes (in %) of cortical BMD (mg/cm) in the tibia at 14% length of the tibia from baseline after 12, 24, and 36 months of treatment with PLC/ALN or Alfa/ALN,measured with pQCT (Stratec XCT2000, Pforzheim, Germany). *At any time point (12, 24, and 36 months) the group differences and differences to baseline were significant (p<0.001). ** The difference to baseline was significant after 36 months (p<0.005). ***The differences to baseline were significant (p<0.05).

Strength Strain Index (SSI) with pQCT

The strength strain index reflects the strength of the bone. Forearm measurements achieved 1.26% (n.s.) increase of SSI in the Alfa/ALN group, and 0.35% (n.s.) in the PLC/ALN group compared to baseline after 36 months. No significant difference between the groups (p=0.278) at the end of the study was recorded.

After 36 months the SSI increased significantly by 1.10% (p<0.0001) at 38% of the tibia length in the Alfa/ALN group and by 0.17% (p=0.362) in the PLC/ALN group. At the end of the study the difference between the groups was statistically significant (p=0.001).

Biochemical measurements

At baseline, 8 patients had slight hypocalcaemia, which was balanced at the time of the recruitment (reference range 2.20-2.65 mmol/L), 6 subjects were in the PLC/ALN group and 2 in the Alfa/ALN group. After 3 years the PLC/ALN group showed 7 subjects with slightly low serum calcium levels

Correlation with study drug	ALFA plus ALN	Placebo plus ALN	Total
None	820 (71.4%)	833 (76.8%)	1653 (74.0%)
Unlikely	316 (27.5%)	242 (22.3%)	558 (25.0%)
Possible	8 (0.7%)	9 (0.8%)	17 (0.8%)
Probable	5 (0.4%)	0 (0%)	5 (0.2%)
Correlation with concomitant medication (Alendronate, calcium)	**ALFA plus ALN**	**Placebo plus ALN**	**Total**
None	786 (68.4%)	795 (73.3%)	1581 (70.8%)
Unlikely	289 (25.2%)	226 (20.8%)	515 (23.1%)
Possible	52 (4.5%)	44 (4.1%)	96 (4.3%)
Probable	19 (1.7%)	18 (1.7%)	37 (1.7%)
Definite	3 (0.3%)	1 (0.1%)	4 (0.2%)

Table 4. Evaluation of adverse events in both groups, related or not to the study medication or concomitant medication (no significant difference between the groups).

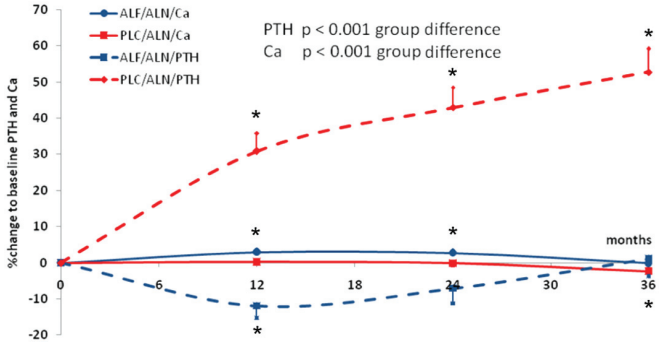

Figure 6. Changes (in %) of serum calcium (contiuous lines) and PTH (dashed lines) in Alfa/ALN group (blue) and PLC/ALN group (red) during the study period. At any time point (12, 24, and 36 months) the group differences of calcium and PTH were significant (p<0.001). Compared to baseline calcium increased in the Alfa/ALN group after 12 and 24 months; no significant difference after 36 months. In the PLC/ALN group calcium did not change significantly after 12 and 24 months treatment, but decreased significantly (p<0.001) after 36 months. PTH increased significantly (p<0.001) at any time point in the PLC/ALN group and decreased significantly (p<0.001) after 12 months of treatment in the Alfa/ALN group. No significant changes in the latter group after 24 and 36 months.

whereas the Alfa/ALN group showed 3 subjects.

Whilst calcium serum level increased in the Alfa/ALN group in the first 24 months, PTH decreased concurrently. After 36 months, the calcium level was non-significantly decreased (-0.29%; p=0.589) in the Alfa/ALN group. In the PLC/ALN group, calcium serum level remained unchanged in the first year but decreased in years 2 and 3 (-3.84%; p<0.0001). The difference between the groups was highly significant at the end of the study (p<0.0001). Consequently, the PTH level decreased marginally in the Alfa/ALN group (-2.31%; p=0.573) after 3 years and increased significantly in the PLC/ALN group (48.80%; p<0.0001). The group difference at the end of the study was significant (p<0.0001) (Figure 6). At baseline, 17 subjects showed a slight hyperparathyroidism in the Alfa/ALN group as well as 14 subjects in the PLC/ALN group. After 36 months of treatment with alendronate + 500 mg calcium, 34 subjects exhibited a slight to moderate hyperparathyroidism whereas in the Alfa/ALN group only 6 subjects showed a slightly increased PTH level.

Bone specific alkaline phosphatase (BAP) was suppressed by 35.1% after one year in the Alfa/ALN group and 16.6% in the PLC/ALN group. BAP remained suppressed after 3 years by -23.81% (p<0.0001) compared to baseline in the Alfa/ALN group and by -8.68% (p=0.002) in the PLC/ALN group as well. The group difference was significant (p<0.004) at the end of the study (Table 3).

The strongest decline of serum NTX compared to baseline was -31.77% (p<0.001) in the Alfa/ALN group and -22.62% (p<0.001) in the PLC/ALN group measured after 12 months (Table 3). There was no significant difference between the groups at the end of the study for NTX.

25(OH)D_3 serum level significantly declined in both groups by 25-30% with no significant group difference (Table 3). 1,25$(OH)_2D_3$ increased slightly in the first year and decreased in the third year by -3.80% (p=0.211), in the Alfa/ALN group. In the PLC/ALN group a significant decrease of -12.93% (p<0.0001) after 3 years was measured. The decrease in the PLC/ALN group started in the first year and continued for the duration of 3 years. At the end of the study the group difference was significant (p=0-001).

After three years no change of IGF 1 to baseline and no changes between the groups were found (Table 3).

Evaluation of adverse events (AEs) and serious adverse events (SAEs)

Total number of AEs: 1149 in the Alfa/ALN group and 1084 in the PLC/ALN group (Table 4) and total number of SAEs:

Correlation with study drug	ALFA plus ALN	Placebo plus ALN	Total
None	66 (72.5%)	89 (78.1%)	155 (75.6%)
Unlikely	24 (26.4%)	24 (21.1%)	48 (23.4%)
Possible	1 (1.1%)	1 (0.9%)	2 (1.0%)
Correlation with Alendronate	**ALFA plus ALN**	**Placebo plus ALN**	**Total**
None	64 (70.3%)	87 (76.3%)	151 (73.7%)
Unlikely	26 (28.6%)	25 (21.9%)	51 (24.9%)
Possible	1 (1.1%)	2 (1.8%)	3 (1.5%)

Table 5. Evaluation of serious adverse events (SAEs) in both groups, related or not to the study medication or concomitant medication (no significant difference between the groups).

91 in the Alfa/ALN group and 114 in the PLC/ALN group (Table 5). There was no significant difference between groups for incidence of AEs or SAEs.

An additional analysis of all parameters was performed to examine whether patients with and without prior bisphosphonates treatment influenced the results: this was not the case (data not shown). Due to the excellent recruitment and performance of the study, a study extension was organised in advance during the first year, without unblinding the patients and investigators. The study was extended from one to three years treatment.

Discussion

In the pivotal interventional studies of bisphosphonate treatment, DXA measurements were conducted to quantify bone mass. DXA measures the mass of bone, yet the mass of a given material alone cannot determine the strength of a structure. The validity of using changes in DXA-BMD to infer the effect of antiresorptive treatment reducing vertebral fracture risk has been a topic of many debates over the last decade[16,22,25,28]. Meta-analyses accomplished by several groups in an attempt to resolve this issue have generated conflicting results. Some investigators concluded that larger increases in BMD in women treated with antiresorptive agents are associated with a lower risk of new vertebral fractures[15,25,52], whereas others concluded that greater increases in BMD do not predict greater decline in vertebral fracture risk[2,28,40,41,53,54]. Even more difficult and controversial is the discussion about the effect of peripheral fractures and BMD change[55].

Material property, distribution of mass, architecture and geometry are the determinants of strength. So far we have no data whether different compounds have a more specific influence on different bone compartments. The results of the current study give some answers about additional effects of a combination of two antiresorptive drugs on bone mineral density in different parts of the skeletal system.

In the current study, DXA-BMD of the spine significantly increased in both treatment groups, PLC/ALN and Alfa/ALN, compared to baseline with a significant advantage of the Alfa/ALN over the PLC/ALN group. Compared to other studies[39], where alendronate 70mg once-weekly with 1000 mg calcium and 400 IU Vitamin D daily was administered to postmenopausal osteoporotic women, the increase of spine DXA-BMD was 3.7% after 12 months whereas in the current study it was 2.92% in the PLC/ALN group. Although we have to consider some differences in the subject populations (non-osteopenic patients in the prior work), these effects are comparable to those of the current study. In the Alfa/ALN group the spine DXA-BMD increased by 4.91% after 12 months. In one of the alendronate pivotal studies with postmenopausal women without prevalent fractures and low DXA-BMD (which is similar to our population) with an alendronate admission of 5/10 mg daily study, 82% of the participants were supplemented with 500mg calcium and 250 IU Vitamin D daily[14]. The BMD increase in the first 12 months of this study was about 4.2% compared to baseline, which is slightly lower than in the Alfa/ALN group of our actual study.

Barone et al[1] studied the effect of ALN 70 mg weekly alone versus ALN 70 mg weekly plus 0.5 µg calcitriol daily ($1,25(OH)_2D_3$) on DXA-BMD in postmenopausal women with vitamin D insufficiency, secondary hyperparathyroidism, and a DXA-BMD of the spine or total hip or femoral neck <- 2.5SD T-score. In this study, the increase of DXA-BMD of the spine was 3.7% in the ALN alone group and 6.5% in the ALN + calcitriol group after 12 months of treatment, with the effect between the two groups being statistically significant. Compared to our current results, the effect of the treatment in this prior work was slightly higher (2.92% and 4.91% respectively), which can be explained by some differences in the subject populations. In total hip DXA-measurements neither in our study nor in the cited study was a significant difference between the groups found. Nonetheless, the comparability of these studies is limited because, in the present study, only women with normal vitamin D level at baseline were included.

The pQCT data of the current study provide more insight into the effects of the interventions. The cortical bone density at the radius of the Alfa/ALN group increased significantly (after 3 years +0.44%) whereas the trabecular bone density did not change significantly at the distal radius, although an effect was seen at the distal tibia (Alfa/ALN group +0.66%). Com-

pared to baseline, the cortical density in the Alfa/ALN group increased in all regions as well as the cross sectional cortical area of the tibia (at 38% region). Consequently, the tibial strength-strain-index (SSI) in the Alfa/ALN group was increasing significantly compared to baseline. The group differences were significant after 3 years, but not after 1 year of treatment by an increase of 0.45% (Alfa/ALN group) and a decrease of -0.18% in the PLC/ALN group (after 3y, +0.95% in favor of Alfa/ALN group, p=0.001). Schneider et al[44] published data pointing out a significant increase of SSI by 6.8% compared to baseline in a subgroup of postmenopausal women treated with alendronate 10 mg daily + 500 mg calcium in the FOSIT-study (possibly comparable with our PLC/ALN group) and a loss of -2.6% in the placebo group (calcium 500 mg/d only) (p=0.037 between the groups). However, in the FOSIT-study the measurement was performed in the ultra-distal radius where the cortical bone is substantially thinner than at the 38% region. Nevertheless, this was the first Phase-III study in which the bone strength index was analyzed, but the results show different tendencies.

Very few clinical studies have used pQCT to examine the change of cortical and trabecular bone density separately[44]. Therefore, our knowledge of the detailed modification of BMD at the cortex elicited by an antiresorptive treatment is limited. The increase of the cortical BMD is mostly caused by higher mineralization and diminished of cortical porosity in patients treated with alendronate. This assumption is derived from studies of Roschger et al[38] who published data about bone mineralization density distribution (BMDD) of iliac crest biopsies of postmenopausal women treated with alendronate or placebo. BMDD was measured with quantitative backscattered electron imaging (qBEI)[37]. The authors found a more uniform mineral distribution after alendronate treatment compared to the placebo group. Scanning small-angle X-ray scattering (SAXS) examinations illustrated that the crystal size did not increase after alendronate treatment. The increase of mineralization is associated with an increase of stiffness of the bone and this can partially explain the reduction of fracture incidence due to bisphosphonate treatment. In the current study, Alfa/ALN increased cortical density and thickness significantly in comparison to PLC/ALN. This explains the increase of bone strength (SSI). Potentially, alfacalcidol directly impacts mineralization of osteoid. These effects are more pronounced in the cortical bone which may have an important influence on fracture risk.

To summarise the alfacalcidol/alendronate effects on bone structure and material property: reduced bone resorption and smaller deterioration of trabecular structure (structural property) associated with increase of mineralization and with advanced bone stiffness (material property). These effects of alendronate treatment are signifcantly enhanced by concomitant alfacalcidol administration.

Serum level of NTX was not significantly different at baseline and after three years of treatment between the two groups. Appraising the absolute values at baseline, the NTX concentration in both groups was normal. According to the reference values (12.9-22.7 nmol BCE) in postmenopausal women, this would characterize a normal postmenopausal bone turnover, although 21.3% of the patients had been treated with bisphosphonates before randomization. The level of suppression of this resorption marker in comparison to baseline value at the end of 3 years was 18.8% in the PLC/ALN group and 26.1% in the Alfa/ALN group. Compared to other studies with alendronate treatment, suppression of bone resorption was quite low in the present study[27,39].

The bone specific alkaline phosphatase (BAP) was significantly more suppressed in the Alfa/ALN group (24.5%) compared to PLC/ALN group, with only 7.7% after 36 months of treatment compared to baseline. The combination of these two antiresorptive compounds seems to be more effective than ALN alone. The marginal decrease of BAP in the PLC/ALN group can be explained by the low postmenopausal turnover as well (reference of healthy postmenopausal women 25.0±5 µg/L, in the current study at baseline 11.2±5.4 µg/l).

The definition of normal serum Ca-level by the attending central laboratory was 2.20-2.65 mmol/L. According to these references six women in the PLC/ALN and two in the Alfa/ALN group have been included with a Ca-level below the reference value and none with Ca-levels beyond the reference values in the PLC/ALN group and one (2.66 nmol/L) in the Alfa/ALN group. These values have no clinical relevance. At the end of the study after 3 years of therapy we identified seven women in the PLC/ALN and six in the Alfa/ALN group with a Ca-level below the reference value and none with Ca-levels beyond the reference values in the PLC/ALN group and one (2.77 nmol/L) in the Alfa/ALN group. In the follow-up period very few subjects showed borderline values beyond the upper reference. In none of these cases was any intervention necessary.

Hypercalcaemia was seen under the treatment with alfacalcidol and calcitriol in studies without bisphosphonate treatment. In the present study hypercalcaemia posed no problem because all patients were treated with alendronate having a negative effect on the calcium balance. Similar results were obtained in other studies[21,31]. The addition of alfacalcidol counteracted the decrease of calcium serum level as a consequence of alendronate treatment.

Independent of hypo- or hypercalcaemia, the Ca-level changed according to the influence of alfacalcidol and intestinal Ca-resorption. In the Alfa/ALN group serum Ca-level increased in the first two years and decreased in the third year (-0.18% compared to baseline; group difference was significant p<0.001). Consequently, the PTH-level decreased in the first two years (maximal by -12.0%) and slightly increased in the third year (+1.07%, significant group difference). The serum Ca decline in the third year is not clearly understood. Maybe this is the result of a reduced patient compliance or some other unknown mechanism. In the PLC/ALN group the serum Ca-level decreased by -2.35% at the end of the study and, consequently, PTH increased by 52.72% (group difference p<0.001). This significant increase of serum PTH level in the PLC/ALN group is perhaps responsible for the occasionally observed de-

crease of the bone mineral density, especially in the cortical bone. The question is: how can an ion regulate cellular function, other than through actions on ion channels, or membrane potential? This works via calcium sensing receptor (CaSR) measuring the extracellular Ca-concentration in the parathyroid chief cells as well as other cells involved in calcium homeostasis, particular the kidney[12]. Changes of calcium concentration enhance or decrease PTH secretion.

A synergy with alendronate in reducing fractures may lie in the pleiotropic effects of alfacalcidol on bone remodeling, on the musculoskeletal, endocrinological, immunological and neurological systems[1,42,43]. Alfacalcidol has been demonstrated in preclinical trials (OVX rats), and in combination with alendronate to restore a better osteoblastic/osteoclastic balance and, thus, increase bone strength[26]. The reduced response of alendronate in patients with increased PTH serum levels was restored by a combination with calcitriol[1]. A positive correlation was found between femoral muscle power and function, falls and 1.25 $(OH)_2D_3$ serum levels in the elderly[7,17,18].

Conclusion

The current work is the first study which focused on additional effects of alfacalcidol and alendronate on BMD, bone strength, and the effect on cortical bone density. Combined administration of alendronate (once weekly in the morning) with alfacalcidol (daily in the evening) increases the efficiency of the osteoporosis treatment. Tibia trabecular density, tibia cortical density and the strength of the tibia increased compared to PLC/ALN treatment with these effects being statistically significant. In the forearm cortical density increased as well. The antiresorptive effect of treatment was mainly explained by preventing secondary hyperparathyroidism. Both cortical mineralization and cortical thickness increased with the addition of alfacalcidol. Hypercalcaemia did not occur in the combination of alfacalcidol and alendronate.

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