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ORIGINAL ARTICLE

Incidence and bone biopsy findings of atypical femoral fractures

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Abstract Bisphosphonates are widely used in the treatment of osteoporosis. It has been suggested that bisphosphonate treatment may be associated with atypical femoral fractures (AFFs), severely suppressed bone turnover rate, and decreased mineralization. We studied bone properties using bone quantitative histomorphometry and Fourier transform infrared spectroscopic imaging (FTIRI) on patients with AFFs. Further, the incidence of AFFs was estimated. Patient records of Kuopio University Hospital, Finland from January 2007 to June 2009 were reviewed to identify all patients who had sustained and had been operated for AFF ($n = 8$). The incidence of AFFs among patients on bisphosphonates was 0.61 fractures/1,000 patients per year, compared to 0.0067/1,000 per year among untreated patients. The patients that underwent bone biopsy ($n = 4$) were postmenopausal women (aged 55.5–81.1 years) who had been treated with bisphosphonates for over 4 years. Histomorphometry revealed low trabecular bone volume. Bone formation and resorption parameters tended to be low. Trabecular bone single labels were detected in one patient in the region of interest. In the extended label search, trabecular bone double labels were found in two patients. Based on FTIRI results, higher phosphate-to-amide I ratio and collagen maturity were found compared to normal samples. The heterogeneity of phosphate-to-amide I ratio was low. Overall incidence of atypical femoral fractures is low. The poor fracture resistance in some patients on long-term bisphosphonate-therapy could be explained by low bone formation, and changes in bone composition, i.e., higher degree of mineralization, increased collagen maturity, and decreased heterogeneity of the degree of mineralization.

Keywords Bisphosphonates · Bone histomorphometry · FTIRI · Trabecular bone · Iliac crest

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Introduction

Antiresorptive drugs, i.e., bisphosphonates, are widely used in the prevention and treatment of osteoporosis [1–4]. Zoledronic acid has been shown to reduce the risk of vertebral fractures by 70 % and the risk of hip fractures by 41 % in postmenopausal women [5]. In recent years, there have been reports of subtrochanteric femur fractures with atypical fracture morphologies characterized radiographically occurring during osteoporosis therapy with bisphosphonates [6–10]. However, the incidence of atypical

femoral fractures (AFFs) seems to remain very low. The incidence among a Swedish population of patients on bisphosphonate therapy was estimated to be less than 1/1,000 [11] whereas another study found the combined rate of subtrochanteric or diaphyseal femoral fractures to be 2.3 per 10,000 patient-years [12]. Specific criteria for AFFs have been defined by the American Society for Bone and Mineral Research (ASBMR) Task Force Report [6].

A severely suppressed bone turnover rate has been reported among a few patients with AFFs both in the iliac crest and at the fracture site by bone histomorphometry [6, 10]. However, severely suppressed bone turnover is not specific to patients with AFFs but has been reported even without bisphosphonate exposure in postmenopausal women without a history of fracture [13, 14]. Fourier transform infrared spectroscopic imaging (FTIRI) can be used to study the composition of bone [15]. Lower heterogeneity, i.e., more narrow distribution of collagen maturity and crystal size/perfection, has been observed at the fracture site in women ($n = 20$) who have sustained typical ($n = 14$) or atypical ($n = 6$) proximal femoral fracture after long-term bisphosphonate treatment compared with non-treated controls ($n = 20$) [16].

We wanted to know whether the incidence of AFFs in our hospital's catchment area would differ from the estimates by others. Moreover, as presented by the ASBMR Task Force Report, there is a need for bone histomorphometric data from patients with AFFs [6]. Therefore, we studied bone remodeling in the iliac crest bone biopsies using bone histomorphometry, and the composition of bone using FTIRI on four patients who had suffered AFF(s).

Materials and methods

Patients

All femoral fractures with the diagnosis code S72.2, S72.3 or S72.4 (ICD10 clinical coding system), or patients aged >49 years with fractures treated with a femoral intramedullary nail, were identified from Kuopio University Hospital Patient Discharge Registry (Fig. 1). Patient records and X-rays of all identified patients were reviewed. Kuopio University Hospital takes care of all surgically treated trauma patients in its catchment area of 248,000

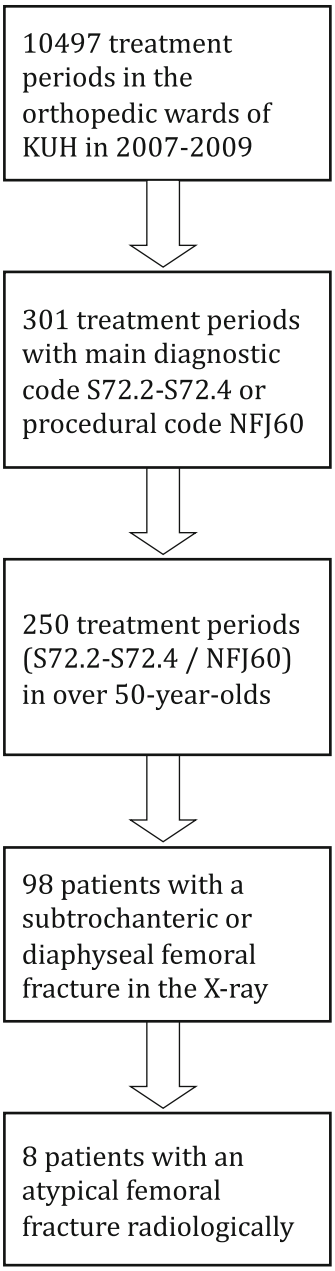

Fig. 1 The identification of patients with atypical femoral fracture. ▶ All femoral fractures, or patients aged >49 years with fractures treated with a femoral intramedullary nail (NOMESCO procedural code NFJ60), were identified from Kuopio University Hospital (KUH) Patient Discharge Registry. All pre-operative radiographs were reviewed

A femoral diaphyseal fracture was defined as a fracture between 5 cm distal to the lesser trochanter and the junction of the middle and distal third of the femur. The patients who met the major criteria for AFF defined by ASBMR were identified (Shane *et al.*. 2010) including one patient who had fallen from 0.5m high stack of wood. Fractures caused by underlying malignancy were excluded. The prevalence data on continuous treatment with bisphosphonate medication (ATC codes M05BA: Bisphosphonates and M05BB: Bisphosphonates, combinations) in the catchment area of KUH was provided by the Finnish Social Insurance Institution's prescription database (Furu *et al*. 2010). The database covers all dispensed prescription medication in Finland. Statistics Finland, a public authority specifically established to produce statistics, provided the population data for the area of interest. All clinical data were acquired from medical records with information on co-morbidities, duration of bisphosphonate treatment, surgical treatment of fracture, and outcome.

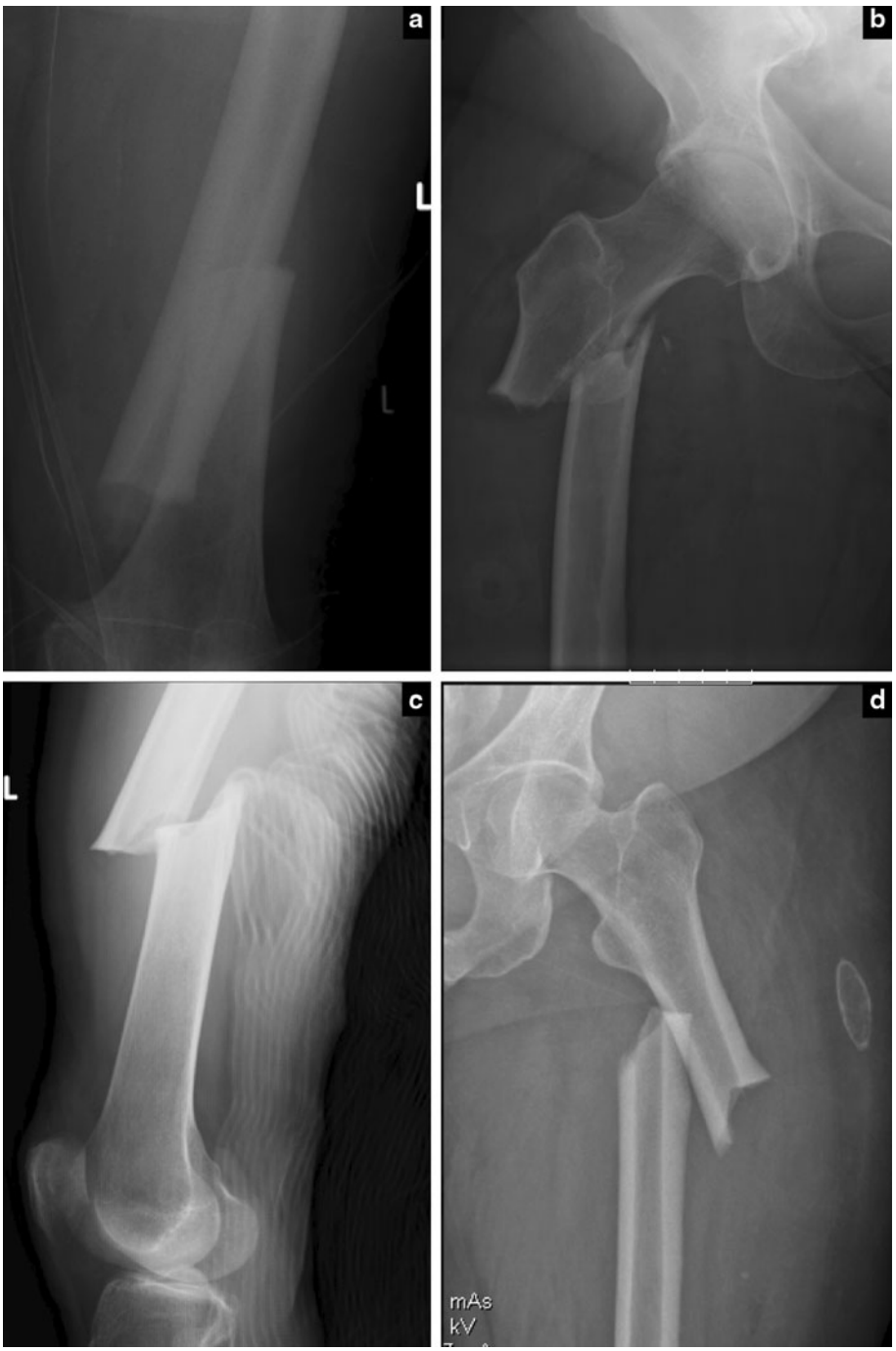

Fig. 2 Key features of atypical femoral fractures in two patients (**a**–**d**). **a** Patient #1, anterior-posterior view; **b** Patient #2, anterior-posterior view; **c** Patient #3, lateral view; **d** Patient #4, anterior-posterior view. Fractures were classified according to the major criteria defined by American Society for Bone and Mineral Research (ASBMR Task Force Report, JBMR 2010)

inhabitants in Eastern Finland. The reviewed time period extended from January 2007 to December 2009. The AFF was identified in radiographs according to radiologic criteria defined by ASBMR (Fig. 2) [6]. All patients were contacted via telephone to verify the data. The patient information is given in Table 1. Register data from Statistics Finland and the Social Security Institute's pharmacologic database were used to know the exact population and amount of bisphosphonate users in our hospital's catchment area during the study period.

The records of the patients that underwent bone histomorphometric evaluation at the Kuopio University Hospital, Kuopio, Finland due to an AFF were identified at the same time (n = 7). Three of these samples had to be excluded from our report (1 malignancy, 1 cortical bone sample, 1 with long time, i.e., 3.8 years from AFF to biopsy). Thus, findings from the remaining patients ($n = 4$) are reported (Table 2). Iliac crest bone biopsies (diameter 7.5 mm, height 10–20 mm) were taken from a standardized site located 2 cm below the anterior superior iliac spine

Table 1 Postmenopausal women with atypical femoral fractures. Bone biopsies were collected from patients #1–4

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6	Patient #7	Patient #8
Age at the time of the presentation (years)	81.1	66.7	66.1	55.5	89.9	58.3	79.3	72.4
Sex	F	F	F	F	F	M	F	F
OP therapy and duration before atypical fracture[a]	Alendronate for 4 years	Alendronate for 6 years	Alendronate for 5 years	Ibandronate latest, BPs for over 10 years	No BPs	No BPs	Alendronate for 10 years	Alendronate for over 5 years
Prior fracture history	No	No	Ankle fracture ×2	Car accident in 1988 (pelvic fracture, bilateral diaphyseal femur fractures)	No	No	No	No
Documentation of delayed healing	No	No	No	Yes	No	Yes	No	No

F female, *M* male, *OP* osteoporosis, *BP* bisphosphonate

[a] The dose of alendronate was 10 mg/day until 2008, which was then changed to weekly dose of 70 mg. The dose of ibandronate was 150 mg monthly

using Rochester bone biopsy trephine. All patients had undergone fluorochrome double labeling by receiving tetracycline 1,500 mg/day in two separate 2-day courses before the biopsy was taken. The interlabel time was 10 days. The samples were fixed in 70 % ethanol for at least 48 h before being embedded in polymethylmetacrylate (PMMA). The study was approved by the local healthcare authorities and the local ethics committee (Research Ethics Board, Kuopio University Hospital, permission 57/2007).

Bone histomorphometry

Bone histomorphometry (Bioquant OsteoII®, Bioquant Image Analysis Corporation, Nashville, TN, USA) was performed on 5 μm thick sections stained with modified Masson Goldner trichrome stain by bright light microscopy. Unstained sections (thickness 5 μm) were used for polarized light and fluorescence microscopy. For each sample, two sections were randomly selected and analyzed with bone histomorphometry. Regions of interest (ROI) were selected to ensure that only trabecular bone was measured, i.e., no cortical or subcortical bone. The tissue area ranged from 14.0 to 25.4 mm^2 depending on the size of the sample. All samples were measured by two microscopists (I.T. and X.T.) using magnification of 200×. The mean values of two observers for each parameter are reported except for the tissue value that is presented as a sum. The nomenclature, abbreviations, and units follow the recommendations by the American Society for Bone and Mineral Research (ASBMR) [17]. Reference values for postmenopausal women were obtained from Recker et al. [18] (mean ± standard deviation) for comparisons. Because of scarce labeling in ROI, a total of four unstained sections were analysed under fluorescence microscopy to further study fluorochrome labeling [19].

Fourier transform infrared spectroscopic imaging (FTIRI)

For FTIRI analysis, 3 μm sections were cut with a microtome (Polycut S®, Reichert-Jung, Germany). The samples were placed on ZnSe windows, and measurements were conducted with a PerkinElmer instrument in transmission mode (Perkin Elmer Spotlight 300®, Waltham, MA, USA). A spatial resolution of 6.25 μm, a spectral resolution of 4 cm^{-1}, and 8 repeated scans were used for data collection. The absorption spectra were recorded between 2,000 and 800 cm^{-1} wavenumbers. The background was corrected by measuring the spectrum from a clean site of the window with the same measurement parameters, but using 75 repeated scans. Based on the bright light microscope image, three to five trabeculae per sample were selected and imaged. The PMMA spectrum was subtracted from every bone spectrum to account for the differences in PMMA penetration into the trabeculae as described previously [15, 20]. Age- and sex matched normal samples ($n = 4$, aged 42–75 years, all females), which were part of our previous study, were selected for comparison [21] together with two additional samples ($n = 2$, aged 51–58 years, both females). Altogether six samples were used for comparisons ($n = 6$, aged 42–75 years, mean age

Table 2 Postmenopausal women with atypical femoral fractures. Bone biopsies were collected from patients #1–4

	Patient #1	Patient #2	Patient #3	Patient #4
Age (years)	81.1	66.7	66.1	55.5
Trauma	(i) Fell down in the forest from a standing position in 2006 (ii) In May 2009, felt sudden pain in thigh and fell down	Felt weakness in her right leg while getting up from a chair, fell down on her right hip, and was unable to walk after the trauma	Fell down from a stack of firewood, 0.5 m high, hurting her left thigh in June 2009	(i) A femoral fracture in 2006 (ii) In September 2009, the patient in post-operative care at the surgery ward. Turned over in her bed at night, and experienced extreme pain in left thigh. A radiograph revealed a fracture (iii) In August 2011, a re-fracture after a low-energy fall indoors
Fracture site	(i) Diaphyseal, right side (ii) Diaphyseal, left side	Subtrochanteric fracture, right side	Diaphyseal, left side	(i) Subtrochanteric, right side (ii) Diaphyseal, left side (iii) Right side
Treatment of fracture	(i) Intramedullary nail (ii) Intramedullary nail	Cephalomedullary nail	Intramedullary nailing	(i) Cephalomedullary nail (ii) Intramedullary nail
Healing of fracture	(i) Without complications (ii) Callus formation 3 months after fracture		Callus formation on control radiograph 3 months later	(i) A postoperative infection, a delay in the fracture union. Re-nailing in December 2006 (ii) Delayed healing of femoral fracture and a progressing angulation of the fracture (11°). Re-medullofixation with autogenous bone transplantation in August 2010 (iii) In December 2011 fracture healing had progressed as expected
BP and discontinuation of BP	Alendronate in treatment of suspected osteoporosis since 2005. Discontinuation at time of fracture in 2009. Calcium and vitamin D supplementations were continued in treatment of osteoporosis	Alendronate for 6 years prior to fracture. Discontinuation at the time of fracture. Calcium and vitamin D supplementation were started after the fracture	Alendronate for 5 years. Discontinuation at the time of the fracture	BPs for over ten years, ibandronate the most recent. Discontinuation at the time of the fracture in 2009
Concomitant diseases	Hypertension, osteoporosis, and glaucoma	Coronary artery disease, asthma, and osteoporosis	Otherwise healthy	Migraine, asthma, oesophagitis, and depression. In 1988, severely injured in a car accident, and sustained a pelvic fracture and femoral fractures on both sides. Immobilization-induced osteoporosis developed
Medication	Diltiazem, bimatoprost eye drops, and amiloride/hydrochlorothiazide when required	Amiloride/hydrochlorothiazide, fluticasone/salmeterol	N/A	Data unavailable
Bone biopsy	An iliac crest bone biopsy during operation in 2009. A tetracycline-labelled biopsy 4.9 months later[a]	An iliac crest bone biopsy 2.6 years after fracture	An iliac crest bone biopsy 11.8 months after fracture	An iliac crest bone biopsy during operation. A tetracycline-labelled biopsy 2 months later[b]

BP bisphosphonate, *N/A* not applicable

[a] Bone biopsy findings at the time of the fracture reported except for dynamic bone histomorphometry based on the second bone biopsy

[b] Bone biopsy findings based on the second bone biopsy are reported due to better sample quality

59.0 years). The additional samples were obtained from cadavers with no metabolic bone disease. Only ethanol was used to store the samples. Measurements were performed similarly, with the exception that in the previous study four repeated scans were used.

The bone mineral content was assessed based on the peak areas of phosphate (900–1,200 cm^{-1}) and carbonate (850–890 cm^{-1}) peaks [22]. The collagen content was assessed based on the amide I peak area (1,585–1,720 cm^{-1}). A linear baseline correction was performed for each peak before the analyses. Bone composition parameters were calculated based on previous literature [15]. The phosphate-to-amide I ratio estimates the degree of mineralization, and has been shown to correlate with the ash content of the bone [15, 23]. Carbonate-to-phosphate ratio has been suggested to reflect the level of carbonate substitution into the hydroxyapatite crystal. Additionally the carbonate-to-amide I ratio was calculated [24]. This reflects the carbonate content in bone. Carbonate accumulates slowly with age, and therefore high turnover could lead to low carbonate-to-phosphate and carbonate-to-amide I ratios [21]. Collagen cross-linking ratio, i.e., collagen maturity, was determined as the ratio between the intensities at 1,660 and 1,690 cm^{-1} [16]. It has been suggested to reflect the degree of mature to immature collagen cross-links [25]. Spatial heterogeneity of each compositional parameter was assessed within each sample. The values for each pixel was calculated and used to create a histogram using Matlab® (version 7.6 Mathworks Inc.). Thereafter, a Gaussian curve was fitted to the histogram, and the full-width-at-half-maximum (FWHM) of the Gaussian curve was determined as a measure of heterogeneity [26, 27].

Results

Among the trauma patients treated in our hospital from January 2007 to December 2009, we identified eight patients with atypical fractures of femur and two of them had bilateral fractures (not simultaneously), i.e., totally 10 fractures. Six of these patients had been treated with bisphosphonates for several years prior to the AFF (Table 1). The mean age of these patients at the time of the fracture was 71.2 years (55.5–89.9 years). The mean duration of bisphosphonate-therapy before the fracture was 7 years (4–10 years).

In the years 2007–2009, the mean population in the catchment area of our hospital aged 50 years and over totalled 103,932 inhabitants, of which 55,539 were women. During these years, the average number of patients on continuous bisphosphonate therapy on the area was 4,379. We calculated the annual occurrence of AFFs to be 3 per year. Two patients (with unilateral fractures) had not been on bisphosphonate treatment. The incidence of fractures in bisphosphonate-treated patients was 2.67 fractures per year and there were 4,379 patients on bisphosphonates in the catchment area of our hospital. The incidence was therefore 2.67/4,379 per year = 0.61/1,000 per year for bisphosphonate users (95 % CI 0.13/1,000–0.92/1,000), compared to 0.0067/1,000 (95 % CI −0.0026/1,000 to 0.016/1,000) per year for untreated patients.

The patients ($n = 4$) that underwent bone histomorphometry were postmenopausal women (aged 55.5–81.1 years). Trabecular bone volume (BV/TV) and trabecular thickness were low in 3 patients, osteoid surface (OS/BS) and osteoblasts surface (Ob.S/BS) low in 2 and mineralizing surface (MS/BS) low in all 4 cases. Further, erosion surface (ES/BS) was low in 3 cases. These findings suggest low bone formation in 3 out of 4 patients. For Patients #1 and #4, the tetracycline labelled biopsies were taken five and 2 months after the first unlabelled biopsy, respectively. By fluorescence microscopy, single labels were detected in the trabecular region of interest in only Patient #3 (MS/BS, 0.8 %) who also had an increased amount of osteoid (OV/BV, 7.1 %) and a higher fraction of osteoblasts of bone surface (Ob.S/BS 10.9 %) (Table 3).

Since there were no labels in regular trabecular ROI, extended label search was performed. Patient #1 had one short single label in cortical bone but no trabecular bone labelling. Patient #2 had one double label in cortex together with one double label in trabecular bone in one section. Another section had a cortical single label. Patient #3 had one double label in trabecular bone and single label on cortex in one section. Cortical single label in two sections was found for Patient #4, and the other section of these included two short single labels in trabecular bone.

The bone composition measured by FTIRI showed a higher degree of mineralization (phosphate-to-amide I ratio) in 3 of our 4 patients (Fig. 3a). Carbonate-to-phosphate ratio was within the normal range (Fig. 3b). Higher carbonate-to-amide I (Fig. 3c) and collagen cross-link ratios (Fig. 3d) were found in 3 of our 4 patients with AFFs compared to normal samples. Hence, the collagen cross-link maturity might be higher in patients with AFFs. The heterogeneity of phosphate-to-amide I ratio was lower for patients with AFFs compared with normal samples (Fig. 4a), whereas the heterogeneity of carbonate-to-phosphate ratio (Fig. 4b) and carbonate-to-amide I ratio were within the normal range (Fig. 4c).

Discussion

We identified eight patients with ten atypical fractures of femur among the patients older than 49 years treated for femoral fractures at Kuopio University Hospital during the

Table 3 Bone histomorphometry findings in four patients with atypical femoral fractures

	Patient #1	Patient #2	Patient #3	Patient #4	Mean value	Reference value[a]
Trabecular bone volume (BV/TV, %)	19.5	*13.6*	*13.7*	*9.6*	*14.1*	16.3–26.1
Osteoid volume (OV/BV, %)	1.1	0.8	7.1	1.9	2.7	0.8–2.9
Osteoid surface (OS/BS, %)	*6.9*	*3.7*	35.8	9.3	13.9	8.0–20.6
Erosion surface (ES/BS, %)	*1.3*	*1.8*	2.1	*1.8*	*1.8*	2.0–6.0
Osteoblast surface (Ob.S/BS, %)	2.3	*0.6*	10.9	*0.7*	3.6	1.2–7.6
Osteoclast surface (Oc.S/BS, %)	0.0	0.2	0.6	0.0	0.2	0.0–1.4
Mean osteoid thickness (O.Th, μm)	8.3	9.2	8.0	*6.1*	7.9	7.2–11.4
Trabecular thickness (Tb.Th, μm)	117.9	*75.5*	*79.6*	*57.5*	*82.6*	104.5–160.3
Mineralizing surface (MS/BS, %)	*0.0*	*0.0*	*0.8*	*0.0*	*0.2*	2.9–11.1
Mineral apposition rate (MAR, μm/day)	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	0.45–0.61
Extended label search	Cortical single label, no trabecular bone labeling	Cortical and trabecular double label	Cortical single label and trabecular double label	Cortical and trabecular single label		
Mean wall thickness (W.Th, μm)	42.7	37.9	48.7	*26.7*	39.0	26.9–34.9

Findings below the reference values are italicized

Tissue volume (TV, mm^2) was determined using the whole field of view including both trabeculae and bone marrow. Bone volume (BV, mm^2) includes both mineralized and unmineralized bone volumes. Osteoid surfaces (OS/BS, %) were recognized as unmineralized seams on the bone surfaces, osteoid thickness (O.Th, μm) was measured, and osteoid volume (OV/BV, %) was calculated. Bone cells, osteoblasts and osteoclasts, were determined as a fraction of bone surface (Ob.S/BS, %; Oc.S/BS, %). Trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, mm^{-1}), and trabecular separation (Tb.Sp, μm) were calculated. Wall thickness (W.Th, μm) was measured under polarized light. Dynamic indices were defined using fluorescence microscopy. Mineralizing surfaces (MS/BS, %) were measured and the mineral apposition rate (MAR, μm/day) was defined as the interlabel width on double labels divided by the number of days between fluorochrome labeling

[a] Reference values for postmenopausal women were obtained from Recker et al. [18]

time period from January 2007 to December 2009. Six of these patients were bisphosphonate users. We were able to estimate the annual incidence of these fractures in patients on bisphosphonates in our hospital's catchment area to be 0.61/1,000 patients. Bone histomorphometry and bone compositional parameters of four postmenopausal women at the iliac crest were available. Bone histomorphometry revealed low trabecular bone volume in 3 of the 4 patients, and in the trabecular region of interest, proper tetracycline single labels were detected in only one patient. Furthermore, higher degree of mineralization and an increased collagen cross-link ratio were observed among these patients by FTIRI. All four patients had used bisphosphonates for at least 4 years prior to the fracture. None of our patients suffered from diabetes or rheumatoid arthritis, and none of them had had glucocorticoids or proton pump inhibitors.

Our results are in line with previous estimates concerning the incidence of AFFs during the bisphosphonate therapy. The method of our estimate is similar to Schilcher et al., who found the incidence of AFFs to be less than 1/1,000 among bisphosphonate-treated patients in Sweden [11]. Black et al. [12] performed secondary analysis of three large randomized trials originally designed to prove the efficacy of bisphosphonates in the prevention of osteoporotic fractures. They suggested the incidence to be somewhat lower, 2.3 per 10,000 patient-years. However, the patient material of those treatment-trials was probably somewhat different from that found in everyday practice. This may affect their estimate of incidence. Danish researchers have estimated the occurrence of AFFs using cross-sectional and matched control cohort studies. In the report by Abrahamsen et al. [9] the overall incidence of subtrochanteric femoral fractures was similar among alendronate users as among patients with hip fractures who had not had bisphosphonate therapy. We suggest that the incidence of AFFs during bisphosphonate therapy as the complication seems to be relatively infrequent. Several large-scale trials have shown that bisphosphonate therapy significantly prevents fractures in patients with osteoporosis [3, 5]. Furthermore, bisphosphonates have shown to improve hip structural parameters [28].

There is only limited data of bone histomorphometric findings among patients with AFFs available. Previous studies suggest severely suppressed bone turnover rate both in the iliac crest and in the fracture site among a few patients with AFFs [6, 10]. Three of our patients had low bone formation based on static histomorphometry parameters and all had low bone turnover rate based on dynamic measures. Lack of tetracycline double labels in biopsy has

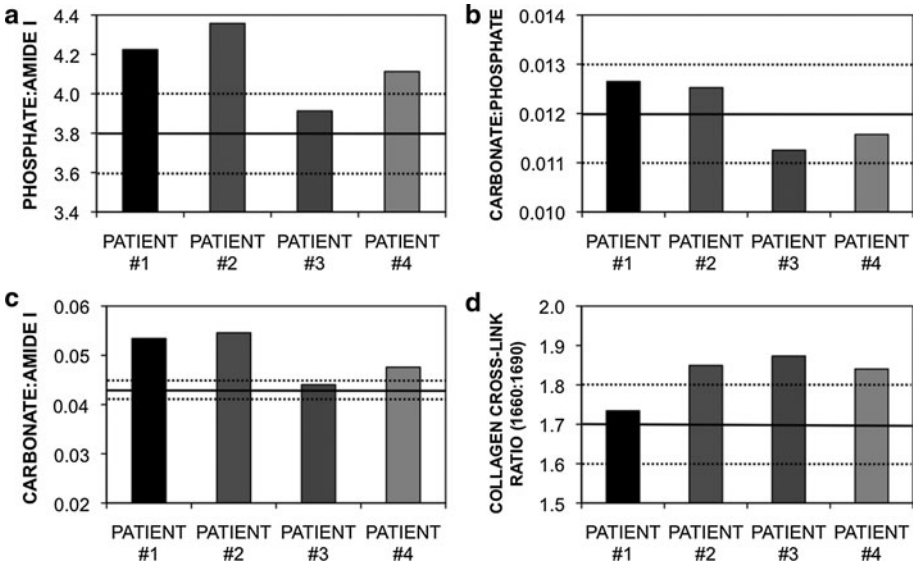

Fig. 3 Bone compositional findings in patients with atypical femoral fractures. Increased degree of mineralization was found in three patients (**a**). The degree of carbonate substitution into the hydroxyapatite crystals was within the normal range (**b**). The carbonate-to-amide I ratio (**c**) and collagen cross-link ratio (**d**) tended to be above the normal range. Age- and sex matched normal values ($n = 6$) are presented as mean (*solid lines*) ± standard deviation (*dashed lines*) for each parameter

been reported in patients with AFFs but similar findings have also been observed among bisphosphonate-treated patients, who have not sustained femoral fracture with atypical morphology [29, 30] or even in untreated postmenopausal women [13, 14]. Tetracycline labels were visible for only one of our patients in the trabecular ROI but almost a year had passed between the AFF and the biopsy. In the extended label search, however, two patients had double labels in trabecular bone. Cortical single label was found in the remaining two samples of which the other patient had also trabecular single label. Therefore, mineralization seems to be low although not absent. However, if only mineralization was low and bone formation was normal, thicker osteoid seams would follow, and this was not the case in our patients. This suggests that both low turnover and low mineralization may coexist in some of our cases. Osteoporotic trabecular bone volume has been found among patients with AFFs [6] with which our results coincide. The low trabecular bone volume in combination with low bone formation could explain the decreased fracture resistance of our patients. Bisphosphonates suppress bone turnover, and in relation to that, the resorption surface in our cases was low in three patients and on the lower end of the normal range in one patient. The high carbonate-to-amide I ratio might also be reflected in the low turnover. The low labeling by tetracycline without osteoid accumulation could rather be explained by low bone formation than by bone mineralization defect only.

Changes of bone material composition at the fracture site among patients with AFFs have been reported in a recent study; all women included were postmenopausal and on bisphosphonates [16]. Greater heterogeneity of mineral crystallinity in trabecular bone was found in patients with AFFs compared to patients with typical femoral fracture morphology but no other differences between these fracture types in trabecular bone was found [16]. Our patients tended to have a higher degree of mineralization. Our results coincide in previous findings in patients with AFFs using different techniques [6] and in low turnover osteoporotic bone by FTIRI [31]. We found increased collagen maturity in patients with AFFs. This has also been found in osteoporotic bone [32] and in alendronate-treated postmenopausal women [33] whereas another study confirmed no difference in alendronate-treated women compared with non-treated controls [26]. Increased collagen maturity in trabecular bone, however, has shown to be associated with increased risk of fractures [34]. Thus, altered collagen maturity in our patients may explain the greater propensity for developing AFFs.

Lower heterogeneity of phosphate-to-amide I ratio was found among patients with AFFs. This is in line with earlier studies on postmenopausal women treated with bisphosphonates [26]. The decreased heterogeneity of mineralization has been suggested to be related to the decreased bone remodeling, and therefore, caused by the antiresorptive therapy [16, 35]. More homogenized mineral content of the bone has been suggested to lead to more brittle bone [35]. Our findings are in line with the previous studies and may explain the higher propensity to develop low energy fractures.

Our study is limited by the low number of both fractures and patients with biopsy data. Since the samples were studied retrospectively, there were also limited data of biochemistry and densitometry available. A relatively long time had passed since the fracture for Patient #2 prior to biopsy. Although Patient #3 had fallen from a stack of wood that was 0.5 m high, she is included to the study because the other characteristics of AFF were present. The

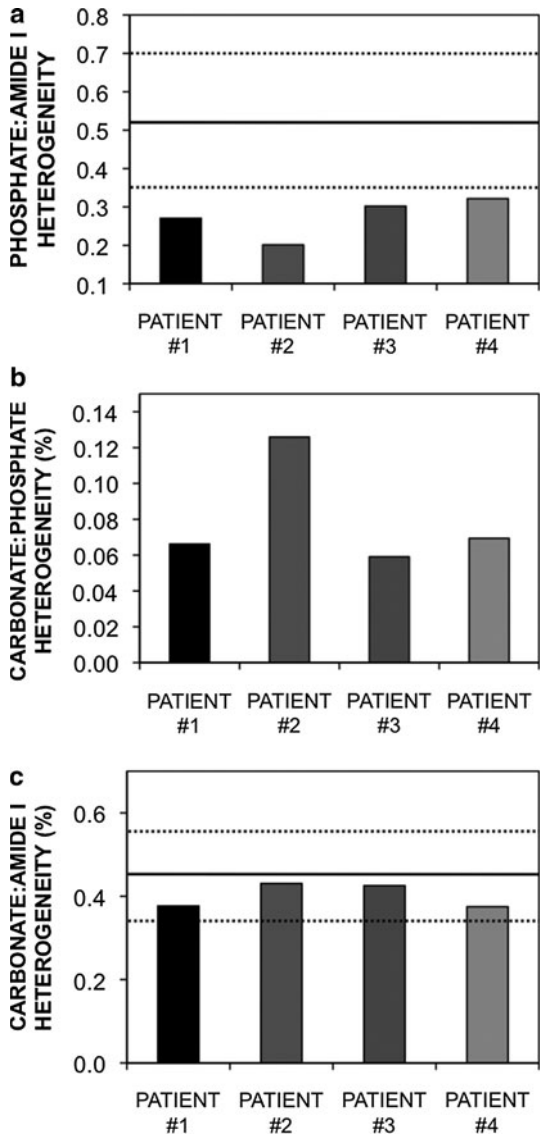

Fig. 4 Heterogeneity of bone compositional parameters. The heterogeneity of phosphate-to-amide I ratio tended to be lower for patients with atypical femoral fractures compared with normal samples (**a**). The heterogeneity of carbonate-to-phosphate ratio (**b**) was within the normal range. Further, the heterogeneity of carbonate-to-amide I ratio (**c**) was within the normal range. Age- and sex matched normal values ($n = 6$) as mean (*solid lines*) ± standard deviation (*dashed lines*) (except carbonate-to-phosphate ratio: normal range of 0.0–2.0 %)

number of normal samples for FTIRI reference was small. Regardless of these limitations we have presented both histomorphometric and bone compositional abnormalities in these four patients with AFFs.

Although the incidence of AFFs seems to remain low, these fractures have a specific morphology and occur especially as a result of low energy trauma. Bone formation was relatively low in 3 of the 4 patients, as judged by static bone histomorphometry parameters. Low mineralization rate was found due to scarce labeling in the biopsy although double labels were not totally absent. The changes in bone composition, i.e., higher degree of mineralization, increased collagen maturity, and decreased heterogeneity of the degree of mineralization, could explain the atypically low fracture resistance among these patients. Although similar findings have been reported among non-bisphosphonate-treated postmenopausal women, the patients with AFF most likely have some other concomitant factors (e.g., treatment or comorbid conditions) that predispose to these fractures. More studies with bone biopsies are needed to confirm these constituents, and to enhance our understanding of AFFs.

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Conflict of interest None of the authors have any conflicts of interest.

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