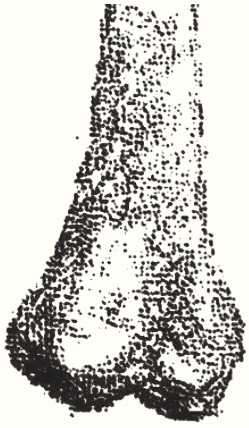
Atypical femur fractures and bisphosphonates

Denise Milou van de Laarschot

Diagnostic imaging, genetic background and medical treatment



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Atypische femurfracturen en bisfosfonaten
Diagnostische beeldvorming, genetische factoren en medicamenteuze behandeling

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GENERAL INTRODUCTION

Atypical femur fractures

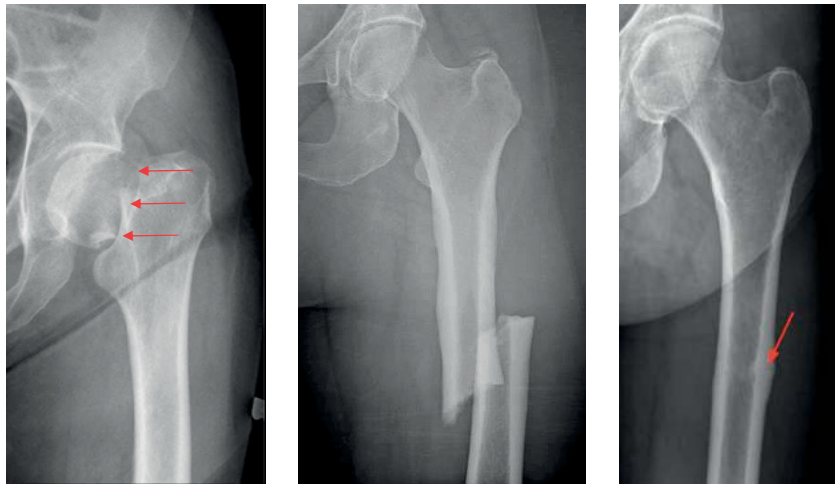
An atypical femur fracture (AFF) is an uncommon, spontaneous fracture of the thigh bone. AFF is considered a serious adverse event of the use of antiresorptive drugs such as bisphosphonates. Bisphosphonates are used by millions of patients worldwide for the treatment of osteoporosis, but also for other metabolic bone disorders and metastatic bone disease. AFFs are associated with bisphosphonate use and the risk increases with longer exposure, but a direct causal relationship has not been proven. The pathophysiology of AFF remains unclear. AFFs are labeled as atypical because of the transverse fracture line without comminution and the nontraumatic presentation of these fractures. Even though AFFs are rare, the risk of this complication leads to fear amongst patients and treating physicians, resulting in nonadherence and suboptimal care, ultimately widening the treatment gap in osteoporosis. If individuals at high risk of this adverse event can be identified, (prolonged) bisphosphonate use could be avoided in those at high risk, whilst improving treatment compliance in all other patients.

Definition

AFF was first described in 2005 in a case series by Odvina *et al*. who reported nine patients on alendronate over three years and experienced atraumatic nonvertebral fractures, including five patients with fractures of the femoral shaft.[1] Since then, numerous case reports and case series appeared on similar femur fractures. In 2010 and 2014, a Task Force from the American Society for Bone and Mineral Research (ASBMR) reviewed the literature on AFF and defined the case definition that is now most commonly used to diagnose AFF.[2,3] Complete and incomplete forms of AFF are distinguished. In the incomplete form, AFFs resemble stress fractures. AFFs have distinct radiological features and – in contrast to the classical hip fractures – do not occur at the femoral neck (**Figure 1**).

The most recent criteria from 2014 state that an AFF is a sub-trochanteric femoral fracture that can occur anywhere along the femoral diaphysis from just distal to the lesser trochanter to just proximal to the supracondylar flare, that must meet four out of five major criteria. These criteria describe the horizontal orientation of the fracture line, the localized cortical reaction, the fracture localization and the absence of loose bone fragments (comminution) or a high impact trauma (**Table 1**).

The transverse configuration of AFFs has been reported as the most sensitive factor to differentiate AFF from typical femoral fractures on conventional radiographs.[6,7]



A. *B.* *C.*

1A: Classical hip fracture located at the femoral neck.[4]
1B: Complete atypical femur fracture.[2]
1C: Incomplete atypical femur fracture with transverse fracture line (arrow).[5]

Table 1. Case definition of AFF as formulated by the ASBMR Task Force 2014.

The fracture must be located along the femoral diaphysis from just distal to the lesser trochanter to just proximal to the supracondylar flare.*
Major features (4 out of 5 required for diagnosis)
The fracture is associated with minimal or no trauma, as in a fall from a standing height or less
The fracture line originates at the lateral cortex and is substantially transverse in its orientation, although it may become oblique as it progresses medially across the femur
Complete fractures extend through both cortices and may be associated with a medial spike; incomplete fractures involve only the lateral cortex
The fracture is non-comminuted or minimally comminuted
Localized periosteal or endosteal thickening of the lateral cortex is present at the fracture site ("beaking" or "flaring")
Minor features (optional)
Generalized increase in cortical thickness of the femoral diaphysis
Unilateral or bilateral prodromal symptoms such as dull or aching pain in the groin or thigh
Bilateral incomplete or complete femoral diaphysis fractures
Delayed fracture healing

* **This definition excludes femoral neck fractures, intertrochanteric fractures with spiral subtrochanteric extension, periprosthetic fractures and pathological fractures associated with primary or metastastic bone tumors and miscellaneous diseases.**

The localized cortical thickening is referred to as "beaking" or "flaring". These terms are used interchangeably, although beaking could be interpreted as the visibility of a

fracture line within a pointy cortical reaction, resembling the beak of a bird. Flaring can be interpreted as the widening of the cortical thickness, in absence of a fracture line.

Minor features are associated with AFF, but not required for the diagnosis (**Table 1**).

There is no general cut-off value available for generalized cortical thickness and its diagnostic value is debatable. When comparing cortical thickness between 59 AFF patients and 218 controls with typical sub-trochanteric fractures, the difference between groups disappeared after adjusting for age.[8] In another study, no difference was found in cortical thickness when comparing patients with low-trauma sub-trochanteric fractures to patients with classical hip fractures, nor when comparing bisphosphonate users to non-users.[9] Similarly, a small case-control study did not find differences in femoral cortical thickness between 43 long-term bisphosphonate users and 12 controls with osteoporosis.[10]

Bilaterality is reported in 19% to up to 44% of the cases.[2,11,12] Sometimes, patients present with simultaneous, bilateral complete AFFs, of which at least seven cases have been documented in literature.[13] Delayed or non-union is a frequent phenomenon and has been reported in 26% to 39% of the cases.[3,11] Delayed union is often defined as insufficient callus bridging six months after the occurrence of a fracture.

AFFs are usually diagnosed using conventional radiography. For incomplete forms of AFF, CT-scanning (computed tomography) may be used to examine the presence or extent of a fracture line and bone scintigraphy or MRI-scans (magnetic resonance imaging) can demonstrate whether it concerns a healed or active fracture with bone marrow edema. AFFs may be visible on DXA (Dual-Energy X-ray Absorptiometry), but its use in the detection of AFFs is not yet established.

AFFs in association with bisphosphonates

AFFs have a low incidence, especially in comparison to typical osteoporotic fractures. For every AFF, over 265 hip fractures occurred in women based on a nationwide Swedish database.[14] The absolute risk of AFF is estimated at 1.8 per 100,000 person years with two years of bisphosphonate treatment, increasing to 113 per 100,000 person years with over eight years of treatment duration.[12,15] In long-term users - defined as 3.5 to 8.5 years - an incidence of 1:1000 has been reported in a registry of two hospitals.[16] The incidence of AFF may be underestimated, because of the lack of a diagnostic code and radiographic signs of AFF may not be recognized, as was reported in an audit of a large acute hospital in Canada where radiologists had only diagnosed AFF in one of 24 patients with AFF out of 193 patients with radiographs of sub-trochanteric fractures.[17]

The relative risk of AFF with the use of any bisphosphonate was 1.7 in a meta-analysis of eleven studies.[18] The relative risk depends strongly on the case definition of AFF, since the relative risk can increase to 11.8 and 28.2 using studies with radiographic adjudication of the AFF with or without use of the ASBMR diagnostic criteria, respectively.[18] It was found in one study that the risk of AFF declines with 70% per year after stopping the treatment.[14] It is not known if this risk reduction also applies to patients who have already sustained an AFF.

In the majority of patients with osteoporosis, the benefits of bisphosphonate treatment outweigh the risk of AFF. The predicted fracture risk for femoral neck fractures without bisphosphonate use is 30 to 100 times as high as the risk of AFF with bisphosphonate therapy.[19] After three years of bisphosphonate use amongst 10,000 patients, the incidence of AFF is 0.8 whilst 1000 fractures are prevented, meaning that one AFF with bisphosphonate use is the price paid for the prevention of 1,200 vertebral and non-vertebral fractures.[20] Moreover, AFFs have so far not been causally linked to bisphosphonates and are also reported in bisphosphonate-naïve individuals, suggesting that other factors must also play a role.[16,21-23] In a nationwide Swedish cohort study, 22 (12.8%) out of 172 AFF cases had no documentation of prior bisphosphonate use.[24] In a Japanese cohort study of 11 AFFs (25%) occurred without exposure to bisphosphonates or denosumab.25 However, it is uncertain whether the information on medication use is complete in these cohort studies.

Crisis in the treatment of osteoporosis

The negative publicity on AFF has been linked to a drastic decline of over 50% in the use of oral bisphosphonates in American women between 2008-2012.[26,27] Even the number of patients with a hip fracture receiving treatment has decreased by around 50% in a decades' time between 2002 and 2011 in the U.S.[28] Subsequently, hip fracture rates amongst women in the U.S. have been higher than predicted in 2013, 2014 and 2015.[29] In the European Union, more than half of the men and women at high risk of fracture were not receiving osteoporosis treatment in 2010, amounting to 1.7 million and 18.4 million persons, respectively.

Also in the Netherlands, both under-treatment of osteoporosis and a decline in the use of bisphosphonates have been noted. The treatment gap in osteoporosis for Dutch men was 52% and 60% for Dutch women in 2010.[30] Dutch public pharmacies dispensed 2.1 million prescriptions of bisphosphonates for osteoporosis to 240,000 patients in 2015 versus 210,000 patients in 2013, which comes down to a decrease of almost 11% in bisphosphonate use in two years' time.[31]

This under-treatment is especially worrisome since osteoporosis is a growing public health problem due to aging of the population, associated with a high disease burden, mortality and financial costs. In 2010, the annual number of fractures in the European Union was 3.5 million and the estimated yearly costs of osteoporosis were 37 billion euros. Both are expected to rise by at least 25% in 2025.[30]

This crisis in the treatment of osteoporosis stresses the need for identification of those at risk for a serious adverse event such as AFF, in order to improve drug compliance and enhance safe use of (long-term) bisphosphonates, preventing both AFFs and typical fractures.

Bisphosphonates: mechanism of action

Bisphosphonates inhibit the bone-resorbing activity by the osteoclasts. These drugs selectively bind to bone mineral, because of their high affinity for hydroxyapatite crystals in the bone. Skeletal uptake is highest at sites with high bone turnover.

The bisphosphonates used in modern medicine are the nitrogen-containing bisphosphonates, which have a different mechanism of action and are more potent than the first-generation, non-nitrogen containing bisphosphonates such as etidronate and clodronate. Nitrogen-containing bisphosphonates block the enzyme farnesyl pyrophosphate synthase in the mevalonate pathway (**Figure 2**). The mevalonate pathway is essential for production of cholesterol, but also other lipids that are involved in the activation of signaling proteins for osteoclast survival. Inhibition of the mevalonate pathway does therefore lead to apoptosis of osteoclasts.[32] Nitrogen-containing

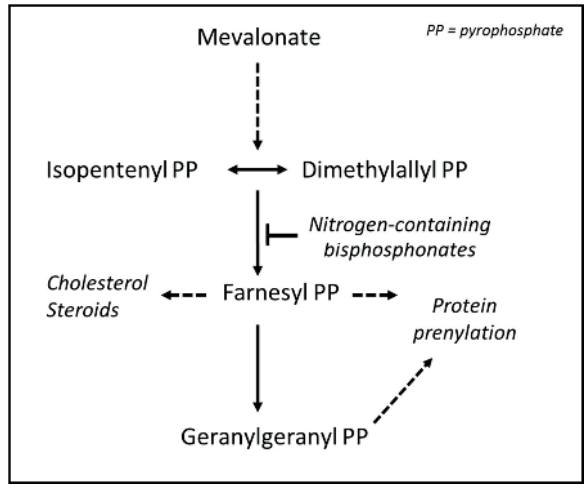


Figure 2. Schematic overview of the mevalonate pathway.

bisphosphonates include alendronate, risedronate, ibandronate, pamidronate and zoledronate. Alendronate has been available since 1995 for osteoporosis and is the most frequently prescribed type of bisphosphonate worldwide, e.g., representing 55% of all bisphosphonate users in the Netherlands.[31] This is an oral drug that must be taken weekly on an empty stomach.

Bisphosphonates are not metabolized, but either built into the bone tissue or excreted in the urine.[33] Consequently, the biological availability of bisphosphonates is difficult to monitor in the urine or blood, but it has been estimated that the half-life of intravenously delivered bisphosphonates in bone is over ten years' time[34] and that bisphosphonates may remain present in the bone for many years even after cessation of therapy.[35-37]

Bisphosphonates are known to improve bone mineral density and consequently reduce fracture risk. Clinical trials have shown that they reduce the risk of non-vertebral fractures by 20-40% and vertebral fractures by 40 to 70% in postmenopausal women.[38] Bisphosphonates even decrease mortality independent of fracture reduction.[39-44] This may be explained by the immunomodulating effects of bisphosphonates, since not only osteoclasts but also macrophages and monocytes have the ability to take up bisphosphonates, although over time the bisphosphonates can only remain present in the body by their binding to calcified tissue. Bisphosphonates have been associated with a wide range of extraskeletal effects, including improved insulin sensitivity, inhibition of the formation of atherosclerotic plaques and reduced cardiovascular disease risk and also favorable effects on breast-cancer recurrence risk and breast-cancer mortality.[45]

Frequent side effects of oral bisphosphonates include gastrointestinal symptoms such as oesophageal irritation and reflux, obstipation or diarrhea. Zoledronate is the most potent type of bisphosphonates and is administered intravenously once per year. Hypocalcemia and an acute phase reaction are known side effects of intravenous bisphosphonates. The latter is possibly the result of pro-inflammatory effects of IL-1, IL-6 and TNFα production by macrophages and osteoclasts.[46]

The optimal duration of treatment with bisphosphonates is controversial, especially since serious adverse events such as AFFs and osteonecrosis of the jaw have been reported, with the first documentation in 2005 and 2003, respectively.[1,47] There is a lack of evidence for overall fracture reduction with bisphosphonate therapy beyond three to five years, although post-hoc analyses in extensions of placebo-controlled trials have shown beneficial effects on bone mineral density and prevention of vertebral fractures with 10 years of alendronate and six years of zoledronate in postmenopausal women with a high fracture risk.[48,49]

Bisphosphonates and bone quality

The cause of AFF is unknown, but impaired bone quality due to bisphosphonate use has been proposed as a key factor in the pathophysiology.

Bisphosphonates induce osteoclast apoptosis, thereby suppressing bone turnover. Potential over-suppression of bone turnover with bisphosphonates can result in increased stiffness of the bone matrix, decreased repair of micro-damage and a more homogeneously mineralized bone.[50-53]

It is hypothesized that these factors combined may eventually lead to the development of a spontaneous fracture such as AFF. This 'frozen bone' theory is not proven, but is supported by the only study in which bone biopsies were performed close to the fracture site of AFF. In this study, the bone tissue taken from 12 postmenopausal women with AFF was hypermineralized and harder compared to bisphosphonate-treated controls with typical fractures.[54]

Other bone biopsy studies in humans show conflicting evidence to whether bisphosphonates lead to impaired quality of the bone. In studies with iliac crest biopsies before and after long-term bisphosphonate use, there was no evidence for hypermineralization.[55,56] There are indications for increased microdamage in the femur in bisphosphonate users[57], but not in the lumbar spine or iliac crest.[58,59] Bisphosphonates restricted the plasticity of the bone through accumulation of non-enzymatic collagen cross-linking in one animal study[60], but in human osteoporotic cortical bone the administration of bisphosphonates was shown to improve mechanical properties.[61]

Even though bisphosphonates may lead to alterations of the collagen and increased amount of microdamage, there is no proof that bisphosphonates can directly cause fractures in humans. There are few animal studies available and whilst bone tissue of sheep and dogs showed reduced fatigue resistance with alendronate, this was not found for zoledronate.[62,63]

Risk factors for AFF other than bisphosphonate use

Other medication

Apart from bisphosphonate use, several other predisposing factors have been associated with AFF. Denosumab is an inhibitor to RANK-ligand and a potent antiresorptive drug. Romosozumab is an inhibitor to sclerostin with an anabolic mechanism of action although it also has antiresorptive effects. The use of both these monoclonal antibodies in the treatment of osteoporosis has been described in relation to the occurrence of

AFF[64], but an epidemiological association between AFF and these drugs is not demonstrated. The role of anabolic therapy with analogs of parathyroid hormone, including teriparatide and abaloparatide, is not determined, although in theory the direct stimulation of osteoblasts by these agents might improve the fracture healing of patients with suppressed bone turnover. Alternatively, hormone replacement therapy, tibolone, selective estrogen receptor modulators (SERMs) or calcitonin may be considered in the medical management of patients with AFF.

Concomitant use of antiresorptives and glucocorticosteroids has been identified as a risk factor for AFF.[2,64] The proportion of corticosteroid users in AFF cohorts has been reported as high as 30%.[65] It is well-known that corticosteroids have detrimental effects on the bone; they directly stimulate apoptosis of osteoblasts and osteocytes amongst other indirect negative effects and prolonged use can induce osteoporosis.[66]

The use of proton pump inhibitors has also been brought forward as a risk factor in some initial case series[2], but the association is dubious and might depend on the frequent concomitant use with corticosteroids and age as confounding factors.

It has been found that the majority of AFF patients have a relatively normal BMD and younger age compared to those with typical femoral fractures[64], which could again be explained by a middle-aged patient population of long-term corticosteroid users that are on bisphosphonates for the prevention – rather than the treatment – of osteoporosis.

Femur anatomy

The often symmetrical presentation of AFFs in both upper legs suggests a critically increased tensile strain on the lateral femoral cortices that might be predisposed by certain femoral geometric properties. Increased curvature of the femoral shaft[67-70] and a more varus hip geometry with decreased femoral neck to shaft angle[71,72] are found to be associated with AFF in some retrospective studies, but not consistently.

Women of Asian ethnicity appear to be at higher risk of AFF than Caucasian women.[11,12,73,74] This might be due to racial differences in femoral geometry, since it is well-known that Asians have a more prominent bowing of the femur[75,76], but also differences in pharmacokinetics and underestimation of the BMD due to smaller bone size, resulting in higher numbers of Asians receiving bisphosphonate treatment.[74] It has also been noted that the preferential localization of AFF appears to differ between Asians and Caucasians, with a predominantly subtrochanteric localization in a Singaporean cohort in contrast with a diaphyseal localization in a Swedish population.[77]

Genetic predisposition for AFF

The rarity of AFF amongst millions of bisphosphonate users suggests an underlying individual susceptibility that could be explained by predisposing genetic factors of relatively low frequency but with strong effects, which is also indicated by the occurrence of AFF in families[78] and in patients with monogenetic bone disease such as osteogenesis imperfecta, hypophosphatasia and osteopetrosis, but without bisphosphonate exposure.[2,16,21-23]

Yet, a more general genetic predisposition to AFF is also plausible given the ethnic differences in AFF incidence, and given that genetic factors determining geometric properties of the hip[79] or determining individual differences in skeletal uptake of anti-resorptive drugs, may increase the risk of AFF. Such more common genetic susceptibility variants for AFF could also impact bone quality due to alterations in collagen-cross linking, impaired osteoclast function or low bone turnover rate, that – in combination with the use of antiresorptive drugs and/or glucocorticosteroids may lead to the development of femoral stress fractures. Thus, both rare as well as more common genetic variants may determine the risk of AFF and constitute its genetic architecture.

To unravel the genetic architecture for AFF, several approaches can be considered ranging from candidate gene testing to genome-wide approaches, looking for rare mutations with string effects up to very common susceptibility variants of individual modest effect but - combined into a polygenic risk score - of substantial effect. Candidate gene studies related to osteoporosis or monogenetic bone disease, but also candidate genes identified from familial forms of AFF, could be used to investigate the presence of known pathogenic mutations or novel susceptibility variants within these bone-related genes. When looking for more rare mutations, Whole-Exome Sequencing (WES) scrutinizes all coding regions of the genome and could identify protein-altering variants in genes that have not yet been linked to bone disease. Whole Genome Sequencing (WGS) would in addition also include studying such rare mutations in noncoding and regulatory regions in the DNA, but these will be more difficult to study than protein altering variants. Finally, when looking for the more common susceptibility variants for AFF Genome-Wide Association Studies (GWAS) with SNP arrays will be more appropriate, but require substantial case series to obtain sufficient statistical power.

Understanding of the genetic background of AFF could enable personalized medicine within the treatment of osteoporosis. If a collection of (common and rare) genetic susceptibility variants for AFF can be identified, patients could be screened by a relatively cheap genetic array test (rather than the more expensive WES or WGS) for a high risk of AFF prior to starting or prolonging bisphosphonate treatment. In those then identi-

fied with a high risk, bisphosphonate treatment can be discontinued and alternative osteoporosis drugs could be prescribed.

Management after AFF

Complete AFFs require surgical intervention. Full-length intramedullary nailing is the recommended choice based on expert opinion. A standard nail size would not protect from new fractures along the femoral diaphysis and it is thought that the chance of surgical material failure is lower than with extramedullary fixation.[80]

Elective surgery is performed in case of an incomplete AFF with risk of deterioration to a complete fracture, because surgical procedures in an acute setting have a higher rate of perioperative complications and worse clinical outcomes.[81-83]

The presence and extent of a radiolucent line, severity of the pain and fracture localization appear to be important hallmarks for the risk of completion of the AFF in a validated prediction tool developed by a Korean research group. Unilaterality of AFF is associated with worse outcomes in this tool, probably because an initial presentation with complete AFF increases the detection rate of incomplete AFFs at the contralateral leg at an early stage, thereby improving the clinical outcome.[84]

Bisphosphonates are usually stopped after AFF has occurred, but it is not clear what the optimal medical management is of patients who are still at a high risk of typical fractures, for example those who are on long-term corticosteroid treatment, have low BMD or have sustained recent vertebral or nonvertebral fractures.

Aims and outlines of this thesis

The aims of this thesis are 1) to investigate the potential of DXA scanning in the prediction and detection of AFF, 2) to study the possibility of identifying genetic predictors of AFF and 3) formulate a statement on the optimal medical treatment of a) fracture healing of the AFF itself and b) osteoporosis after the occurrence of AFF.

Part 1 focuses on the diagnostic imaging of AFF. We hypothesized that the trabecular bone score (TBS), an indirect measure of bone architecture measured on lumbar spine DXA scans, is lower in patients with AFF. We also speculated that hip structural analysis on DXA scans of the femur in patients with AFF would have distinct features at the cross-section of the femoral shaft**. Chapter 2** describes our findings on TBS and HSA in patients with AFF in comparison with controls. **Chapter 3** explores the possibility of medially located AFF rather than the lateral side of the femoral shaft. **Chapter 4** and **Chapter 5** concern the role of DXA scanning in detection of incomplete forms of AFF.

Part 2 discusses the role of genetics in the pathophysiology of AFF. We present one of our patients with monogenetic bone disease and AFF in **Chapter 6**. **Chapter 7** reviews the literature on genetic bone disease in relation to AFF. In **Chapter 8** the results of whole-exome sequencing in two small families of Asian ethnicity with bisphosphonate-associated AFFs are reported. We present the preliminary findings of a whole-exome sequencing study in a Caucasian family with osteoporosis and AFFs in **Chapter 9**.

Part 3 consists of **Chapter 10** that entails a comprehensive review of the use of teriparatide, denosumab and raloxifene in patients with AFFs and recommendations for medical management of patients after the occurrence of AFF in a position statement by the European Calcified Tissue Society.

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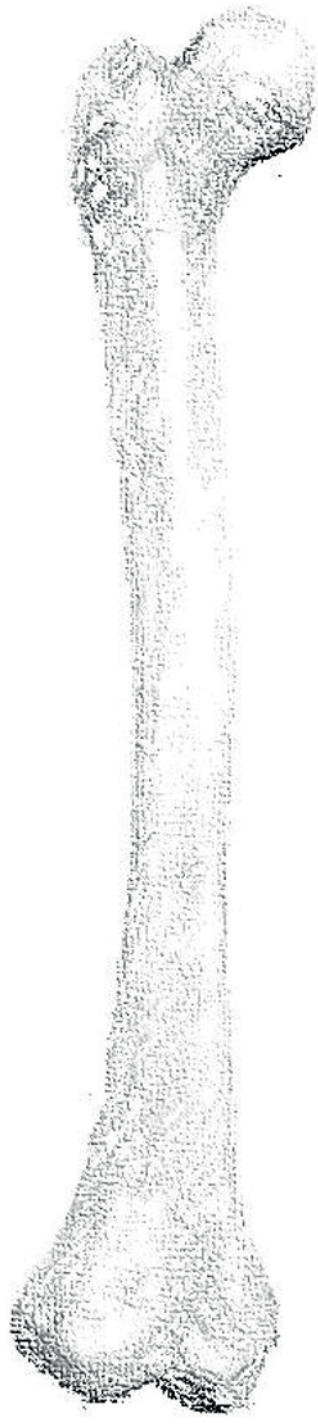

Part 1

Diagnostic imaging of atypical femur fractures

2

Trabecular bone score and hip structural analysis in patients with atypical femur fractures

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ABSTRACT

Introduction: Bisphosphonate use has declined dramatically in recent years, partly because of fear of rare side effects like atypical femur fractures (AFFs). It is therefore desirable to have a diagnostic method to identify those at risk of AFF in order to prevent this serious complication.

Methodology: We compared trabecular microarchitecture and hip geometry between 30 patients with AFF and 141 controls of similar age and sex, using bisphosphonates. Trabecular Bone Score (TBS) and hip structural analysis (HSA) were used to assess trabecular microarchitecture and macroscopic hip geometry from DXA images of the lumbar spine and hip, respectively. General characteristics, TBS and HSA were compared between AFF patients and controls using student T-tests and chi-square statistics. Associations between AFF and TBS and femur geometric characteristics by HSA were adjusted for sex, age, height, weight, ethnicity, duration of bisphosphonate use and glucocorticoid use. Additionally, the analysis of TBS was adjusted for lumbar spine bone mineral density (BMD) and the time difference between DXA scanning and the diagnosis of AFF.

Results: AFF patients had significantly higher body mass index (BMI) than controls, had used bisphosphonates longer and glucocorticoids and proton pump inhibitors more frequently. Sex-specific T-score was significantly higher in AFF patients at the lumbar spine ($p=0.004$), but not at the femoral neck ($p=0.190$) after adjustment for age, height and weight. TBS did not differ significantly between AFF patients and controls. Neither neck shaft angle nor any geometric variables at the femoral shaft measured by HSA differed between AFF patients and controls. At the narrow neck, AFF patients had lower buckling ratio and higher centroid position, consistent with a lower risk of classical fragility hip fractures. The findings at narrow neck and higher BMD might be explained by the fact that the majority of AFF patients used bisphosphonates to prevent glucocorticoid-induced osteoporosis.

Conclusions: Based on our results, TBS and HSA do not appear to have value in detecting patients at risk of AFF.

1. INTRODUCTION

Atypical femur fractures (AFFs) are subtrochanteric or diaphyseal fractures occurring after minimal or no trauma associated with bisphosphonate therapy. To define a fracture as an AFF according to the criteria outlined by the ASBMR Task Force, the fracture must be located along the femoral diaphysis from just distal to the lesser trochanter to just proximal to the supracondylar flare and at least four out of five major features must be present (**Table 1**).[1] The minor features are not required for diagnosis, but have been related to AFFs.

AFFs occur more frequently in patients on bisphosphonate therapy and longer duration of treatment is associated with a higher risk.[1, 2] There is great uncertainty in the magnitude of the relation of bisphosphonates to AFF with relative risks ranging from 0.77 to 69 dependent on duration of treatment[1], with a meta-analysis value of 1.70.[2] The incidence of AFFs in bisphosphonate users is very low, between 3.2 to 50 per 100,000 person-years.[1] Bisphosphonates are the first-line choice for the treatment of osteoporosis because they effectively decrease fracture risk.[3, 4] Partly due to the attention on potential serious complications of bisphosphonates, such as AFFs, the prevalence of oral bisphosphonate use in the United States has declined by more than 50% between 2008 and 2012.[5] This is a disconcerting development as more patients at high risk of osteoporotic fracture remain untreated. It is therefore desirable to have a diagnostic method that is able to identify those at risk of AFF in order to determine which patients should avoid (long-term) use of bisphosphonates. We hypothesize that certain properties of the bone tissue or geometry make a minority of patients susceptible to AFF when treated with bisphosphonates. Fractures usually occur when mechanical stresses due to applied loads exceed the capacity of the bone to withstand them. Fragility fractures occur because load stresses under trauma conditions exceed the material strength. AFFs are located at the femoral shaft where the cortex is quite thick and trauma is not necessarily involved. One would not expect that loading forces during minimal trauma or during activities of daily living cause a fracture on the femoral cortex, unless the tissue composition is altered in a manner that degrades its strength. One of the current hypotheses on the pathogenesis of AFF is that long-term suppression of bone turnover by bisphosphonates causes impaired bone remodeling and increased homogeneity of the bone[6], making the bone more brittle.[7] These changes in bone material properties are thought to increase microcrack accumulation and propagation, and ultimately increased risk of fracture.[6-8]

An ideal measurement would evaluate the bone dimensions (geometry) that govern load stresses as well as the tissue material strength that determines the ability to resist

those stresses. Unfortunately, current measures used to estimate bone tissue material strength require tissue biopsy or microindentation or involve advanced image processing techniques, such as high resolution peripheral quantitative computed tomography (HR-pQCT) based microfinite element analysis.[9, 10] Dual energy X-ray absorptiometry (DXA) based non-invasive measurements are a logical choice for a test of AFF susceptibility, because DXA is already used in the clinic to evaluate osteoporosis and with special software bone microarchitecture and geometry can be investigated. Trabecular microarchitecture can indirectly be assessed by using the Trabecular Bone Score (TBS), which is measured at the lumbar spine.[11-13] Hip Structural Analysis (HSA) is a technique that can be used to determine hip geometry at several locations in the femur.[14, 15] In this case-control study we aimed to compare structural and geometric properties of the bone using TBS and HSA between AFF patients and controls, all using bisphosphonates. We hypothesized that patients with AFF have deteriorated material strength or distinct hip geometry compared to controls, predisposing them to AFF. The results of this study might help to distinguish patients at high risk of developing AFFs by using easily accessible techniques.

Table 1 Major and Minor Features of AFFs according to the ASBMR 2013 case definition

	Major features:
1.	The fracture is associated with minimal or no trauma, as in a fall from a standing height or less.
2.	The fracture line originates at the lateral cortex and is substantially transverse in its orientation, although it may become oblique as it progresses medially across the femur.
3.	Complete fractures extend through both cortices and may be associated with a medial spike; incomplete fractures involve only the lateral cortex.
4.	The fracture is non-comminuted or minimally comminuted.
5.	Localized periosteal or endosteal thickening of the lateral cortex is present at the fracture site (“beaking” or “flaring”).
	Minor features:
1.	Generalized increase in cortical thickness of the femoral diaphysis.
2.	Unilateral or bilateral prodromal symptoms such as dull or aching pain in the groin or thigh.
3.	Bilateral incomplete or complete femoral diaphysis fractures.
4.	Delayed fracture healing.

2. METHODS

We conducted a retrospective case-control study at the Bone Center of Erasmus University Medical Center, Rotterdam, the Netherlands. The study was approved by the Medical Ethical Committee of Erasmus MC.

2.1 Patients

We included patients diagnosed with AFF that were also evaluated with DXA scanner that was calibrated for TBS in our hospital between 2013 and 2016. X-ray images of the fractures were examined to determine if the radiologic characteristics fulfilled the ASBMR 2013 criteria of AFF[1] by two investigators. The patients filled in a questionnaire on medication use and medical history. Patient data at time of AFF and at time of DXA were collected. If several DXA scans had been performed in one patient, the scan closest to the occurrence of AFF was used, either before or after the AFF.

2.2 Controls

Controls were selected from patients undergoing routine DXA for osteoporosis between 2014 and 2015. Patients with confirmed bisphosphonate use and without AFF were included in the control group. If available, extended femur scans by DXA were evaluated in control patients, since this type of imaging appears to be able to identify yet unknown incomplete AFFs.[16-18] General characteristics such as height, weight, smoking status, menopausal status, duration of bisphosphonate use and use of glucocorticoids and proton pomp inhibitors (PPIs) were retrieved from medical records.

2.3 DXA scanning

Posteroanterior (PA) DXA scans of the lumbar spine and total hip were performed by two licensed DXA technicians using a GE-Lunar Prodigy Advance device (GE Healthcare, USA, software 14.10.022) on patients in supine position following manufacturers protocols. BMD of the lumbar vertebrae (L1-L4) and the femoral neck was measured after exclusion of unreliable scans e.g., due to vertebral fractures or surgical material.

2.4 Trabecular Bone Score (TBS)

TBS is an indirect measure of trabecular microarchitecture and is derived by analyzing the pixel gray-level variations in the lumbar spine DXA image.[11-13] A higher TBS indicates better, more dense trabecular microarchitecture, producing many small gray-level variations. TBS was calculated using TBS iNsight software version 3.0.0.0 (Medimaps, Geneva, Switzerland). TBS was determined on PA spine DXA scans of the lumbar vertebrae (L1-L4). Patients with characteristics outside the working range for TBS (BMI 15 to 37 kg/m^2, age>20 years, and height >140 cm) were excluded from TBS analysis.

In a subgroup of seven AFF patients, TBS was calculated based on DXA scans of a previous DXA device that was not calibrated for use of TBS software to give an impression of the structural properties before the fracture in a sensitivity analysis, acknowledging that results may be less reliable.

2.5 Hip Structural Analysis (HSA)

HSA measures bone geometric properties of cross-sections of the bone using two-dimensional DXA-derived images of the hip.[14, 15] DXA measures only the inorganic component of bone mineral and removes all soft tissue and lighter organic elements of bone tissue, like the matrix where the mineral is deposited and the bone marrow. The HSA software generates profiles of the distribution of mineral mass in a line of pixels across the bone axis. Five of these mass profiles spaced ~1 mm apart along the bone axis are averaged and used to determine parameters of hip geometry and strength at that location. Three regions were analyzed: 1) the narrow neck, traversing the narrowest width of the femoral neck, 2) intertrochanteric, along the bisector of the shaft, and femoral neck axes, and 3) femoral shaft, at a distance of 1.5 times minimum neck width distal to the intersection of the neck and shaft axes. The structural parameters used in this paper are described in **Table 2**. HSA measurements were performed by Beck Radiological Innovations Inc.[19]

Table 2 Terminology of hip structural analysis

Neck-shaft angle (NSA): The angle between femoral neck and femoral shaft.
Neck Length (cm): The distance from user defined center of femoral head to intersection of neck and shaft axes
Cross-sectional area (CSA, cm^2): The surface area of bone tissue in the cross-section after excluding soft tissue spaces. CSA is an index of resistance to forces directed along the long axis of the bone.
Cross-sectional moment of inertia (CSMI, mm^4): The distribution of material around the centroid axis. This is an index of structural rigidity to bending in the plane of the image.
Outer diameter (cm): The distance between (blur corrected) outer margins of the cross-section.
Section Modulus (Z, cm^3): An index of bending strength in a cross-section, which derives from CSMI by dividing it by the distance from the centroidal axis to the edge of the section.
Endocortical Diameter (cm): Estimate of inside diameter of cortex.
Average cortex (cm): An estimate of the mean cortical thickness. This is calculated by assuming that 60, 70 and 100% of the measured bone mass is in the cortex for narrow-neck, intertrochanteric and shaft, respectively.
Buckling ratio (BR): The ratio of the outer radius to the wall thickness. This parameter is only important for thin-walled tubes; therefore it is only relevant for the narrow neck and intertrochanteric regions. If the BR exceeds a factor of about 10, the cross-section is susceptible to local buckling and loses strength.
Centroid position: The distance from the location center of mass to the medial cortical margin divided by the outer diameter. This is an index of symmetry of the mass in the cross-section.

2.6 Statistical analysis

Patient characteristics and DXA variables are expressed as mean (SD) or percentages. All data were normally distributed. Student T-tests were used to compare continuous variables between the groups and chi-square statistics for categorical variables. Differences in age and duration of bisphosphonate use between time of AFF and time of DXA were calculated using paired sample T-tests. Analysis of covariance (ANCOVA) was used

to compare adjusted means of BMD, TBS and HSA. Associations between AFF and BMD, TBS and femur geometric characteristics by HSA were adjusted for sex, age, height and weight.[20, 21] Additionally, the analysis of HSA and TBS were adjusted for ethnicity[22], duration of bisphosphonate use and use of glucocorticoids and the analysis of TBS was also adjusted for lumbar spine BMD and time difference between acquisition of the DXA scan and the diagnosis of AFF.[23, 24]

HSA statistical analyses were not corrected for multiple testing. Sex-stratified analysis was performed since especially bone geometry is sex-specific. Correlations were calculated using Pearson (partial) correlation. A two-tailed significance level of 5% was used. The Statistical Package for the Social Sciences (SPSS) ® version 21 (IBM Corp., Armonk, New York) was used for statistical analysis.

3. RESULTS

3.1 Patient characteristics

In total 30 patients with radiologically confirmed AFFs and 141 control patients of similar age and sex were included. Patient characteristics at time of AFF and fracture characteristics of the AFF group are described in **Table 3**. The fracture location was subtrochanteric in 12 cases (40%) and diaphyseal in 18 cases (60%). 24 of 30 patients with AFF (80%) had experienced complete fractures and 13 patients (43.3%) had bilateral AFFs. All patients had a history of bisphosphonate use. The mean age and duration of bisphosphonate use of the AFF patients at time of DXA were significantly higher than at time of AFF, p=0.002 and p=0.022 respectively. On average AFF patients were 1.9 years older and had used bisphosphonates 9.5 months longer at time of DXA compared to time of AFF, meaning that DXA measurements were usually obtained after the occurrence of AFF and that bisphosphonates were not always stopped at the time of AFF.

Patient characteristics at the time of the DXA are described in **Table 4** for both the AFF group and the control group. Data on BMD at the lumbar spine were missing in one control patient and at the femoral shaft in five AFF patients. Patients with AFF had a higher BMI (p=0.015) and had used bisphosphonates longer than control patients (p<0.001). Mean duration of bisphosphonate use prior to DXA in the AFF group was 9.8 (SD 4.6) years, ranging from five months to 18.3 years. In the control group the mean duration was 6.0 (SD 3.8) years, ranging from 4.5 months to 17.9 years. The most frequently prescribed bisphosphonate was alendronate in both the AFF (80%) and the control (68.8%) group. Glucocorticoid use for more than three months (p=0.031) and PPI use (p=0.010) were more prevalent among AFF patients, 56.7% and 76.7% in AFF patients vs. 35.5%

and 51.1% in controls, respectively. Unadjusted sex-specific T-scores were significantly different between cases and controls at the lumbar spine (p<0.001) and borderline significant at the femoral neck (p=0.050), with higher T-scores in AFF cases. After adjustment for age, height and weight, sex-specific T-score at the lumbar spine remained significantly higher (p=0.004) in patients with AFF than in controls, while at the femoral neck this difference was not significant (p=0.190). In 124 of 141 (87.9%) controls no signs of an incomplete AFF were visible on extended femur scans by DXA, which has been a screening method in bisphosphonate users in the Bone Center of Erasmus MC since June 2014.[18]

Table 3 Patient characteristics[a] at time of first atypical femur fracture and fracture characteristics of the AFF group

AFF patients (n=30)	At time of AFF	At time of DXA	p value
Age (years)	61.2 (13.5)	63.1 (12.8)	0.002
Bisphosphonate use (months)	107.5 (55.1)	117.0 (55.0)	0.022
Prodromal pain present, n (%)	15 (50%)		
Location			
Subtrochanteric, n (%)	12 (40%)		
Diaphyseal, n (%)	18 (60%)		
Morphology			
Complete, n (%)	24 (80%)		
Incomplete, n (%)	6 (20%)		
Bilateral, n (%)	13 (43.3%)		

[a] *Mean (SD) unless otherwise noted.*

AFF=atypical femur fracture; DXA=dual energy x-ray absorptiometry; BMI=body mass index;

3.2 Trabecular Bone Score

TBS was calculated in 24 AFF patients and 135 controls. Six AFF patients and six controls were excluded from TBS analysis, because parameters fell outside the working range of TBS. TBS was significantly correlated with age (r=-0.227, p=0.004) and lumbar spine BMD (r=0.216, p=0.006). There was no relation between TBS and BMI. TBS comparisons between AFF cases and controls are displayed in **Table 5**. Mean TBS was not statistically different between the AFF group and the control group (p=0.647). No significant difference was found after adjustment for sex, age, height, weight, ethnicity, spine BMD, duration of bisphosphonate use, glucocorticoid use and time difference between DXA scanning and diagnosis of AFF.

Table 4 Patient characteristics[a] at time of DXA of the AFF group and the control group

	AFF (n=30)	Controls (n=141)	p value
Female, n (%)	20 (66.7%)	80 (56.7%)	0.316
Age, years	63.1 (12.8)	59.4 (14.3)	0.189
Height, cm	164.9 (10.6)	168.2 (10.4)	0.122
Weight, kg	78.5 (22.7)	72.1 (15.5)	0.150
BMI, kg/m²	28.6 (6.5)	25.4 (4.6)	0.015
Caucasian, n (%)	26 (86.7%)	123 (87.2%)	0.933
Postmenopausal, n (%)	17 (85.0%)	68 (85.0%)	1.000
Current smoking, n (%)	5 (16.7%)	24 (17.0%)	0.963
Bisphosphonate duration, months	117 (55)	71.5 (45)	<0.001
Alendronate, n (%)	24 (80.0%)	97 (68.8%)	0.220
Glucocorticoid use more than 3 months, n (%)	17 (56.7%)	50 (35.5%)	0.031
PPI use, n (%)	23 (76.7%)	72 (51.1%)	0.010
BMD L1-L4, g/cm²	1.127 (0.213)	0.981 (0.177)	<0.001
BMD L1-L4[b], g/cm²	1.101 (0.181)	0.986 (0.177)	0.002
BMD L1-L4, T-score	-0.566 (1.753)	-1.804 (1.460)	<0.001
BMD L1-L4 [c], T-score	-0.848 (1.508)	-1.745 (1.462)	0.004
BMD femoral neck, g/cm²	0.863 (0.115)	0.798 (0.156)	0.051
BMD femoral neck[b], g/cm²	0.848 (0.142)	0.801 (0.140)	0.133
BMD femoral neck , T-score	-1.408 (0.798)	-1.880 (1.145)	0.050
BMD femoral neck [c], T-score	-1.551 (1.06)	-1.855 (1.033)	0.190

[a] *Mean (SD) unless otherwise noted.*
[b] *Adjusted for age, sex, height and weight*
[c] *Adjusted for age, height and weight*
AFF=atypical femur fracture; BMI=body mass index; PPI=proton pump inhibitor; BMD=bone mineral density.

A sensitivity analysis in a subgroup of seven AFF patients showed that TBS calculated from a previous DXA scan closer to the time of diagnosis of AFF, but from a DXA machine that was not calibrated for TBS, was higher compared to the more recent scans used in the main analysis in all cases.

Table 5 Comparison of mean TBS (SD) between patients with and without atypical femur fractures

	AFF (n=24)	Controls (n=135)	p-value
TBS	1.211 (0.154)	1.198 (0.124)	0.647
TBS[a]	1.225 (0.127)	1.195 (0.128)	0.296
TBS[b]	1.242 (0.152)	1.192 (0.128)	0.143

AFF=atypical femur fracture; TBS=trabecular bone score
[a] *Adjusted for sex and age*
[b] *Adjusted for sex, age, ethnicity, height, weight, spinal bone mineral density, duration of bisphosphonate use, glucocorticoid use and time difference between DXA scanning and diagnosis of AFF*

3.3 Hip Structural Analysis

HSA was analyzed in 23 AFF patients and 137 controls. HSA could not be performed in seven AFF patients and four controls, because no DXA of the hip was available (four AFF patients), the presence of a hip prosthesis (one AFF patient), poor positioning (one AFF patient) or the DXA file could not be converted to HSA format (one AFF patient and four controls).

The results of the HSA are described in **Table 6**. At the femoral shaft there were no significant differences between cases and controls, regardless of adjustment for potential confounders. At the narrow neck region of interest, the BMD (p=0.034), average cortex (p=0.034) and centroid position (p=0.047) were higher in the AFF group, while the buckling ratio (p=0.015) was lower in the AFF group. After adjustment for sex, age, height, weight, ethnicity, duration of bisphosphonate use and glucocorticoid use, AFF patients had a significantly lower buckling ratio (p=0.036) and a higher centroid position (p=0.012), whilst the differences in BMD (p=0.14) and average cortex (p=0.143) were no longer significant.

Similarly, at the intertrochanteric region of interest, the buckling ratio was significantly lower (p=0.039) and the centroid position was significantly higher in the AFF group (p=0.017). After adjustment, only the difference in centroid position remained significant (p=0.014).

3.4 Sex-stratified analyses

For TBS, we found no differences between AFF cases and controls when stratified by sex (data not shown).

For HSA, we found in men with AFF an increased centroid position at the narrow neck region compared to controls (p=0.015).In women with AFF we found a lower buckling ratio both at the narrow neck (p=0.030) and the intertrochanteric region (p=0.047) and increased centroid position at the intertrochanteric region (p=0.026) compared to controls. No other differences were found.

Table 6 Comparison of crude and adjusted means of HSA parameters between patients with and without atypical femur fractures

	Crude mean (SD)		Adjusted mean (SD)[a]	
	AFF (n=23)	**Control (n=137)**	**AFF (n=23)**	**Control (n=137)**
NSA (°)	129.84 (5.59)	131.56 (5.56)	129.15 (2.22)	131.68 (13.91)
Neck length (cm)	5.44 (0.74)	5.50 (0.74)	5.46(0.64)	5.50 (0.60)
Narrow Neck Region				
BMD (g/cm²)	0.74 (0.13)*	0.67 (0.14)*	0.72 (0.14)	0.67 (0.13)
CSA (cm²)	2.23 (0.72)	2.08 (0.49)	2.17 (0.42)	2.09 (0.39)
CSMI (mm⁴)	2.02 (0.72)	1.97 (0.77)	1.95 (0.53)	1.99 (0.50)
Outer diameter (cm)	3.17 (0.37)	3.26 (0.33)	3.17 (0.24)	3.26 (0.22)
Section Modulus (cm³)	1.16 (0.32)	0.09 (0.34)	1.13 (0.25)	1.10 (0.23)
Endocortical diameter (cm)	2.89 (0.37)	3.00 (0.34)	2.89 (0.26)	3.00 (0.25)
Average cortex (cm)	0.14 (0.03)*	0.13 (0.03)*	0.14 (0.03)	0.13 (0.02)
BR	12.55 (2.80)*	14.67 (3.95)*	12.78 (3.83)*	14.63 (3.60)*
Centroid position	0.46 (0.03)*	0.45 (0.02)*	0.47 (0.02)*	0.45 (0.02)*
Intertrochanteric Region				
BMD (g/cm²)	0.71 (0.11)	0.65 (0.15)	0.68 (0.14)	0.66 (0.13)
CSA (cm²)	3.81 (0.70)	3.54 (0.94)	3.64 (0.75)	0.57 (0.70)
CSMI (mm⁴)	11.64 (3.64)	10.99 (4.25)	10.98 (2.66)	11.10 (2.49)
Outer diameter (cm)	5.66 (0.48)	5.71 (0.53)	5.66 (0.36)	5.71 (0.34)
Section Modulus (cm³)	3.59 (0.85)	3.29 (1.06)	3.41 (0.72)	3.32 (0.67)
Endocortical diameter (cm)	5.09 (0.48)	5.18 (0.52)	5.11 (0.39)	5.18 (0.36)
Average cortex (cm)	0.28 (0.05)	0.26 (0.06)	0.27 (0.06)	0.27 (0.06)
BR	11.62 (2.40)*	13.24 (3.58)*	12.08 (3.54)	13.16 (3.32)
Centroid position	0.44 (0.03)*	0.42 (0.02)*	0.44 (0.02)*	0.42 (0.02)*
Femoral Shaft Region				
BMD (g/cm²)	1.19 (0.18)	1.12 (0.23)	1.15 (0.19)	1.13 (0.18)
CSA (cm²)	3.57 (0.63)	3.37 (0.78)	3.43 (0.55)	3.40 (0.52)
CSMI (mm⁴)	3.49 (1.21)	3.25 (1.10)	3.33 (0.72)	3.28 (0.68)
Outer diameter (cm)	3.16 (0.31)	3.14 (0.25)	3.15 (0.20)	3.14 (0.19)
Section Modulus (cm³)	2.11 (0.54)	1.98 (0.54)	2.02 (0.35)	2.01 (0.33)
Endocortical diameter (cm)	2.33 (0.36)	2.36 (0.29)	2.35 (0.30)	2.35 (0.28)
Average cortex (cm)	0.42 (0.07)	0.39 (0.09)	0.40 (0.08)	0.40 (0.07)
BR	4.03 (1.00)	4.33 (1.12)	4.19 (1.12)	4.30 (1.05)
Centroid position	0.49 (0.01)	0.49 (0.01)	0.49 (0.01)	0.49 (0.01)

* $p<0.05$

[a] *Adjusted for ethnicity, sex, age, height and weight, duration of bisphosphonate use and glucocorticoid use. NSA and centroid position were only adjusted for ethnicity, sex, age, duration of bisphosphonate use and glucocorticoid use.*

AFF=atypical femur fracture; NSA=neck shaft angle; BMD=bone mineral density; CSA=cross sectional area; CSMI=cross sectional moment of inertia; BR=buckling ratio.

4. DISCUSSION

This retrospective case-control study used TBS and HSA to assess bone quality and hip geometry in patients with AFFs in comparison with controls using bisphosphonates of similar age and sex. TBS did not differ between patients with AFF and controls. Also, the HSA showed no significant differences at the femoral shaft, which is the fracture location of AFFs, nor differences in neck shaft angle. Some parameters by HSA showed a trend towards more favorable geometry in AFF patients at the femoral neck and the intertrochanteric region, the site of classical hip fractures.

To our knowledge, this study is the first to compare TBS between AFF patients and controls using bisphosphonates. Although TBS is measured at the lumbar spine, lower TBS has been shown to predict osteoporotic fractures of the hip.[25, 26] We expected TBS to be decreased in AFF patients, because the fracture characteristics of AFFs (non-traumatic fracture with a transverse fracture line) are compatible with reduced bone material strength. Also, a low trabecular bone volume was detected in bone biopsies of AFF patients using histomorphometry.[27] Moreover, the AFF group had used glucocorticoids more frequently and during a longer period of time, which is associated with reduced TBS.[28, 29]

Our finding on TBS analysis is consistent with an earlier study on trabecular bone microarchitecture using HR-pQCT, which showed no differences between 20 AFF patients and 35 postmenopausal women using long-term bisphosphonates without AFF.[30] In another study, change of TBS over time was analyzed in response to teriparatide treatment in 14 patients with AFF. Pretreatment baseline TBS measurements varied widely among the individuals, and did not change after two years of teriparatide.[31]

As mentioned above, it is possible that TBS of the spine does not accurately reflect trabecular architecture at the femur. It is also conceivable that AFF is mostly related to abnormalities in cortical bone structure, since the femoral shaft contains more cortical than trabecular bone and AFFs are characterized by periosteal thickening of the cortex [1], possibly to compensate for an impairment of cortical material strength. This is supported by a study using in vivo microindentation to asses material properties of bone tissue in six patients with AFF.[32] In this study, a non-significant trend was found towards deterioration of material properties of the tibia in AFF patients. Further research is needed to evaluate cortical bone quality in AFF patients by HR-pQCT scanning or microindentation.

We expected to find differences in geometry at the femoral shaft, being the fracture localization of AFFs. However, HSA in this region was not different between cases and controls. This finding is consistent with a Chinese study by Chou *et al.* on HSA among 31 patients with AFF compared to 31 sex- and age-matched long-term bisphosphonate users without AFF.[33] However, the results of this paper are not generalizable, since the study population was predominantly Chinese[34] and it is known that Asians have different femoral neck structure than Caucasians.[22, 35] Possibly, no differences in hip geometry at the femoral shaft measured by HSA exist between AFF patients and controls using bisphosphonates or differences are very small and can only be detected in a larger sample size.

Previous studies suggested that AFF patients have a more varus neck shaft angle of the femur based on classical X-rays.[36-38] We found no difference in neck shaft angle between AFF patients and controls, similar to the aforementioned Chinese study by Chou *et al.* [33] This could be due to differences in positioning of the femur or in methodology, using a software algorithm on DXA versus manual measurement on X-rays.

Although we were primarily interested in bone geometry at the femoral shaft, we also evaluated the narrow neck and the intertrochanteric region. At the narrow neck, we found a lower buckling ratio in AFF patients, consistent with the increased generalized cortical thickness of the femur reported in AFF patients. The buckling ratio is defined as the ratio of the outer radius to the wall thickness. A lower buckling ratio relates to a lower susceptibility of the bone to buckling (bending). Furthermore, the centroid position was higher in AFF patients at both the narrow neck and intertrochanteric regions of interest. Centroid position is a parameter of symmetry of the bone. A higher centroid position (>0.5) indicates a more laterally located center of mass, whilst a lower (more medial) centroid position is associated with increased risk of classical hip fractures.[39] This, combined with the lower buckling ratio suggests that patients with AFF have more favorable hip geometry in the narrow neck region compared to controls. This may be explained by better baseline hip geometry in AFF patients using bisphosphonates for prevention of glucocorticoid-induced osteoporosis, instead of treatment of osteoporosis. Indeed, 56.7% of AFF patients used glucocorticoids for more than three months compared to 35.5% of control patients. Also, AFF patients were treated significantly longer with bisphosphonates than controls. Bisphosphonate use for one and two years was shown to improve geometric parameters assessed by HSA[40, 41], although it is not known if parameters keep improving after longer duration of treatment.

The findings of more favorable bone geometry are in line with our finding that the T-score at the lumbar spine was higher in AFF patients than in controls (p=0.004), also

after adjusting for age, height and weight. A relatively high BMD in patients with AFF has been reported in previous studies[32, 42], but is unlikely to be merely explained by long-term use of bisphosphonates since BMD increases only during the first three years of bisphosphonate use.[43]

4.1 Strengths and limitations

Important strengths of our study include the radiographic adjudication of AFFs to assure the diagnostic criteria and the use of extended femur scans by DXA to minimize the possibility of yet unknown incomplete AFFs in controls. Also, we used a relatively large number of control patients with confirmed bisphosphonate use and all DXA scans were made using the same machine by two licensed DXA technicians.

The main limitation of our study is that in 27 of 30 AFF patients DXA scans were included that were made after the diagnosis of AFF, in 15 patients even more than six months later, due to the retrospective design of this study. We have adjusted for the time difference between the moment of DXA scanning and diagnosis of AFF. Yet we cannot rule out that TBS would have been lower in AFF cases at the time of AFF, since we did not perform TBS measurement just prior to the diagnosis. A subgroup analysis in seven AFF patients with available DXA scans prior to the atypical fracture, showed that TBS was not lower, but even higher compared to TBS after the fracture. Nevertheless, this analysis may not be reliable, because these older DXA scans were made on a DXA machine that was not calibrated for TBS software. A lower TBS prior to the AFF is also less likely because TBS is known to decline during aging. Possibly TBS might also have decreased over time because of diminished mobility after the occurrence of AFF.

Furthermore, HSA has some limitations. First, HSA has large variabilities due to differences in femur rotation[44], which makes it difficult to distinguish small differences in dimensions from differences due to position. Secondly, hip geometry is three-dimensional, but HSA is based on a two-dimensional DXA image. Lastly, the image quality of the DXA scans may in some cases be insufficient to determine the exact edge margins of the bone.[19]

Finally, the AFF group had used bisphosphonates longer and glucocorticoids more frequently than the control group, but adjusting for these covariates did not change the results of the TBS analysis and the HSA parameters at the femoral shaft.

5. CONCLUSIONS

This case-control study showed no differences in trabecular microarchitecture measured with TBS between patients with AFF and controls using bisphosphonates without AFF. Also, hip geometry assessed by HSA did not differ at the femoral shaft between cases and controls. Based on the results of this study, trabecular microarchitecture and femoral shaft geometry appear not to be related to the occurrence of AFFs in patients using bisphosphonates. TBS and HSA may thus not have value in detecting patients at risk of developing AFFs. However, further research is needed to evaluate TBS and HSA in larger prospective studies and in interaction with other potential risk factors like genetic susceptibility.

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3

“Atypical” atypical femur fractures and use of bisphosphonates

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ABSTRACT

Background. Atypical femur fractures (AFFs) present a rare but serious condition associated with use of bisphosphonates. Underlying mechanisms and clinical risk factors remain unclear. According to the diagnostic criteria formulated by the ASBMR, a lateral localization of an AFF is required.

Case history. We present a patient who developed bilateral leg pain while using an oral bisphosphonate and aromatase inhibitor in the course of adjuvant treatment for breast cancer. Initially she was diagnosed with bone metastases and received radiotherapy on the right femur. However, the bilateral periosteal reactions of the subtrochanteric femur are highly suggestive of AFFs. Our case meets all criteria for AFF except that she presented with lesions at the medial side of the femur. Therefore they could be best described as "atypical" atypical femur fractures.

Discussion. Since the pathogenesis of AFFs is not fully understood, we cannot rule out that AFFs also occur in the medial femur or in other weight-bearing bones. Hence we propose that medial stress reactions belong to a spectrum of atypical fractures associated with use of antiresorptive drugs. The localization may depend on yet unknown biomechanical factors.

Conclusion. We propose that these periosteal reactions of the subtrochanteric femur are in fact AFFs with uncommon medial localization and could hence be considered "atypical" AFFs. We recommend being alert of AFFs in patients with bone pain and medial subtrochanteric lesions. More epidemiological studies are needed to investigate the occurrence of both medial and lateral AFFs and to gain more insight into its frequency and pathogenesis.

1. BACKGROUND

Bisphosphonates are highly effective for prevention of osteoporotic fractures and are therefore used by millions of patients worldwide for treatment of osteoporosis. A recent concern is that especially long-term use of bisphosphonates is associated with the occurrence of atypical femur fractures (AFFs).[1-4] This rare but severe condition involves a low-energetic subtrochanteric fracture, which often affects both legs and shows delayed healing.[5,6]

Although AFFs occur more often in patients using bisphosphonates, no causal relationship has yet been demonstrated. Occasionally AFF have been observed in bisphosphonate-naïve individuals.[4] At present underlying mechanisms and specific clinical risk factors remain unclear. It has been suggested that AFFs are stress or insufficiency fractures.[5,6] Unlike classical stress fractures that are seen in athletes, it is thought that AFFs originate from the lateral cortex of the femur.[8] A lateral localization of AFFs is even required to fulfill the diagnostic criteria as formulated by the American Society for Bone and Mineral Research (ASBMR).[5,6]

We report a case of what could be best described as an “atypical” AFF, since it meets the ASBMR criteria except for medial instead of lateral localization.

2. CASE HISTORY

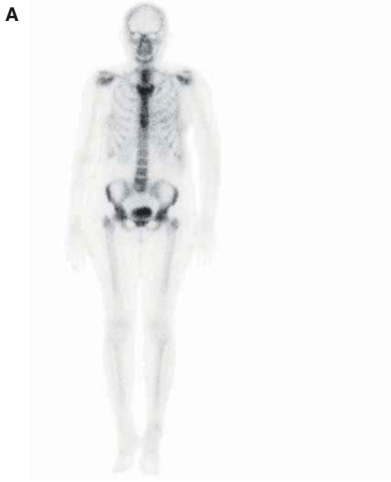
A 50-year-old woman of Iraqi descent was treated elsewhere for stage III ductal mamma carcinoma. She underwent mastectomy with axillary lymph node dissection in January 2008. In July 2008 she completed six regimens of adjuvant TAC-chemotherapy (docetaxel, adriamycin, cyclophosphamide). She was subsequently treated with local radiation therapy of the breast and tamoxifen. After three months she switched to anastrozol because of side effects. Given the use of this aromatase-inhibitor a DXA-scan was performed in June 2009, which showed osteoporosis (T-score lumbar spine -2.5 SD, T-score left femur neck -1.3 SD). She was prescribed calcium and vitamin D at first, followed by alendronate 70 mg weekly in April 2010. She had several clinical risk factors for osteoporosis, including inadequate calcium intake, positive family history for osteoporosis and low BMI (<17 kg/m2) during adolescence. Evaluation of her bone status is shown in **Table 1.**

Table 1: General characteristics and laboratory measurements

Clinical and laboratory findings[i]		Reference
Age (yrs)	50	-
Ethnicity	Asian	-
Weight (kg)	61	-
Length (cm)	151	-
Body Mass Index (kg/m²)	26.8	20-25
25-OH-vitamin D (nmol/l)[ii]	49	50-120
P1NP (µg/l)	10	19-102
Total alkaline phosphatase (U/l)	44	0-97
Bone-specific alkaline phosphatase (µg/l)	8	0-22.4
Serum C-terminal telopeptide (µg/l)	0.08	0-0.56

[i]All bone markers were measured one year after the patient had received radiation therapy and eight zoledronic acid infusions. [ii]Whilst on vitamin D suppletion

In October 2010 she presented with pain in her left hip without any prior trauma. A previous bone scan taken in 2008 during staging of breast cancer was normal (**Figure 1A**). Now, a radiograph of the pelvis showed no abnormalities (**Figure 1B**), nor did CT and MRI of the pelvic area. In contrast, a new bone scintigraphy demonstrated increased uptake at the medial side of the proximal left femur (**Figure 2A**). Also, a focus of increased uptake in the ribs was noted. A repeated radiograph of the left femur in April 2011 was initially interpreted as normal. However, in retrospect, it showed localized cortical thickening at the site of the hotspot (**Figure 2B**). In February 2011 she developed pain in her right groin and upper leg as well. At this time a bone scan also displayed a hotspot at the



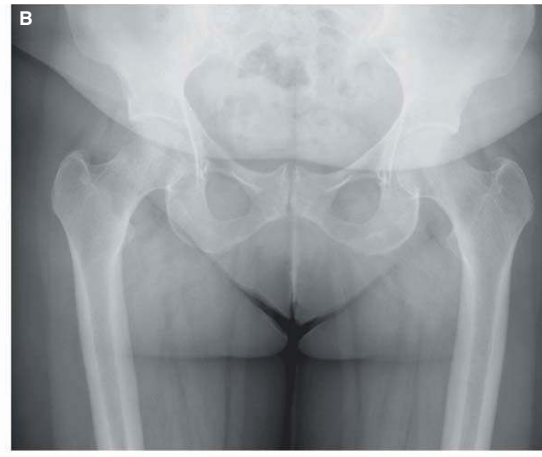


Figure 1A **Figure 1B**

1A: Bone scintigraphy during screening without abnormalities in January 2008.
1B: Radiograph of pelvis showing no abnormalities in October 2010.

medial side of the right proximal femur (**Figure 3A**). MRI revealed a signal abnormality in the right femur corresponding with the location of increased uptake. A diagnosis was made of metastatic bone disease from primary breast cancer. There were no signs of metastases to other organs on abdominal and chest CT. She received radiation therapy once on the right upper leg in May 2011 and she was further treated with i.v. zoledronic acid 4 mg monthly. A radiograph taken of the right femur in September 2011 appeared normal at first, but in hindsight it also showed discrete localized cortical thickening at the medial aspect of the femur (**Figure 3B**).

In January 2012 the aching in both upper legs had considerably worsened. Another bone scan was performed on which the hotspot on the right side had increased with a linear uptake (**Figure 4A**). On MRI the radiologist reported bilateral periosteal reactions at the same level as the hotspots on bone scintigraphy. The diagnosis of bone metastases was then called into question.

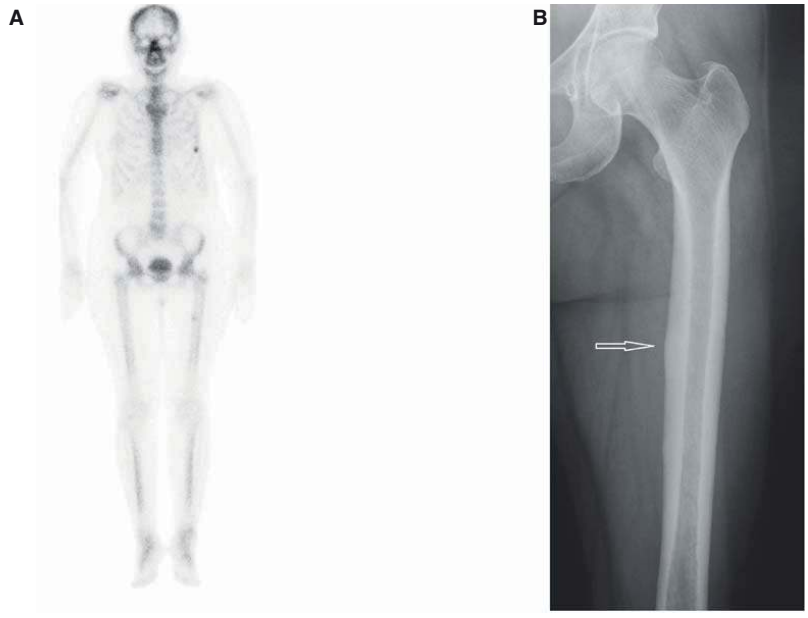


Figure 2A **Figure 2B**

2A: Bone scintigraphy with a hotspot at the medial side of the left femur in November 2010.
2B: Radiograph of the left femur, in retrospect showing localized cortical thickening at the medial aspect of the femur (arrow) in April 2011.

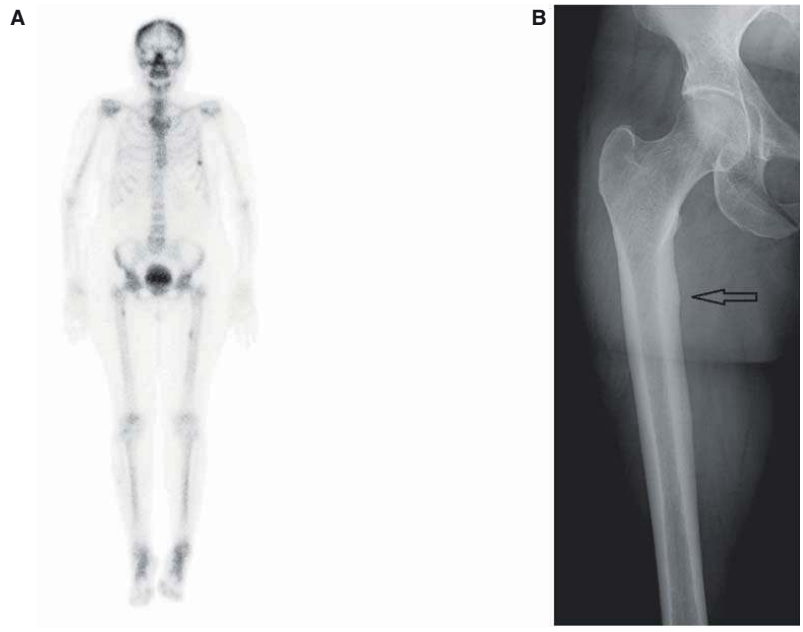


Figure 3A **Figure 3B**

3A: Bone scintigraphy showing a new hotspot at the medial side of the right femur in March 2011.
3B: Radiograph of the right femur, in retrospect showing localized cortical thickening at the medial side (arrow) in September 2011.

First of all, it was remarkable that conventional radiographs consistently did not show any lesions that one would expect in metastatic bone disease. Furthermore, the abnormalities on bone scintigraphy, MRI and radiographs had an uncharacteristic localization for disseminated breast cancer. Moreover, tumor marker CA15-3 that was elevated preoperatively was within normal ranges during follow-up. Hence it was proposed that the patient might have insufficiency fractures. Zoledronic acid was discontinued after eight infusions in total.

In February 2012 an incomplete oblique fracture line was observed on radiographs of the right femur without signs of consolidation **(Figure 4B**). After she was referred to the Bone Center of our Hospital, we made the presumptive diagnosis of bilateral 'atypical' atypical femur fractures associated with the use of bisphosphonates. She was prescribed strontium ranelate, whereupon the pain in both legs diminished and the fracture line healed slowly over the course of six to eight months.

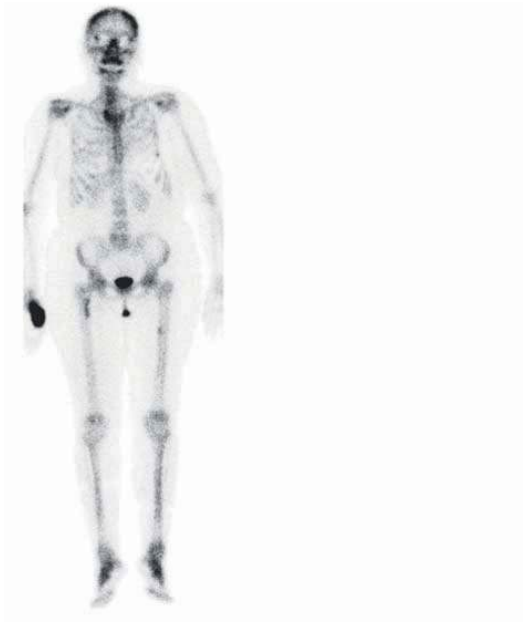


Figure 4A

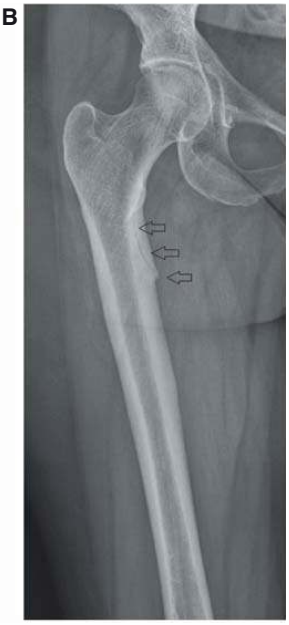


Figure 4B

4A: Bone scintigraphy showing increased linear uptake in the right femur in January 2012.
4B: Radiograph of the right femur showing an incomplete fracture line originating from localized cortical thickening at the medial side (arrows) in February 2012.

3. DISCUSSION

We present a patient who developed sequential bilateral leg pain while using an oral bisphosphonate and an aromatase inhibitor in the course of adjuvant treatment for breast cancer.

Based on increased uptake in both femora at the medial side on a bone scan and a localized abnormality on MRI of the right femur, she was diagnosed with bone metastases. Subsequently she was treated with zoledronic acid and local radiation therapy on her right leg. Only several months after the hotspots were noted on the bone scans, a localized periosteal reaction became visible on plain radiographs and MRI of the femora. Apart from the medial localization, this case fulfills all of the major ASBMR criteria for an AFF: a subtrochanteric, non-comminuted, incomplete fracture, non-traumatic and with localized periosteal reaction at the fracture site.[5,6] Moreover, our patient displayed all four minor criteria: she experienced prodromal pain in the groin and thigh, radiographs showed increased generalized thickness of her femoral cortices, she had bilateral symptoms and delayed healing of the fracture. The fracture line that became visible after radiation on the right leg had a predominantly oblique orientation instead of the more

classical transverse orientation. This may be explained by the effects of the previous radiotherapy. The ASBMR criteria specifically exclude pathological fractures related to primary or metastatic bone tumors. Although the existence of bone metastases was not definitively excluded by a bone biopsy, the disease course makes the existence of bone metastases highly unlikely. The small focus in the ribs remained stable on bone scans during follow-up and was therefore interpreted as degenerative disease.

The pathophysiology of AFFs is not fully understood. Because of the periosteal reaction and transverse orientation, AFFs are considered stress or insufficiency fractures. Strictly, these are two different fracture types.[5,9] Stress or "fatigue" fractures result from abnormal loading in an individual with normal bone quality. In contrast,insufficiency fractures involve poor bone quality and normal loading. Exercise-induced stress fractures are preferentially located along the medial femur, as a result of excessive medial compression force during weight bearing.[8] It is hypothesized that AFFs are the result of failure in bone with abnormal microarchitecture due to increased tensile forces on the lateral femoral side. A link between AFFs and biomechanical factors is apparent considering the often bilateral occurrence with a parallel fracture location in the contralateral femur. It has been suggested that a greater curvature of the femora or malalignment of the mechanical axes predispose patients to an AFF at the lateral side.[10]

Bone quality is most likely compromised in patients with AFF, since these patients are usually prescribed bisphosphonates or denosumab. These antiresorptive drugs are indicated when bone strength is reduced. However, the use of these antiresorptives might eventually have a negative impact on bone quality by inhibiting repair mechanisms within the bone. It is known that bisphosphonates change the bone matrix composition, leading to highly mineralized collagen. This may result in a generalized suppression of bone turnover.[11-14] Additionally, bisphosphonates accumulate at sites of high bone remodeling including sites of stress fractures. This could affect the intracortical repair of a developing stress fracture and ultimately lead to a complete fracture.[5,6,11,15]

We present a patient with unusual spontaneous periosteal reactions of both medial femora. Only recently a case was reported very similar to our patient. It illustrated a medial defect of the subtrochanteric femur without prior trauma in an 81-year-old woman with groin and thigh pain.[16] This patient had used the bone resorption inhibitor denosumab. Comparably, the lesion was initially not observed on femoral radiographs. These cases raise the question whether it is correct that the definition of AFF includes only fractures originating from the lateral sides of the femoral cortex. It is conceivable that the localization where these fractures develop are dependent on femur shape or other factors related to bone geometry. Based on the current diagnostic criteria, all

medially located fractures are a priori not labeled as AFFs. This approach may overlook fractures with atypical features and a medial localization.

Insufficiency fractures related to bisphosphonate-induced suppression of bone turnover have also been reported at sites other than the femoral diaphysis, including the pelvis, ankle, metatarsals and long bones such as the humerus, fibula and tibia.[17,18]

Recently, a case was described of a patient on long-term bisphosphonate therapy who presented with a diaphyseal tibial insufficiency fracture. Again, this case fulfills all the major criteria except for the location. The authors discussed the need for greater awareness of the possibility of atypical fractures at other sites than the femur, especially in weight-bearing bones.[18] Likewise we suggest that one should be aware that also fractures on the medial side of the femur may be considered atypical fractures associated with bisphosphonate use. If not recognized as such, a misdiagnosis like metastatic bone disease in our patient can be a problematical result.

Based on our case and the case on a medial fracture after denosumab use, we suggest that more attention should be given to the potential presence of AFFs arising from the medial cortex as well as from the lateral cortex, both in clinical practice and in research. If more of such cases are identified, this may lead in the future to a modification of criteria for AFF as developed by the ASBMR task force. We propose that both medial and lateral fractures may result from suppression of bone turnover in patients with already compromised bone strength with the exact fracture localization depending on local biomechanical or other, yet unidentified, factors.

4. CONCLUSION

We report a bilateral periosteal reaction of the subtrochanteric femur in our patient treated with bisphosphonates and an aromatase inhibitor suggestive of AFFs, though not meeting the current diagnostic criteria because of its medial localization. We propose that these fractures are in fact AFFs with an uncommon medial localization and could hence be considered “atypical” AFFs.

Although a causal association has not been demonstrated in our patient nor of AFF in general, we cannot rule out that medial stress reactions belong to a spectrum of atypical fractures that are associated with treatment with antiresorptive drugs. The localization may depend on yet unknown biomechanical factors. We recommend being alert of AFFs in patients with bone pain and medial subtrochanteric lesions. More epidemiological

studies are needed to investigate the occurrence of both medial and lateral AFFs and to gain more insight into its frequency and pathogenesis.

Declaration of interest: There is no potential conflict of interest.
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4

Screening for atypical femur fractures using extended femur scans by DXA

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ABSTRACT

Atypical femur fractures (AFFs) are a rare but serious complication associated with the use of antiresorptive drugs such as bisphosphonates. Assessment of incomplete AFFs on extended femur scans by Dual X-ray Absorptiometry (DXA) may prevent the development of complete fractures. The aim of this study was to evaluate the potential of extended femur scans by DXA as a screening tool for incomplete AFFs. From June 2014 until September 2016 extended femur scans were routinely performed in all consecutive patients undergoing DXA scanning who had used bisphosphonates or denosumab at any given moment in the previous year. When "beaking" was found, defined as a localized periosteal or endosteal thickening of the lateral cortex, a radiograph of the femur was performed to confirm incomplete AFF. Beaking was detected in 12 out of 282 patients (4.3%) with extended scans of both femora. In nine patients (3.2%) beaking corresponded with the radiological presence of incomplete AFFs, of whom four already had an X-ray made because of a previous complete AFF of the other leg. Five patients (1.8%) were newly diagnosed with six yet unknown incomplete AFFs. No additional X-ray was performed in two patients because of loss of follow-up. Beaking was explained by known soft tissue calcifications in one patient. The positive predictive value of beaking on extended femur scan was 83.3% in our study.

Three cases in whom the new diagnosis of incomplete AFF has affected medical and surgical treatment are further discussed to illustrate the relevance of early detection.

We conclude that extended femur scans by DXA can detect incomplete AFFs in patients on antiresorptive treatment and should therefore be considered a clinically relevant screening tool since early identification of AFFs has therapeutic consequences.

INTRODUCTION

Bisphosphonates are a key element in the treatment of osteoporosis and used by millions of patients worldwide. Clinical trials have shown that they improve bone mineral density (BMD), prevent vertebral fractures and reduce the risk of hip and other non-vertebral osteoporotic fractures in postmenopausal women.[1-4] They are also known to decrease mortality independently of fracture reduction.[5-7]

Since the introduction of bisphosphonates in clinical practice two decades ago, there have been reports of several serious adverse events possibly related to the use of bisphosphonates such as osteonecrosis of the jaw[8], atrial fibrillation[9] and atypical femur fractures (AFFs).[10] AFFs are rare fractures located below the lesser trochanter that occur after minimal or no trauma associated with the use of bisphosphonates. Patients may experience prodromal groin or thigh pain. AFFs often occur bilaterally and may show delayed fracture healing.[11] Although AFFs are considered an adverse effect of (long-term) treatment with bisphosphonates, no causal link has been demonstrated yet and they are reported in bisphosphonate-naïve patients as well. AFFs have also been reported amongst patients on newer anti-osteoporosis drugs such as denosumab.[11] In a large Swedish cohort study with radiographic adjudication of fracture characteristics amongst women 55 years of age or older with a femoral fracture, incidence rates of AFF for bisphosphonate-users were 55 per 100,000 person-years compared to 1 per 100,000 person-years for bisphosphonate-naïve patients.[12] Thus, the absolute risk of AFF remains very low and is 30- to 100-fold less than that for osteoporotic hip fracture among untreated persons at risk.[3,11] Hence in most patients the benefits of bisphosphonate treatment outweigh the risk of AFF.

Recent studies however suggest that the safety concerns on bisphosphonates have most likely contributed to a drastic decline of more than 50% in use of oral bisphosphonates between 2008 - 2012 in the United States, possibly related to the negative publicity on bisphosphonates in the (social) media.[13,14] The ASBMR, supported by several other organizations in the field of bone health, has recently called out a “crisis in the treatment of osteoporosis”, partly related to this fear of side effects such as AFF.[15,16] Treating physicians need to reassure their patients, encourage them to continue treatment and thus maintain therapeutic compliance. The potential occurrence of AFF in bisphosphonate treatment might be monitored by a screening tool for early detection of incomplete forms of AFF.

A case description in 2010 first suggested that an extended femur scan on Dual-Energy X-ray Absorptiometry (DXA) may visualize incomplete forms of AFF by showing localized

cortical thickening.[17] Subsequently, in 2013 a study amongst 257 patients using bisphosphonates for five years or more demonstrated that these scans were able to detect the presence of incomplete AFF.[18] Although a new software to identify AFFs has already been developed for some DXA scanners, there is still little scientific evidence available on the clinical utility of these extended femur scans by DXA.[19]

The aim of this study was to evaluate the value of extended femur scans as a screening instrument of incomplete AFFs in patients using short- or long-term antiresorptive therapy who underwent routine DXA scanning for therapeutic evaluation in the Bone Centre of Erasmus MC. We report the detection rate of incomplete AFFs on extended femur scans performed over a period of 2.25 years. We compare our findings on DXA with conventional X-rays of the femur. Lastly we describe the consequences for individual patients by highlighting three cases as examples of the clinical relevance of this screening technique in patient care.

METHODS

Patients

This retrospective study was performed in the Bone Centre of Erasmus MC, a tertiary referral centre for complex and rare diseases in the field of calcium and bone metabolism. Extended bilateral femur scans were routinely made in all patients undergoing DXA scanning for BMD assessment when they had used oral or intravenous bisphosphonates or denosumab at any given moment in the 12 months prior to the DXA scan. Medication use was verified by checking the medical records and confirmed by the patients during the appointment. The duration of treatment with antiresorptive drugs was not relevant for the decision to make the femur scan, meaning that also patients who had recently started antiresorptive treatment (e.g. prescribed by the referring physician) were included. We analysed the results of the scans since 1st of June 2014 until 1st of September in a total period of 2.25 years. The Medical Ethical Committee of Erasmus MC approved this study.

Extended femur scan

Dual-energy extended femur scans were performed by two licensed DXA technicians using the same DXA machine: GE Lunar Prodigy, software 14.10.022. The extended femur scans depict the lesser trochanter down to the supracondylar flare. The tissue types were neutralized for the entire image and lines from Region of Interest were removed to properly evaluate the femur scan. All extended femur scans were evaluated by the performing DXA technician whilst scanning and subsequently assessed by one physi-

cian. Femur scans were assessed on beaking (also called flaring), which is defined as localized periosteal or endosteal thickening of the lateral cortex, by visual inspection. If beaking was visible on DXA and evaluation of previous X-rays or other medical images did not explain this abnormality, an additional X-ray of the femur was ordered to confirm the presence of incomplete AFF. Incidental findings such as irregularities of the medial cortex are reported as well as they may lead to additional diagnostics. All medical records of the patients were checked for the occurrence of a complete or incomplete AFF in the past based on the available clinical correspondence and/or radiographs of the femora. In all cases of radiologically confirmed incomplete AFF it was evaluated whether patients had prodromal symptoms.

Exclusion

Extended femur scans were not assessed for beaking in patients who had surgical material in the femur due to a total hip replacement or an intramedullary rod. Patients with surgical material in both femora were therefore excluded from this study.

RESULTS

Study population

Extended femur scans of both legs were performed in 282 consecutive patients on antiresorptive therapy undergoing DXA scanning between the 1st of June 2014 and the 31th of August 2016.

Patient characteristics are summarized in **Table 1**. Patients with incomplete AFF on DEXA were nine years older and on longer duration of antiresorptive treatment compared to patients without AFF, but statistical testing for significant differences was not performed due to lack of power considering the small number of patients with incomplete AFF.

Beaking

Beaking of the femur was visible in 12 patients (4.3%) and bilaterally present in two patients. **Figure 1** shows an overview of findings in patients with beaking on extended femur scans.

Five patients with beaking already had X-rays available of the femur. Four of these patients were diagnosed with incomplete AFF just prior to the extended femur scan, since they all had sustained a complete AFF of the contralateral leg and thus were at increased risk of AFF. One patient was known since many years to have calcifications of the soft tissue on a pelvic X-ray that explained the false impression of beaking. These small

Table 1. Clinical characteristics of the study population.

Patient characteristics	Incomplete AFF on DXA (n=9)	No AFF (n=267)*
Female (%)	8 (88.9)	165 (61.8)
Age, median (IQR)	70.1 (64.3;74.8)	61 (51;69)
Length in cm, median (IQR)	160.5 (158;166)	167 (161;175)
Weight in kg, median (IQR)	65 (60.4;78.5)	70 (60;81.9)
BMI kg/m^2, median (IQR)	25.7 (25;27.1)	25 (21.7;28.3)
BMD lumbar (L2-L4) T-score, median (IQR)	-0.2 SD (-2.5;1.1)	-1.8 SD (-2.7;-0.6)
BMD femoral neck T-score, median (IQR)	-1.8 SD (-2.1;-1.2)	-1.9 SD (-2.5;-1.4)
BMD lumbar (L2-L4) Z-score, median (IQR)	1.5 SD (-1.8;2.9)	-0.8 SD (-2.0;0.1)
BMD femoral neck Z-score, median (IQR)	0.1 SD (-0.5;0.5)	-0.9 SD (-1.3;-0.1)
Alendronate use (%)	7 (77.8)	171 (64)
Ibandronate use (%)	1 (11.1)	25 (9.4)
Risedronate use (%)	1 (11.1)	82 (30.7)
Pamidronate use (%)	1 (11.1)	18 (6.7)
Clodronate use (%)	0	1 (0.4)
Zoledronate use (%)	1 (11.1)	36 (13.5)
Denosumab use (%)	2 (22.2)[i]	26 (9.7)
Duration of treatment years, median (IQR)	8.5 (6.5;10)	5 (3;7)
Corticosteroid use > 3 months (%)	5 (55.6)	125 (46.8)
Postmenopausal state (% of females)	8 (100)	142 (86.1)
Current smoker (%)	0	51 (19.1)
Former smoker (%)	4 (44.4)	67 (25.1)

* Excluding six patients with a former history of complete AFF
[i] Both patients have used alendronate prior to denosumab therapy

calcifications were suggestive for local fat necrosis due to an old trauma or injections and not suspicious for underlying pathology.

In five patients additional radiographs of the femur were ordered that confirmed the presence of six incomplete AFFs that had not been documented before. One patient with a new diagnosis of incomplete AFF had a previous record of a complete AFF of the other leg.

Two patients did not have follow-up investigation and therefore the diagnosis of AFF cannot be excluded nor confirmed. This involved a patient who had no prodromal symptoms and had already stopped bisphosphonates. Consequently, the treating physician decided not to perform additional diagnostics. The other patient died 10 days after the extended femur scan due to an unrelated disease.

Thus, of all 12 patients with beaking on the extended femur scan, nine patients (3.2%) eventually had the diagnosis of radiologically confirmed incomplete AFF in 10 femora, two patients lacked further investigation and one patient had soft tissue calcifications that explained the beaking. Six incomplete AFFs in five patients (1.8%) were newly diagnosed. In all known four cases of incomplete AFF on X-ray in the study population, beaking was visible on the scan of the affected femur.

Only two out of nine patients with incomplete AFF had spontaneously reported prodromal pain, whilst two patients acknowledged complaints after specific inquiry about prodromal symptoms.

Coincidental findings

Irregularities of the femoral cortex were noted in 15 patients which could be explained by previous surgery or femoral fractures. In three patients discrete focal irregularities of the medial cortex were seen and additional plain radiographs were performed. In none of these radiographs signs of a stress fracture or any other abnormality were seen except for a slight irregularity of the medial cortex that was interpreted by the radiologist as physiological in these patients. Three patients had unreliable extended femur scans due to motion artefacts. One patient had scans that could not be properly evaluated due to a technical error. One patient had a notable generalized increase in cortical thickness that corresponded with known Paget's disease; therefore, no additional X-ray was performed.

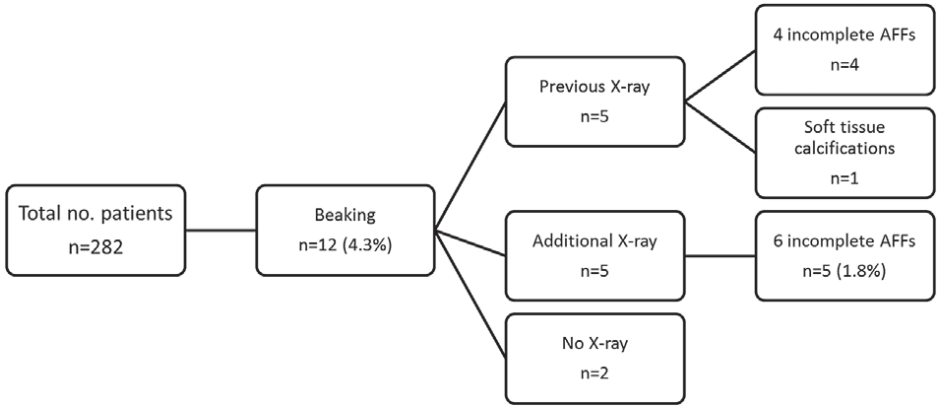

Figure 1. Overview of findings in patients with beaking on extended femur scans.

Clinical cases

Three cases of newly discovered incomplete AFF on extended femur scan that illustrate the clinical relevance of a timey diagnosis of AFF will be discussed in more detail.

CASE A

In December 2015, a 79-year-old woman underwent a DXA scan for follow-up of post-menopausal osteoporosis. She had used denosumab since one year and previously alendronic acid for almost a decade in a time span of 16 years. Other medication included a proton pump inhibitor and an inhaled corticosteroid. 25OHD was within the normal range. Bone markers (bone-specific alkaline phosphatase, beta-CTx and P1NP) were normal four years prior to the diagnosis of AFF, but were not available at a later time except for alkaline phosphatase which remained normal. Extended femur scans indicated localized cortical thickness of the lateral cortex on both femora, suspicious for AFFs (**Figure 2A** and **2B**). An X-ray confirmed the presence of bilateral incomplete AFFs without a visible fracture line (**Figure 2C**). Although she did not spontaneously report any complaints, when specifically inquired about prodromal symptoms she acknowledged that she had had pain in both hips for some time especially during walking and exercise. Antiresorptive treatment was then discontinued. She declined alternative medication such as teriparatide or a SERM and was solely treated with calcium and vitamin D supplementation. She was referred to an orthopedic surgeon who proposed conservative treatment. One year later the pain in the hips had greatly diminished and follow-up X-ray of the femora remained unchanged.

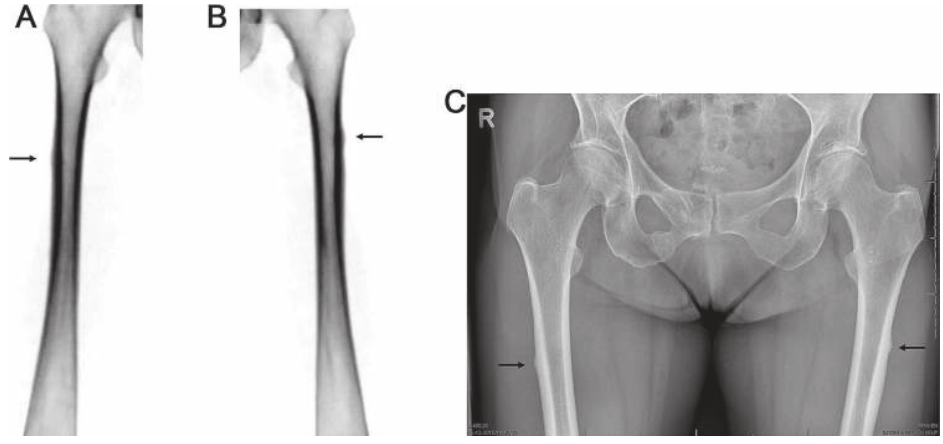

Figure 2A and 2B. Bilateral beaking on the extended femur scan (case A).
Figure 2C. Bilateral incomplete AFFs confirmed on X-ray (case A).

CASE B

In August 2015, a 74-year-old woman underwent a DXA scan for follow-up of osteopenia. She had been extensively treated with various bisphosphonates from 2007 until January

2015, including zoledronic acid and pamidronic acid infusions for presumed metastatic bone disease from breast cancer. This diagnosis was later retracted. Use of other medication included an aromatase inhibitor since seven years and previous use of a SERM during two years. 25OHD and alkaline phosphatase were within the normal range. She had undergone a total hip replacement on the right side in 2005 because of osteoarthritis. Since 2012 she had complaints of pain in her right upper leg. In 2014 an X-ray showed a stress fracture located near the stem of the total hip replacement which thus did not fulfill the ASBMR criteria of AFF. She later also developed pain in her left leg which was attributed to an altered walking pattern due to her problems with the right leg. An extended femur scan in August 2015 showed beaking of the left femur for which additional radiography was performed that confirmed the presence of an incomplete AFF (**Figure 3**A and **3B**). In February 2016 a lucent fracture line appeared in the AFF (**Figure 3C**) of the left leg that progressed over time whilst the pain increased. To prevent a complete AFF she underwent surgery with prophylactic placement of an intramedullary rod.

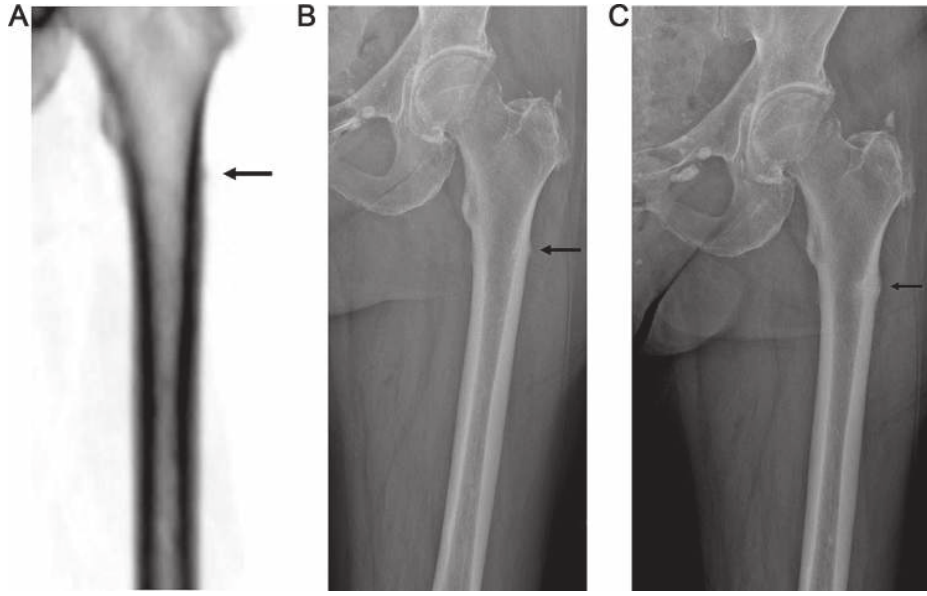

Figure 3A and 3B. Incomplete AFF of the left femur confirmed by X-ray (case B).
Figure 3C. Progression of incomplete AFF on X-ray (case B).

CASE C

In August 2016 a 75-year-old woman underwent a DXA scan for follow-up of osteoporosis with vertebral fractures for which she had been treated with alendronic acid in the past during 12 years. In 2010 she had received teriparatide injections during two years.

Since 2013 she had used denosumab. Other medication included acetylsalicylic acid, insulin and hydrocortisone for more than 30 years, the latter as replacement for primary adrenal insufficiency. At the end of teriparatide treatment, P1NP was within the normal range and bone-specific alkaline phosphatase was slightly elevated. 25OHD was within the normal range. The extended femur scan demonstrated beaking of the distal femoral diaphysis of the right leg (**Figure 4A**). An X-ray confirmed an incomplete AFF without a visible fracture line (**Figure 4B**). Bone scintigraphy SPECT/CT showed a hotspot with increased uptake at the fracture site (**Figure 4C**). She had no pain in the upper legs. She was advised to discontinue denosumab and switch to strontium ranelate or raloxifene.

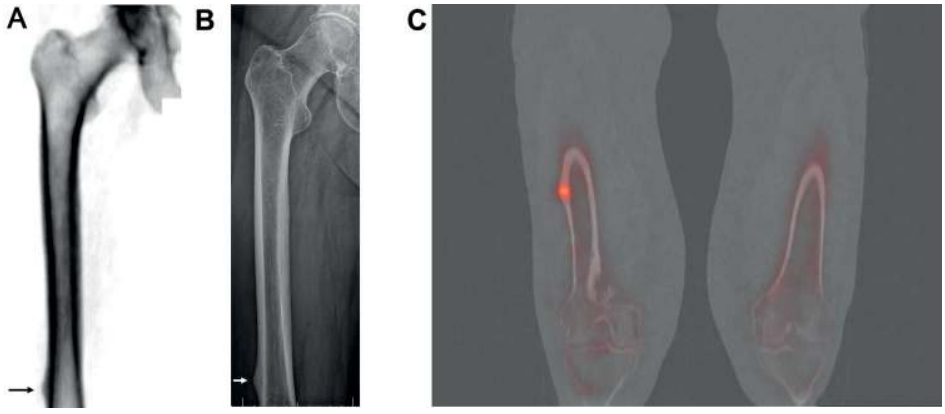

Figure 4A and 4B. Incomplete AFF of the right femur confirmed by X-ray (case C).
Figure 4C. Bone scintigraphy SPECT/CT-scan (512 MBq Tc-99m-HDP) with hotspot at the site of the incomplete AFF of the right femur (case C).

DISCUSSION

Because of the dramatic decline in use of bisphosphonates in recent years, which is partly attributed to the occurrence of rare side effects such as AFFs, it is important to timely diagnose incomplete forms of AFF. In this study the presence of localized thickening on the lateral cortex (beaking) was investigated on extended femur scans by DXA. The findings were compared with conventional X-rays of the femora. In 282 patients using antiresorptive drugs bilateral extended femur scans were performed when a DXA scan was ordered for therapeutic evaluation. Ten incomplete AFFs in nine patients (3.2%) were detected on the extended femur scan, including six yet unknown incomplete AFFs in five patients (1.8%). Two patients with beaking had no additional X-ray of the femur; it remains unclear whether this was due to an incomplete AFF as well. One patient had false positive scans for beaking of the femora because of known calcifications of the soft tissue. This gives a positive predictive value of beaking on extended femur scans of

83.3% for incomplete AFFs when the scans of two patients without available X-rays are excluded.

The relevance of these findings are illustrated by three clinical cases of incomplete AFF that were newly discovered as a result of screening with extended femur scans. These cases show how early detection of incomplete AFF can potentially prevent the development of complete fractures, either by discontinuation of antiresorptives or preventive surgery.

It is generally advised when patients have potential prodromal symptoms to perform conventional radiography with or without magnetic resonance imaging, computed tomography scans or bone scintigraphy to make a diagnosis of AFF. However, not all patients with incomplete AFFs are symptomatic or spontaneously report symptoms.[20] A standard pelvic X-ray does not usually involve the entire femoral diaphysis so that AFFs can be missed. In a Swedish nation-wide study it was noted that 104 out of 129 cases of AFF (81%) had a fracture localization at the mid-shaft, defined as 8 cm below the lesser trochanter, compared to 25 cases of AFF with a subtrochanteric localization.[21] Extended femur scans can easily be implemented as a screening tool for incomplete AFFs when a follow-up DXA is performed for therapeutic evaluation and they are not limited to symptomatic patients. Moreover, DXA scans cause very low exposure to radiation compared to conventional radiography.[22,23] We estimate that the effective radiation dose is ~0.37 µSv of a unilateral dual-energy extended femur scan with a maximum length of 33.6 cm compared to ~10 µSv of one anterioposterior X-ray of the femur.[24]

Based on our findings, extended femur scanning by DXA appears to be a clinically relevant screening tool for incomplete AFFs. In our study, all previously diagnosed incomplete AFFs were clearly visible on the extended DXA-scan. Furthermore six yet unknown incomplete AFFs were detected and confirmed on X-ray and subsequently had therapeutic consequences for the patients involved. When prolonged treatment with anti-osteoporotic medication is necessary, it is reassuring for physicians and patients that the possibility of an incomplete AFF at that moment is minimized by extended femur scan by DXA. However, it should be kept in mind that prospective studies on the natural course of established incomplete AFFs are lacking and that it is also unknown if and how soon AFFs still may develop when there is absence of beaking at this moment. Early detection of an incomplete AFF by extended femur scan will necessitate decision-making for preventive surgery versus conservative treatment. In a recent study by Min and colleagues a novel scoring system was proposed to predict the occurrence of a complete fracture amongst patients with incomplete AFF.[25] A score of nine or more indicates a high risk of an impending complete fracture and warrants prophylactic fixation. Our

case B who underwent preventive surgery, had a score >10, in contrast to case A and C with scores of eight and seven respectively, who were treated conservatively.

A possible disadvantage of screening by DXA might be anxiety in some patients when attention is paid to these very rare side effects. On the other hand, these potential side effects should already have been discussed at the start of treatment with emphasis on the very rare occurrence of such events. Another disadvantage is the slightly extended time of the scanning procedure. A regular hip DXA takes place in 44 seconds. Extended femur scans of both upper legs in one patient take approximately one minute and 50 seconds. Repositioning the patients' legs is not required. Evaluation of beaking by visual inspection only requires a few seconds. Although separate extended femur scans were performed in addition to regular hip DXA images in our study, previous literature suggests that one extended femur scan would suffice to both measure BMD and detect potential incomplete AFFs.[26,27] Since very subtle cortical irregularities cannot with certainty be discarded as AFF, screening by DXA may lead to unnecessary X-rays as was the case in three of our patients with focal irregularities of the medial cortex. These findings suggests that the lateral cortex should be the main focus when evaluating the extended femur scan for AFFs, which are by definition located on the lateral cortex, although medially located AFFs have previously been reported.[28,29]

Our results are in line with the few studies on this topic so far. A case description in 2010 first suggested that an extended femur scan on DXA corresponds with radiologic characteristics of AFF on a bone scintigraphy, computed tomography and conventional radiography.[17] Another case report and two retrospective studies have since confirmed that an incomplete AFF can be observed on DXA images.[18,30,31]

In a study by McKenna and colleagues, 257 patients underwent extended femur scans that were assessed on beaking.[18] Patients were 50 years or older and on bisphosphonate treatment for over five years. They reported abnormalities in 19 patients. In seven patients (2.7%) there was radiographic evidence for AFF, resulting in a positive predictive value of 37%. Our study found a similar proportion of patients with an incomplete AFF confirmed on X-ray (3.2%), but a more favorable positive predictive value (83.3%). Our study population is considerably different, because all patients were included regardless of age, type of antiresorptive drug and duration of treatment. As the relative risk of an AFF increases with longer duration of treatment, the incidence of AFF is most likely higher when restricting inclusion to older patients and those on long-term bisphosphonate treatment. However, the occurrence of AFFs is also described in bisphosphonate-naïve individuals and in patients on a short period of bisphosphonate treatment.[32] One of our patients had developed an AFF after just one year of treatment with oral

bisphosphonates. Also, we have diagnosed previously an AFF in an adolescent boy at the age of 18.[33]

A study conducted by Kim and colleagues retrospectively examined the presence of localized cortical thickening on hip DXA images that were performed in patients before they underwent surgical intervention for AFF.[31] They found localized cortical thickening in 20 out of 33 DXA images of the affected side, six DXA images failed to include the site of the future AFF and the remaining seven DXA images showed negative DXA results. This could mean that either the possibility of false negative DXA scans exists, or that beaking was not (yet) present in patients that later developed AFF.

Currently, manufacturers of DXA scanners are either developing new software for screening for incomplete AFFs or already have it on the market in order to screen for incomplete AFFs automatically.

LIMITATIONS

A limitation may be that single energy femur scans are possibly of higher image quality compared to the dual energy scans that were performed in our study. Single energy scans are not available from the Lunar DXA machine at our institution. Another weakness is that false negative findings on DXA cannot be ruled out, because conventional radiographs of both femora were often not available. Also there may have been a retrospective bias in evaluating the extended femur scan, in patients that had already been diagnosed with incomplete AFF prior to the DXA. Still it is encouraging that all incomplete AFFs on X-ray were also visible on extended femur scans and vice versa. Furthermore we did not use a cut-off value to quantify the beaking, since the required software to detect focal cortical thickness was not available at our institution. The interpretation is therefore based on visual inspection and thus dependent on personal experience. Also, our study was not set up to analyze inter- and intra-reader reliability. Beaking was already detected by the DXA technician during the scanning procedure. Lastly, our findings on prevalence of AFF are not representative of the general population since this study was conducted at the Bone Centre of Erasmus MC, a tertiary referral centre for complex and rare bone diseases.

STRENGTHS

Our study confirms the findings in a previous study that extended femur scans by DXA are able to detect incomplete forms of AFF and extends these findings to a broader spectrum of patients since subjects were included regardless of age, duration or type of antiresorptive treatment. Importantly, most patients with incomplete AFF did not spontaneously report prodromal symptoms, meaning that the current practice to investigate for AFF only in those patients with pain is most likely not adequate.

CONCLUSION

In summary, 10 incomplete AFFs in nine patients were identified on extended femur scans by DXA including six incomplete AFFs in five patients that were yet unknown, in a total study population of 282 patients on antiresorptive treatment with variable duration. Although we cannot exclude that some early changes may have been missed, based on these findings extended femur scanning by DXA appears to be able to detect incomplete AFF in patients on antiresorptive treatment with negligible radiation exposure and without additional costs when DXA is performed for follow-up evaluation. Extended femur scans by DXA could be considered a clinically relevant screening method since early identification of AFFs has therapeutic consequences.

In our opinion, at least patients who meet one of the following criteria should be screened with extended femur scans by DXA: patients who have already sustained an AFF in the past, bisphosphonate users that (upon specific inquiry) report pain in the hips, groin or upper legs, patients who have used antiresorptive treatment for over five years or have other risk factors of developing an AFF such as long-term use of glucocorticosteroids.[11,34] Based on our results, the safety and low expenses of extended femur scans, it might be appropriate to perform this scan in every patient who is having a routine DXA and is using antiresorptive therapy. For patients who discontinued antiresorptive therapy because of an incomplete AFF, follow-up by either DXA or conventional X-rays is needed in order to learn more about the natural course of these fractures and improve insight for therapeutic guidelines for incomplete AFFs. Nonetheless, there is a need for a comprehensive analysis of the costs and benefits of screening by DXA and a prospective study should be performed in patients without AFF on a DXA scan to evaluate if and when some patients may still develop an AFF in the future.

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5

Detection of atypical femur fractures

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ABSTRACT

The 2019 International Society for Clinical Densitometry (ISCD) Position Development Conference Task Force for monitoring with dual-energy X-ray absorptiometry (DXA) identified detection of atypical femur fractures (AFFs) as an important topic and established this working group to answer key questions in this area. The authors conducted a systematic review of the literature and deliberated on proposed ISCD positions, which were then reviewed by an external expert panel and vetted at the 2019 ISCD Position Development Conference in Kuala Lumpur on March 23, 2019. This paper summarizes the final ISCD positions and the rationale for supporting these positions. Default-length femur imaging or extended-length femur imaging as well as full-length femur imaging (FFI), both single-energy and dual-energy scans, by DXA can detect abnormalities in the spectrum of AFF. It is important to visually inspect all DXA scans of the hip and femur, and report on findings of focal periosteal and endosteal thickening at the lateral cortex (grade: Good, A, W). FFI is the preferred DXA scan mode for detecting abnormalities in the spectrum of AFF. The FFI report should state the absence or presence of abnormalities in the spectrum of AFF. If focal thickening is present on the lateral cortex, the report should state whether a lucent line is seen (grade: Fair, C, W). The ISCD recommends considering the use of bilateral FFI in patients who are currently or have been in the past year on potent anti-resorptive therapy (i.e., oral or intravenous bisphosphonate or subcutaneous denosumab therapy) for a cumulative period of three or more years, especially those on long-term glucocorticoid therapy (grade: Fair, B, W). More research is needed to determine the role of repeat testing and the optimal time interval for follow-up DXA scans, whether an automated measuring tool would perform better than visual inspection, whether FFI would change patient management and outcomes, and the cost-effectiveness of FFI.

INTRODUCTION

The role of dual-energy X-ray absorptiometry (DXA) systems in the recognition of incomplete atypical femur fracture (iAFF) was first described by McKiernan, who published serial DXA images of default-length femur field showing iAFF progression in a single case from 2004 through 2009.[1] Subsequently, reports have demonstrated that default-length femur field DXA images have identified iAFFs (or abnormalities in the spectrum of AFF) in advance of progression to complete AFFs.[2-4] That DXA has the potential for early detection of AFFs means DXA systems may have a role in the prevention of complete AFFs.

The use of DXA systems for the early detection of AFFs was one of the topics selected by the International Society for Clinical Densitometry (ISCD) Board of Directors, Scientific Advisory Committee, and Executive Committee for the 2019 ISCD Position Development Conference (PDC). Selection was based on clinical relevance, perceived value of an Official Position given limited evidence or conflicting opinions, and probability of reaching consensus. Potential topics were ranked according to their importance. Our task force group drafted the initial position statements based on a systematic review and quality assessment of the literature, and deliberation within our group. These statements were then reviewed by the Steering Committee of the 2019 ISCD PDC and an external Expert Panel, and deliberated at the 2019 ISCD PDC on March 23, 2019. In this paper, we define the terminology used, discuss methods for developing our position statements, summarize the final approved 2019 ISCD position statements, and identify current knowledge gaps.

DEFINITIONS

Atypical Femur Fractures

We use the American Society for Bone and Mineral Research (ASBMR) Task Force Definition for AFFs.[5] AFFs are low trauma stress fractures with characteristic radiographic findings and are located below the lesser trochanter of the femur all the way distal to the supracondylar flare. Complete AFFs are minimally comminuted; iAFFs start from the lateral cortex and extend toward the medial cortex. Since they are stress fractures, they often develop over time with prodromal thigh or groin pain, especially with or after weight-bearing activities, and have focal periosteal or endosteal thickening at the lateral cortex on imaging prior to a complete fracture.

Focal periosteal or endosteal thickening at the lateral cortex is one of the ASBMR defining criteria for an iAFF. However, not all focal thickenings are iAFFs; some may be old scars

from prior healed iAFFs. Often it is unclear whether the focal periosteal or endosteal thickening observed with DXA is an active lesion or not; this necessitates further imaging such as plain radiographs, computed tomography (CT), magnetic resonance imaging (MRI), or bone scintigraphy. The term "beaking" refers specifically to focal periosteal thickening with a radiolucent line in the middle, resembling a bird's beak. Beaking on DXA images or plain radiographs indicates an active iAFF. All the above abnormalities are considered abnormalities in the spectrum of AFF (**Figure 1**). Although generalized cortical thickening has been described in patients with AFFs, this feature is nonspecific and is associated with smaller diameter of the femur. For this manuscript, we will only consider focal periosteal and endosteal thickening at the lateral cortex, with or without beaking, as abnormalities in the spectrum of AFF.

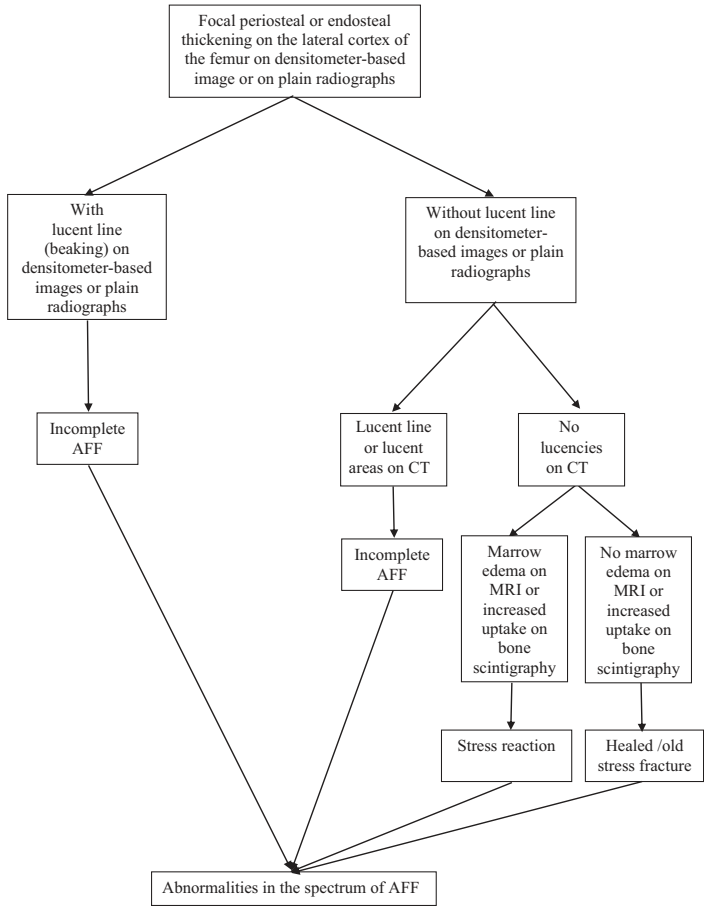

Figure 1. Classification of abnormalities in the spectrum of AFF.

Full-Length Femur Imaging

We use the term "full-length femur imaging" (FFI) to denote the densitometer-based imaging of the full length of the femur, from the lesser trochanter to the supracondylar flare at the knee (**Figure 2**). This is in contrast to the default-length femur imaging that is routinely used for obtaining total hip and femoral neck bone mineral density (BMD), and extended-femur imaging (extending the length of the region of interest of the routine hip BMD scan to cover more of the shaft of the femur; *see* **Supplementary Appendix**—A: Technology Overview); neither the default-length nor the extended-length can cover the whole femur. Contemporaneous BMD measurement occurs with both default-length imaging and extendedlength imaging without affecting the BMD measurement[6,7], whereas FFI is separate from BMD measurement.

Figure 2. Densitometer-based full-length femur imaging (FFI). **(a)** Single-energy scan showing beaking (arrows). **(b)** Dual-energy scan showing focal cortical periosteal and endosteal reactions at the lateral cortex (arrow; image: courtesy of Diane Krueger). **(c)** Image from densitometer-based full-length femur imaging (FFI).

Positioning for all these scans follow manufacturers' positioning of the hip with internal rotation using hip positioning devices. This positioning may result in an unexpected effect on the appearance of the lateral aspect of the femur. When the femur is internally rotated, the insertion point of the gluteus maximus along the linea aspera is now brought into view along the lateral cortex. If this is particularly prominent, then it may give the appearance of a thickened lateral cortex or a localized thickening on the periosteal aspect of the lateral cortex[3], also known as a tug lesion. A tug lesion has an intact periosteum underlying the "bump" whereas a focal cortical thickening, which is consistent with abnormalities in the spectrum of AFF, does not (**Figure 3**). A tug lesion

also has a smoother contour compared to the more pointed contour of a focal cortical thickening.

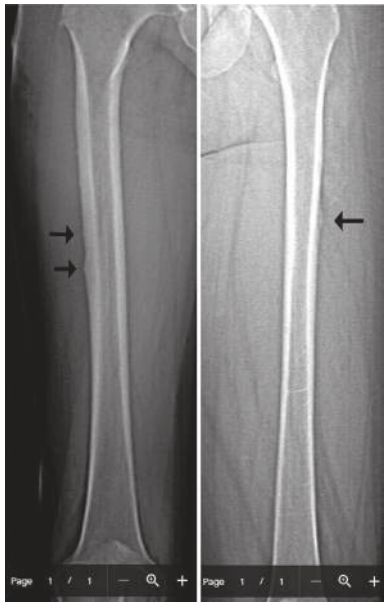

Figure 3. Single-energy full-length femur images showing focal cortical thickening (left) versus tug lesion (right).

METHODOLOGY

A systematic review was performed to inform expert opinion when creating these Official Positions. This systematic review of observational studies was conducted in accordance with guidance documents provided by the Cochrane Collaboration and a modified RAND/ UCLA Appropriateness Method, as per previous PDC statements.[8,9] A search strategy was performed from inception to December 5, 2018 using MEDLINE and EMBASE. The following search terms were used:

(x-ray OR radiography OR radiograph* OR densitometry OR dual-energy x-ray absorptiometry OR DXA OR DEXA OR bone density OR bone mineral density OR BMD) AND ("Femoral Fractures"/ OR (((femur* OR femoral* OR subtrochant* OR atypical*) ADJ6 (fracture*))).ab,ti.) AND (prevalence or incidence or risk factor* or predictor* or sensitivity or specificity or positive predictive value* or negative predictive value* or false positive* or false negative*).* The search strategy was limited to human evidence; no other restrictions applied. Task force members were consulted regarding additional studies or abstracts and a hand search of the bibliographies of included publications was also performed.

Using the inclusion/exclusion criteria outlined below, two reviewers (JS and DML) conducted study selection by screening the titles and abstracts generated from the MEDLINE and EMBASE searches, and then reviewed full texts of articles deemed potentially appropriate for inclusion. Any disagreements were resolved by consensus. Available abstracts presented at ASBMR, ISCD, WCO-IOF-ESCEO (World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases), and ECTS (European Calcified Tissue Society) since 2010 were searched for and reviewed.

Eligible studies included those that examined screening/ detection of iAFFs using radiography or DXA-based femur imaging in female and/or male adults, and included information on any of the following:

(i) Incidence/prevalence.
(ii) Operational characteristics (true and false positives, true and false negatives, sensitivity, specificity, number needed to screen, positive predictive value, negative predictive value).
(iii) Clinical criteria related to iAFF detection using DXAbased femur imaging (e.g., age, sex, glucocorticoid use, thigh or groin pain, use of antiresorptive drugs).
(iv) Imaging features of iAFFs obtained from DXA-based femur imaging.
(v) Requirements for repetition of DXA-based femur imaging for screening among at-risk patients.

Given the limited research in this area, we included all study designs and all types of data providing information on the above-mentioned variables (qualitative and quantitative). Studies that were (1) exclusively using other imaging modalities such as bone scintigraphy, (2) describing features of AFFs and iAFFs, or (3) assessing complete AFFs were excluded.

The following data were extracted from the included studies: country, study design, inclusion/exclusion criteria, type of imaging, sample size, setting, mean age, percentage of females in the sample, study duration, and information related to the above-mentioned variables (operational characteristics, clinical criteria, imaging features, and repeat imaging). Given the limited data, findings were not pooled.

Quality assessment of studies was independently performed by two reviewers (JS and DML) using guidance from the Ottawa Newcastle Scale for cohort studies.[10] Disagreements were resolved by a third reviewer (AMC). As per previous ISCD PDCs, the quality of the evidence supporting each statement was provisionally rated as being good, fair, or poor, where:

"Good" evidence was from two or more well-designed prospective studies (randomized controlled trials or high quality observational studies).

"Fair" evidence was judged to be sufficient to determine effects on outcomes but limited by the number, quality, or consistency of the available studies.

"Poor" evidence was judged to be insufficient to determine effects or consequences of implementing the statement on outcomes, due to the number of available studies, flaws in their design or conduct, major gaps in the chain of evidence, or conflicting evidence.

Figure 4 and **Table 1** show the results of our systematic review (see **Supplementary Appendix** for additional data). Thirty-one full-text articles and abstracts were assessed for eligibility and of those, 13 were excluded.[11-23] Thirteen full manuscripts and five abstracts were included to answer our questions below.[1-4,6,7,24-35]

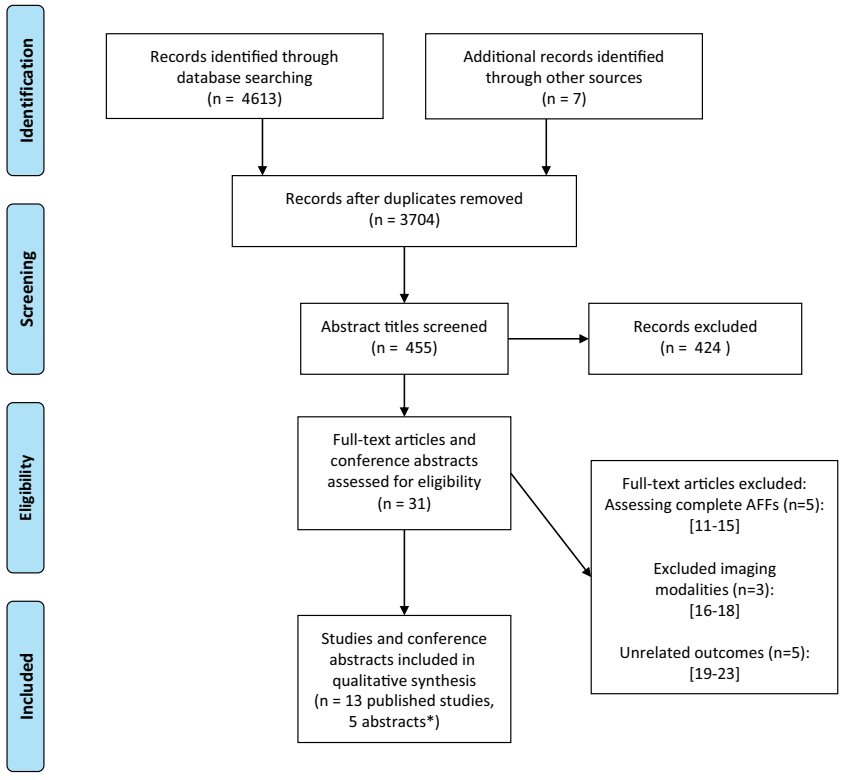

Figure 4. PRISMA flow diagram of our systematic review.
*May not have completely captured all abstracts. Abstract databases for the following meetings were assessed: ISCD (2010-2018), ASBMR (2010-2018), IOF/ESCEO (2015-2018), ECTS (2016-2018), Endocrine Society (2010, 2011, 2013-2018), RSNA (2013,2014, 2016-2018)

KEY QUESTIONS

Our overarching question was, "Should screening using DXA-based femur imaging be preferred over no screening in individuals at risk of AFF?" Specifically, our subquestions were as follows:

1. Can DXA systems detect iAFFs or abnormalities in the spectrum of AFF?
2. What densitometer-based test should be used for the detection of abnormalities in the spectrum of AFF, and how should it be analyzed, interpreted, and reported?
3. In which patient population should densitometerbased FFI be used to screen for abnormalities in the spectrum of AFF?

ISCD OFFICIAL POSITION 1

Femur DXA images should be reviewed for focal cortical abnormalities in the spectrum of AFF (grade: Good, A, W).

Rationale

The first DXA recognition of iAFF was on a DXA unit using default-length femur imaging.[1] An iAFF can either be directly visualized or delineated by edgedetection software at the time of default-length DXA, before it progresses to a complete AFF.[3] Kim *et al.* conducted a retrospective study of 33 patients with radiographic evidence of either complete AFF or iAFF, all of whom had an ipsilateral default-length femur field DXA scan performed on average 11 months (range 0.24–24) prior to conventional radiographically proven AFF.[4] Abnormalities in the spectrum of iAFF were evident in 61% of subjects, even though there was limited visualization of the femur due to default-length femur imaging.

Since the default-length femur image only views the immediate subtrochanteric region distal to the lesser trochanter, it has limited potential to capture all iAFFs. In order to enhance detection of iAFF, McKiernan *et al*. advocated for lengthening the femur view, since called extended-length femur imaging.[6] Default-length femur imaging for DXA acquisition starts below the lesser trochanter. The DXA operator has the option of extending distally the default length of the femur field, while ensuring that the global region covers an adequate quantity of soft tissue superior to the neck of the femur.[6,7] The maximum adjustment that the DXA operator can make in the femur field length is determined by the factory configuration, which varies slightly according to the manufacturer: ranging from about 21 cm to 24 cm, covering about half the proximal femur to the mid-diaphysis where most AFFs occur (*see* "technical overview" in **Supplementary Appendix**).[6,7]

Given that most cases of complete AFFs appear to have a prodromal phase of iAFFs and that there is a substantial morbidity gap between complete and iAFFs, the identification of iAFFs before progression to complete AFFs is a sensible undertaking.

DISCUSSION

It is apparent that DXA systems can detect abnormalities in the spectrum of AFF regardless of whether one uses default-length femur imaging (as part of routine BMD examination), extended-length femur imaging or FFI. Therefore, an image generated by any DXA system at the time of the hip examination has the potential to detect abnormalities in the spectrum of AFF. This observation reinforces the best practice of image inspection at the time of femur BMD acquisition, analysis, and reporting, especially in those who have been on long-term potent antiresorptive therapies.

ISCD OFFICIAL POSITION 2

When using DXA systems to detect abnormalities in the spectrum of AFF, scanning methods that generate bilateral full-femur length images (FFI) should be used. The FFI report should state the absence or presence of abnormalities in the spectrum of AFF. If a focal cortical thickening is present on the lateral cortex, the report should state whether a lucent line is seen. Consider additional imaging when clinically appropriate (grade: Fair, C, W).

RATIONALE

Although AFFs are mostly in the proximal and middiaphyseal areas of the femur, they can be in the distal femur as well. In Singapore (n = 75), 48% of the AFFs are subtrochanteric, while in Sweden (n = 151) only 17% are subtrochanteric.[36] In the Ontario AFF cohort (n = 350), approximately 80% were mid-diaphyseal, 20% proximal, and <1% distal fractures.[37] While default-length and extended-length femur imaging with DXA can detect abnormalities in the spectrum of AFF, they can also miss abnormalities outside of the field of view. FFI is fast and safe with very low radiation exposure (*see* **Supplementary Appendix** for details), and the entire length of the femur can be visualized with good quality images. FFI is now an approved feature on certain densitometers (namely GELunar and Hologic) in the United States and Canada.

The 2013 revised diagnostic criteria by ASBMR state that an AFF is a fracture that can occur along the femoral cortex from just distal to the lesser trochanter down to the supracondylar flare, meeting at least four out of five major criteria: (1) no or minimal trauma, (2) a predominantly transverse fracture line originating from the lateral cortex, (3) involvement of the lateral cortex only for iAFFs, while complete AFFs extend through both cortices and may have a medial spike, (4) no or minimal comminution, and (5) localized periosteal or endosteal thickening of the lateral cortex present at the fracture site, called "flaring" or "beaking" (if with a fracture line).[5] It is this final criterion that is pertinent to the early detection of abnormalities in the spectrum of AFF using a densitometer.

Traditionally, these focal cortical thickenings have been identified using conventional radiography[24], but recent studies have shown that it is possible to detect focal cortical thickening on the lateral cortex with or without a fracture line by densitometers using dual-energy or single-energy scans.[3,4,25,26,33,38,39] These are often identified by visual inspection. Cases of iAFFs with only focal endosteal thickening may occur[4,19]; therefore, exclusive assessment of periosteal changes is insufficient and the endosteal cortex should be included. In a small cohort of 45 healthy volunteers, 12 patients with a recent diagnosis of osteoporosis and 43 patients on long-term (>five years) bisphosphonate therapies, no cases of focal cortical thickening or beaking were identified on X-rays of the femur.[40]

If focal cortical thickening is identified by visual inspection, either with or without a lucent line, then additional imaging is warranted. A plain radiograph of the femur should be the first-choice diagnostic test if no lucent line is seen on FFI, because plain radiographs are easily accessible and economical. If the FFI or plain radiograph identifies beaking, with an incomplete fracture line being visible, then the diagnosis of an iAFF is clear. When a fracture line is visible, either on the densitometer-based images or on plain radiographs, we recommend CT imaging, so that the depth of the fracture line and the extent of the crack around the circumference can be ascertained. When no fracture line is visible on plain radiograph, then MRI or bone scintigraphy can be used to assess whether there are stress changes (marrow edema on MRI or increased uptake on bone scintigraphy), differentiating between an active iAFF lesion and an old scar from a healed iAFF.

These additional imaging studies may also differentiate iAFF from other phenomena that may lead to false positive scans on FFI, such as calcifications of the muscles or vasculature, post-traumatic changes, postoperative irregularities of the cortex, or a prominent gluteal tuberosity of the linea aspera.[25,26] Incidental findings on DXA scanning should be

reported only when this may have clinical consequences, such as suspicion of a bone tumor, but it should be stated that DXA images are not adequate for making a radiological diagnosis. Reported incidental findings on DXA have been related to conditions like Paget's disease of bone, vascular calcifications, an intraosseous lipoma, avascular necrosis, enchondroma, and osteochondroma.[3,25,26,33] These incidental abnormalities on DXA can also be an indication for additional imaging.

DISCUSSION

If the intent is to detect abnormalities in the spectrum of AFF, then FFI is recommended over both default-length femur imaging and extended-length femur imaging. Only about half the length of the femur in adult women is visualized by the extended-length femur field imaging.[6] The principal limitations of default-length and extended-length femur imaging are as follows: limited length of femur imaging, lower quality of images, and slower scan times. Although there may be theoretical advantages of single energy FFI (e.g., Hologic SE-Femur) versus dual-energy FFI (eg, GE Lunar), there are not enough data to differentiate the two modes of FFI.

In keeping with the ASBMR criteria for AFF, the aim of FFI is to identify focal cortical thickening on the lateral cortex of the femur, including both the periosteal and the endosteal surfaces. Direct visualization of the image is the preferred approach to interpretation. The choice rests between viewing the image at the time of image acquisition, viewing a printed copy at the time of reporting, or visualizing on physician viewer later. The interpretation of FFI is a subjective assessment by the reviewer. Although this is a subjective assessment, it has good reliability. The reliability of defining the presence or absence of iAFF has been evaluated by Kim *et al*.[4] Using three observers, who examined 232 DXA images either with iAFF (n = 33) or without AFF (n = 199), interobserver agreement was tested using the quadratic-weighted k statistic. Agreement was excellent with k statistics of 0.945, 0.864, and 0.865. Matters relating to the diagnostic accuracy of DXA in the detection of abnormalities in the spectrum of AFF are reviewed in more detail in the "technical overview" in **Supplementary Appendix**.

Although direct visualization is our current recommendation for the identification of abnormalities in the spectrum of AFF, new approaches may make identification easier and faster. Recognition of focal cortical thickening has been automated using the GE-Lunar enCORE software, which was designed to detect focal thickening on the lateral cortex (what the company called "beaking index" or BI), but not fracture lines. A clinically relevant cut-off value of the "beaking" width (mm) measured by the AFF software has

yet to be established. A recent retrospective review of 95,495 hip DXA scans at a single center resulted in 258 scans with BI >1 mm, but none had abnormalities in the spectrum of AFF by visual inspection.[31] In addition, the spatial resolution of dual-energy scans may be inadequate to depict fracture lines, although the presence of lucent clefts has been visualized using both dual-energy scans[4] and single-energy scans.[34]

The remaining ASBMR criteria may not be useful for the interpretation of FFI scans. Generalized increase in cortical thickness of the femur (when the width of the lateral cortex plus the width of the medial cortex exceeds 50% of the diameter of the femur) is a minor feature of the ASBMR criteria and it does not seem to be a specific finding for AFF patients. This feature may reflect the size of the femur (the smaller the femur, the greater the proportion for cortical thickness) or may rather be a sign of long-term bisphosphonate use, since generalized cortical thickening occurred in 121 femurs of 81 women on longterm antiresorptive treatment (32%) using single-energy FFI screening for AFF.[33] In this study, only two cases of abnormalities in the spectrum of AFF were established- and neither had generalized increase in cortical thickness. The concept of generalized cortical thickness associated with either bisphosphonate use or AFF is controversial. In a previous study, the difference in cortical thickness between 59 AFF patients and 218 controls with ordinary subtrochanteric fractures disappeared when correcting for age.[41] Similarly, no differences in femoral cortical thickness were observed between 43 long-term bisphosphonate users and 12 osteoporotic controls.[40] Another study did not find differences in cortical thickness between patients with low-trauma subtrochanteric fractures and patients with typical hip fractures, nor between bisphosphonate users and nonusers, and no effect was found in relation to duration of antiresorptive treatment.[42]

AFF is defined as atypical because these stress fractures initiate at the lateral cortex. Typical femur stress fractures (as seen in osteomalacia or marathon runners) start at the medial cortex because compressive forces at the medial cortex are usually much higher than the tensile forces at the lateral cortex.[43] Some reports have described abnormalities on the medial cortex.[44,45] In a study by van de Laarschot *et al*. on screening by DXA, in three out of 282 patients (1%) with bilateral FFI, discrete focal irregularities of the medial cortex were seen, but no abnormalities were found on X-rays in these patients.[26] This observation combined with the very sparse documentation of abnormalities on the medial cortex in AFF patients suggests that screening of the medial cortex is not indicated in routine patient care, although it may be an interesting area to explore in future research.

When reporting FFI, it is clinically relevant to report either the absence or presence of focal cortical thickening. A finding of focal cortical thickening without a lucent line on FFI is not diagnostic of iAFF; rather it is an abnormality in the spectrum of AFF and is an

indication for further imaging, such as with plain radiography. If a lucent line is present, imaging with CT to assess the depth and extent of the fracture line may save costs and time. In the absence of a lucent line on plain radiographs or FFI, then either MRI or bone scintigraphy is warranted to distinguish between an active lesion and a healed scar.

ISCD OFFICIAL POSITION 3

Consider bilateral FFI for detecting abnormalities in the spectrum of AFF in patients who are receiving bisphosphonates or denosumab therapy or discontinued it within the last year, with a cumulative exposure of three or more years, especially those on glucocorticoid therapy (grade: Fair, B, W).

Rationale

The 2010 and 2013 ASBMR Task Force reports highlighted key risk factors for AFFs, such as duration of bisphosphonate therapy, female sex, Asian descent, rheumatoid arthritis and glucocorticoid use. Six studies (five papers, one abstract) have assessed the prevalence of abnormalities in the spectrum of AFF in patients on bisphosphonate therapy using densitometer-based femur imaging or plain radiographs with different inclusion criteria (**Table 1** shows the five studies that are published as full papers).[3,24-26,29,33] Prevalence varies from 0% to 10.3%, with the highest reported prevalence in a study of patients with autoimmune diseases on glucocorticoid therapy.[29] The zero-prevalence study was explained by declining prevalence in AFF in that region as well as having a small sample size (n = 173).[25] In addition, several studies have shown that the risk increases with duration of bisphosphonate therapy, especially after three to five years.[46,47]

Most patients with complete AFFs had prodromal symptoms in hindsight, thus considering prodromal symptoms as a risk factor for selecting patients for screening may be reasonable. Kim *et al*. showed that detection rates for focal cortical thickening on the lateral cortex were higher for DXA imaging alone compared to prodromal symptoms of hip or groin pain alone, but detection rates were highest when DXA imaging was performed in patients with prodromal symptoms.[4]

Table 1 Prevalence of abnormalities in the spectrum of AFF using densitometer-based imaging and conventional radiography

Study	Country	Study population	Inclusion criteria	Sample size	Women (%)	Imaging modality	Prevalence (%)
La Rocca Vieira, 2012[24]	USA	Patient-based; patients with osteoporosis and metabolic bone diseases	Asymptomatic (no pelvic, hip, or thigh pain) Taking bisphosphonates for ≥3yr	100	93	Plain radiographs	2.0
McKenna, 2013[3]	Ireland	Community based; at time of presentation for routine DXA	Age >50 yr Taking bisphosphonates for >5yr	257	92.6	DXA (extended-length femur)	2.7
Sato, 2016[29]	Japan	Patient-based; patients with autoimmune diseases on glucocorticoid therapy	Taking bisphosphonate and prednisolone according to the 2005 guidelines on glucocorticoid-induced osteoporosis	116	91	Plain radiographs	10.3
McKenna, 2017[25]	Ireland	Community based; at time of presentation for routine DXA	Age >50 yr Taking bisphosphonates for >5yr	173	96	FFI: SE-femur scan	0
van de Laarschot, 2017[26]	The Netherlands	Tertiary care patients; used BPs or denosumab the year prior	Used bisphosphonates or denosumab in the previous year	282	62.7	FFI: DXA femur field extended to full length	3.2

BP= bisphosphonates; FFI= full-length femur imaging; SE-femur=single-energy femur scan.

DISCUSSION

To define the patient populations that should be screened for abnormalities in the spectrum of AFF using densitometer-based FFI, we need to first determine the yield (or prevalence of abnormalities), then estimate the effectiveness (or the number needed to screen to prevent a complete AFF), and then measure the cost-effectiveness of such a strategy. Unfortunately, there are limited data in this area. The only data in the published literature are on the yield, while no studies have examined effectiveness or cost-effectiveness of screening.

Researchers have noted more women than men developing AFFs, but this may be a function of the number of women vs men on potent antiresorptive therapy. Based on the available data, longer duration of therapy increases the risk of developing AFFs.[5,46-48] Although AFFs can occur in individuals who were not exposed to any antiresorptive therapies, the risk dramatically increases after years of oral bisphosphonate therapy.[49] There are limited data on intravenous zoledronate. For denosumab, there have been nine case reports, five clinical trials, and one prospective study that have reported AFFs in individuals on denosumab.[50] In the Ontario AFF cohort study, 41 individuals out of a total of 355 (11.5%) were on denosumab prior to sustaining an AFF.[37] All except one had prior bisphosphonate exposure.

Since 80% or more patients with complete AFFs had prodromal symptoms, it makes intuitive sense that screening those with prodromal symptoms would result in a higher yield, as shown by Kim *et al*.[4] However, that difference is small, and currently available data does not support screening only those with prodromal symptoms (*see* **Supplementary Appendix Table**), likely because aches and pains are common in this population, and often not due to impending AFFs. The lack of merit as a criterion for screening should not lead the clinician to discount suspected prodromal symptoms in a patient on long-term potent antiresorptive therapy. If a clinician has a suspicion, then they should proceed with the imaging modality that is deemed appropriate for the circumstances.

Other risk factors, such as Asian descent, comorbidities such as diabetes mellitus, cancer, rheumatoid arthritis, and glucocorticoid therapy, are also associated with AFFs. Except for patients with autoimmune diseases on glucocorticoid therapy, data on the yield of screening specific populations are lacking.[29] For patients with a diagnosed AFF, it is important to screen the contralateral femur, as two-thirds of patients with AFFs have bilateral AFFs.[37]

A negative FFI may improve drug compliance by the patient and may decrease hesitancy of the treating physician to prescribe antiresorptive therapies, although it is unclear how often and when an AFF may develop in the absence of focal cortical thickening. A positive FFI permits timely detection of an iAFF; this is clinically pertinent because it may decrease the chances of progression to a complete AFF by interventions such as decreasing weightbearing activities and discontinuing antiresorptive therapy. While the risk of AFF declines 70% per year when antiresorptives are stopped[51], it is not clear whether this reduced risk also applies to patients who already have abnormalities in the spectrum of AFF. While the absence of these abnormalities may reassure patients to continue to adhere to their antiresorptive therapies and their physicians to continue to prescribe antiresorptive therapies, those false positive reports of incidental findings may lead to unnecessary and expensive medical imaging tests in addition to the anxiety for the patient.

KNOWLEDGE GAPS AND FUTURE RESEARCH

Based on our systematic review of the literature, our knowledge of AFFs and the performance of densitometer- based femur imaging, using FFI with visual inspection is a reasonable tool for detecting abnormalities in the spectrum of AFF. This is an emerging area of research and there are many unanswered questions, including but not limited to: (1) whether a DXA-based measuring tool (e.g., “beaking index”; femoral shaft density, diameter, or cortical width in hip structural analysis; or bowing angle of femur shaft) should be used to better identify risk of AFF, (2) the role for repeat scanning and the optimal time interval, (3) whether FFI will alter patient management and improve outcomes, (4) the cost-effectiveness of FFI, and (5) the risk of future AFF in those with abnormalities in the spectrum of AFF that have healed.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jocd.2019.07.003.

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APPENDIX

Technology Overview

This section covers the evolution of the approach to using DXA systems for detecting abnormalities in the spectrum of atypical femur fractures (AFFs): starting with default-length femur field imaging as part of routine femur DXA examination; followed by investigator-led extended-length femur field imaging; then followed by investigator-led full-length femur imaging (FFI); and culminating in manufacturer-led adaptations in DXA technology for FFI. In addition, the diagnostic accuracy of this DXA approach is reviewed.

General Considerations

There are some general considerations that apply to all approaches: femur positioning, direction of scanning, contemporaneous or separate bone mineral density (BMD) measurement, radiation exposure, acquisition time, image resolution, visual inspection of images, and measurement. Universally, positioning adheres to standard manufacturer guidelines deploying hip-positioning devices with internal rotation of the femur. The direction of scanning is from distal femur to proximal femur except for the recently customized feature on the Hologic Horizon series.

Contemporaneous BMD measurement occurs with both default-length imaging and extended-length imaging, whereas imaging is usually separate from BMD measurement in FFI. Radiation exposure is very low for all approaches, as outlined later. Regarding image resolution, default-length femur scans and extended-length femur scans (both acquired with dual-energy imaging) have similar resolution to that seen in routine BMD measurement. Image resolution is enhanced for FFI; some densitometers using single-energy X-ray imaging approximate radiographic-quality.[25] Acquisition times vary from about two minutes for dual-energy X-ray imaging to about 20 seconds for single-energy X-ray imaging.

Default-Length Femur Field Imaging

The first recognition of iAFF was on a DXA instrument using default-length femur imaging.[1] Therefore, an image generated by any DXA system at the time of hip examination has the potential to detect iAFF. This observation reinforces the best practice of advocating image inspection at the time of femur BMD acquisition, analysis, and reporting – most particularly at the time of routine DXA visit in those who are being medicated long-term with antiresorptive agents.

Extended-Length Femur Field Imaging

For routine hip examination, there is a default-length femur field that can be extended. Default-length proximal femur DXA scanning starts below the lesser trochanter. The maximum adjustment that the DXA operator can make in the femur field length is determined by the factory configuration, which varies slightly according to the manufacturer: ranging from about 21 cm to 24 cm.[6,7] The scanning time is about two minutes (McKenna, personal communication). This lengthening of the femur image views the femur down to the mid-diaphysis within which the majority of AFFs occur.[37]

Full-Length Femur Imaging

Only about one-half the length of the femur in adult women is visualized by the extended-length femur field imaging.[6] This is suboptimal because iAFFs may occur at any point along the entire lateral surface of the femur. DXA systems have been adapted by investigators in order to view the entire length of the femur. In addition, manufacturers of DXA systems have responded to the investigator-led developments such that FFI is now an approved standard feature on certain densitometers.

Adapting DXA Systems

Regarding Hologic systems, if the model is capable of single-energy high-definition imaging of the lateral spine for vertebral fracture assessment, then it can be adapted to acquire high-definition imaging of the femur.[25] A rotating C-arm is not needed to acquire the femur image. For lateral spine imaging, the C-arm is positioned at the iliac crest in order to acquire a spine image; for FFI the C-arm is positioned over the distal femur prior to image acquisition.[25] Compared to DXA imaging, McKenna *et al.* have demonstrated that single-energy imaging is about 5-times faster at 20 seconds, has much superior image quality, and visualizes the full length of the femur.[3,25] A limitation is that the femur images are recorded as spinal images in the database.

Regarding GE Lunar systems, van de Laarschot *et al.* have demonstrated that the femur field length can be extended beyond the factory setting up to 33.6 cm with technical assistance from the manufacturers such that the entire extent of the femur is visualized.[26] They neutralized tissue types along the entire image in order to maximise resolution. This mode uses dual-energy X-ray imaging. The duration for a regular hip DXA is about 44 seconds; the duration of FFI is approximately one minute 50 seconds. They identified iAFFs over the entire femur including the lower diaphysis.[26]

Manufacturer Approach: Hologic

Hologic incorporated a feature for iAFF detection in the Horizon DXA series using single-energy imaging that they term “SE Femur”. Compared to earlier models, the Horizon

series has more efficient detectors that enhance resolution giving near-radiographic level of resolution. If the SE Femur is enabled in the configuration, then the DXA operator is prompted to perform SE Femur immediately after the hip DXA examination while maintaining the same position. If SE Femur is not enabled in the configuration, then the operator may choose to select SE Femur as a separate exam from the panel of scan types. If the operator proceeds with SE Femur, then imaging starts at the greater trochanter and proceeds caudally until the supracondylar flare is seen by operator.

The duration of the scan is about 15 sec. The radiation entry dose is about 35 μGy giving an effective radiation dose of about 1.8 μSv (Hologic, personal communication). Regarding evaluation, the image is visualized in the viewer. Software tools allow for alterations in contrast and magnification. In addition, annotations can be added to the image. A ruler can be applied for the sake of measurement. Annotations and measurements can be retained and stored. SE Femur is approved in North America.

Manufacturer Approach: Lunar

GE Lunar introduced a software upgrade (enCORE v17) with an option called "Atypical Femur Fracture". It can be installed on any GE Lunar Prodigy or GE Lunar iDXA systems. The software can be used to analyze retrospectively for iAFF in any prior proximal femur examination that was conducted with either a Prodigy machine or an iDXA machine. It enables visual assessment of the entire femur as well as providing automated measurements of both the medial cortex and the lateral cortex. It is a form of DXA imaging. There is a single scan with two components being merged into one image: a distal component of variable length depending on patient height, and a proximal component with a default femur field length of about 18 cm with a SmartScan feature stopping after the required anatomy is imaged. This single scan with a split sequence permits both imaging of the entire femur and separate BMD assessment of the proximal femur. The rationale for the split sequence is to minimize any potential impact on BMD measurement. The duration of the distal component depends on the DXA model and the mode, ranging from about 45 to 100 seconds. Likewise, the entry radiation exposure is very low varying from nine μGy to 19 μGy using thin or standard mode on Prodigy systems respectively; and it is a little higher on iDXA systems ranging from 38 μGy to 75 μGy. Using the thick mode, entry radiation exposure is about 83 μGy on Prodigy and 329 μGy on iDXA; this corresponds to an effective radiation dose of 0.37 to 15.0 μSv depending on the system and the scan mode (GE Lunar, personal communication).

The AFF option can be selected as part of the dual femur examination. The foot brace ensures that both legs are rotated internally. The distal scan starts in the left leg over the patella and continues proximally. The DXA operator is then prompted for the initiation

of the proximal scan with subsequent BMD measurement. As part of the automated sequencing, upon completion of the left femur exam, the scanner moves to the start position for the right distal femur facilitating completion of the same exam on the right femur.

The analysis option for AFF displays the FFI for visual inspection. For the quantitative analysis, there is automated measurement of cortical width for both lateral and medial cortices. The FFI is the default option but it is feasible to select this quantitative analysis for default-length femur image if only the proximal scan is performed. The threshold for automated identification of a localized cortical reaction is set in advance of the analysis. The manufacturer-recommended default setting is one mm, which they term as the "beaking index". Published research on the validity of the use of this cut-off is awaited. There are three graph options: a beaking profile graph, a lateral cortical width graph, and a medial cortical width graph. Another related feature is the facility to analyze prior scans such that serial trends in cortical profiles can be explored. This AFF option has vast potential both as a diagnostic test and as an investigative tool. This software upgrade is approved in North America.

Diagnostic accuracy of FFI in the detection of abnormalities in the spectrum of AFF

Background

In order to determine the diagnostic accuracy of densitometer-based FFI, the optimal study is to have all subjects undergo both FFI and the gold standard, namely plain radiograph of the femur. This generatesdata about the frequency of errors – namely, false positives (FP) and false negatives (FN) – as well the frequency of correct tests – namely, true positives (TP) and true negatives (TN). With this data, the following estimates can be derived: FP percent, FN percent, TP percent, TN percent; sensitivity; specificity; likelihood ratios; positive predictive value (PPV); and negative predictive value (NPV).

Diagnostic accuracy studies are divided into two types according to design: case control design andcohort design. When the disease is rare, such as is the situation with AFF, a case-control design is practical because it does not require as many participants as a cohort design. A cohort design has the advantage that the full spectrum of iAFF is captured. An acknowledged design feature of cohort studies is selective disease verification whereby subjects only have the reference test when the index test is positive. As applied to DXA testing, this means only referring patients with suspected abnormalities for a confirmatory femur radiograph. Using this type of study design, estimates of diagnostic

accuracy are limited to PPV because only the number of TP and the number of FP are known.[3]

False Positive Percent

The FP percent is pertinent to clinical practice because this indicates the percent of patients who will have unnecessary higher-level imaging in order to exclude an abnormality in the spectrum of AFF. For this reason, particular emphasis in this section is placed on FP percent. It is derived as follows:

$$FP\% = \left[\frac{FP}{TP+TN+FP+FN}\right] * 100.$$

Before reviewing the literature on diagnostic accuracy, it should be noted that the calculation of FP percent of FFI is influenced by factors that alter either the numerator or the denominator. This difference is peculiar to FFI testing for two reasons: there is apossibility of performing FFI in either one femur per patient or two femurs per patient; and there is the possibility of bilaterality of FP. The numerator varies with the degree of unilaterality/bilaterality of FP. The denominator is determined by the number of femurs that are imaged. The denominator for estimating the FP percentper patient is determined by the number of patients in the study.

These differences are explained by the following examples. If 200 patients have both femurs tested (n=400) and there are 10 FP, then the FP percent is 2.5% (10/400). If all the FP are unilateral, then the percent of patients needing femur radiography is 5% (10/200); but if all the FP are bilateral, then the percent of patients needing femur radiography is 2.5% (5/200). Alternatively, if the 200 patients only have 300 femurs tested with 10 FP, then the FP percent is 3.3% (10/300). If all the FP are unilateral, then percent of patients needing femur radiography will still be 5% (10/200); but if all the FP are bilateral, then the percent needing femur radiograph will be still 2.5% (5/200). This distinction becomes evident in the analysis below.

Case control studies

Kim *et al*. were first to report a case control study.[4] Patients with either radiographically-proven iAFF or complete AFF were selected if medical records included prior DXA images on the ipsilateral side (n=33); the DXA scans were performed on average 11 (range, 0.24 to 24) months prior to the diagnosis of AFF. Control patients (n=199) were selected if they had both a DXA image and an ipsilateral femur X-ray within a three month interval; they were deemed to be a representative sample. Their DXA definition of iAFF was based on “focal cortical change”of the lateral cortex at either a periosteal surface or an endosteal surface, or at both surfaces. They did not specify if a fracture line was seen.

Since this was a case control study, the frequency of radiographically-proven AFF was 14.2%, as set by the sample sizes. Using three blinded observers they calculated the following range of estimates: FP percent of 0-0.9%, FN percent of 6.0-6.9%, sensitivity of 51.5-57.6%, specificity of 99-100%, PPV of 92.6-93.3%, and NPV of 92.7-93.5% (**Appendix Table)**.[4] The low sensitivity was likely secondary to the limited view of the default-length femur; they demonstrated that the location of the radiographically-proven AFF was below the default-length of the femur image in 46% of cases. The remaining cases of FN were partly deemed to be consequent upon the limited resolution of the older DXA systems; the retrospective DXA images were acquired by scanners in use from 2004 to 2011. Despite this, the NPV was high.[4] Of note, in a smaller sample (n=19), who had prior DXA of the contralateral femur, the sensitivity for detecting an abnormality at the time of DXA imaging was much lower at 10%, likely due to the same issues above plus only a portion of patients with AFFs have contralateral AFFs.

One case control study (presented as a poster at a prior ASBMR annual meeting) examined the test characteristics of FFI using plain radiographs as the gold standard.[34] The authors found that FFI has a sensitivity of 66% to 75% and a specificity of 91-92%, a PPV of 87-89% and NPV of 73-82%. The presence of focal cortical periosteal and endosteal thickening on the lateral cortex has a positive likelihood ratio of 9.3-10, and the absence of these abnormalities has a negative likelihood ratio of 0.28-0.37.

Cohort studies

There are three cohort studies using either extended-length femur imaging or FFI. All three studies had selective disease verification, whereby femur radiographs were performed in those with suspected abnormality in the spectrum of AFF. So, the studies provide information on TP, FP, and PPV (**Appendix Table**).[3,25,26] McKenna *et al*. in a prospective study selected consecutive patients (n=257) attending for routine DXA examination, who were on bisphosphonate therapy for greater than five years; all patients had bilateral DXA imaging using an extended-length femur field. The radiographically-proven prevalence of abnormality in the spectrum of AFF was 2.7%. The FP percent was 2.3%. Since all of the FP findings were unilateral, then 4.6% of patients had unnecessary femur radiography. Since both TP and FP were counted, the PPV could be derived: it was 37%. By erring in favor of not missing any cases of AFF and thereby minimizing FN, the authors suggested that the NPV would be close to 100%.[3] A limitation of this study was not imaging the full-length of the femur. A second smaller prospective study by McKenna *et al*., using similar criteria for selection of cases (n=173) but adapting the DXA system to FFI using single-energy high-definition feature, did not identify any case of iAFF due to declining prevalence, such that PPVcould not be estimated.[25] The FP percent was 3.4%; since all the abnormalities were unilateral, then 6.9% of patients had unnecessary

femur radiography. FP percent would have been 1.7%, if those cases with an anatomic abnormality related to the linea aspera were excluded.[25]

van de Laarschot *et al*. in a retrospective study selected patients (n=282) attending for routine DXA, who had used either bisphosphonates or denosumab at any time in the preceding 12 months; the median duration of antiresorptive therapy was 8.5 years.[26] All patients had bilateral FFI using an investigator-adapted DXA system. All but two of the positive cases for focal cortical thickening had either review of recent femur X-rays orother imaging modalities or had subsequent femur radiograph. The radiographically-confirmed cases of abnormality in the spectrum of AFF was 3.2%. The PPV was 83.3%. The FP percentwas 0.4%. Since one patient had bilateral FP results, then only 0.4% needed additional radiography.

DISCUSSION

Information on diagnostic accuracy of DXA is confined to five studies. Estimates of FP percent are low ranging from 0.4% to 3.5%. The first study on diagnostic accuracy was a very detailed case-control study by Kim *et al*.[4] The FP percent was very low at 0.4% accounting for a high PPV. The retrospective cohort study by van de Laarschot also had a low FP percent at 0.4%[26]. A FP is clinically important, because a positive FFI will necessitate further imaging tests. As observers get more experienced, FP should decline. For instance, the studies by McKenna *et al*.[3] and van de Laarschot *et al*.[26] had similar percent of patients with radiographically-proven abnormalities in the spectrum of AFF, but the latter study had a much lower FPpercent giving it a much higher PPV (**Appendix Table**).

The FN percent is particularly pertinent because one wants a high degree of certainty that a negative test means absence of AFF. For the Kim study, the FN percent was unacceptably high at 6.5%. This was explained both by inadequate visualization of the femur due to using default-length femur imaging and by poor resolution of older DXA systems. The three prevalence studies[3,25,26] could not comment on FN percent because they did not subject everyone to plain radiographs, only those who screened positive. Given the low prevalence of iAFF, it is expected that FN percent will be very low, which is the desired outcome.

One case control study did show that FFI has moderate to high sensitivity and high specificity for detecting abnormalities in the spectrum of AFF, but it was only presented in abstract form.[34] More studies are needed in this area, preferably prospective studies that perform femur radiographs on all participants. Calculations regarding diagnostic

accuracy should incorporate adjustments for: the number of femurs tested, the number of patients studied, and the frequency of bilaterality. If this detailed information is not given, then it will limit comparisons between studies.

In conclusion, the FP percent is low, and it is likely that FP will decline as experience in reporting images is acquired. Given the combination of low prevalence, extremely low FN percent, and low FP percent, the following can be expected: an extremely high NPV, and modest PPV.

Appendix Table. Diagnostic accuracy of DXA systems in the detection of abnormalities in the spectrum of AFF based on confirmatory X-rays

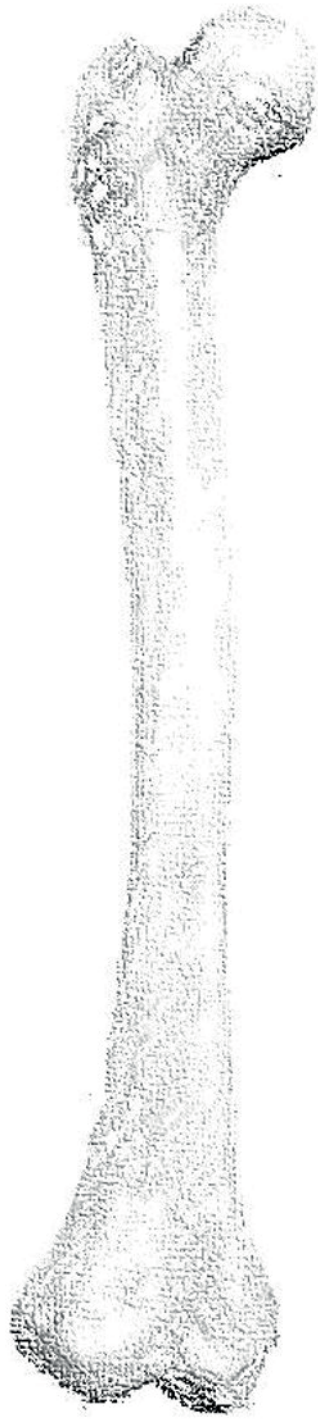
Study	Study Design	Sample size	Imaging modality	DXA abnormality (%)	FP (%)	FN (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	X-ray abnormality (%)
Kim, 2014[4]	Case control	29 cases (33 hips by DXA) 199 controls (199 hips by DXA)	DXA (default-length femur imaging)	8.2[a]	0.4[a]	6.5[a]	55 [a]	99 [a]	95 [a]	93 [a]	14.2
McKenna, 2013[3]	Prospective cohort	257	DXA (Extended-length femur imaging) [b]	7.4	2.3	n/a	n/a	n/a	37	n/a	2.7
McKenna, 2017[25]	Prospective cohort	173	Single-energy (FFI)	6.9	3.5	n/a	n/a	n/a	n/a	n/a	0
Van de Laarschot, 2017[26]	Retrospective cohort	282	DXA (FFI)	4.3	0.4	n/a	n/a	n/a	83	n/a	3.2

FP, false positive; FN, false negative; PPV, positive predictive value; NPV, negative predictive value;

Note: since the design of the three cohort studies had selective verification of iAFF, the following can only be estimated: TP percent, FP percent, and PPV (see text). The Kim study is based on the number of femurs imaged. For the three cohort studies, the prevalence of DXA abnormalities and X-ray abnormalities are based on the number patients and not on the number of femurs, but FP%, TP%, and PPV% are all based on the number of femurs imaged.

[a] Estimates are based on average results of three observers.

[b] Scan length of the DXA image was extended to 22 cm.



Part 2

Genetics in relation to atypical femur fractures

6

Atypical femur fracture in an adolescent boy treated with bisphosphonates for X-linked osteoporosis based on PLS3 mutation

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ABSTRACT

Long-term use of bisphosphonates has raised concerns about the association with Atypical femur fractures (AFFs) that have been reported mainly in postmenopausal women. We report a case of an 18-year-old patient with juvenile osteoporosis based on X-linked osteoporosis due to a PLS3 mutation who developed a low trauma femoral fracture after seven years of intravenous and two years of oral bisphosphonate use, fulfilling the revised ASBMR diagnostic criteria of an AFF. The occurrence of AFFs has not been described previously in children or adolescents. The underlying monogenetic bone disease in our case strengthens the possibility of a genetic predisposition at least in some cases of AFF. We cannot exclude that a transverse fracture of the tibia that also occurred after a minor trauma at age 16 might be part of the same spectrum of atypical fractures related to the use of bisphosphonates. In retrospect, our patient experienced prodromal pain prior to both the tibia and the femur fracture. Case reports of atypical fractures in children with a monogenetic bone disease such as Osteogenesis Imperfecta (OI) or juvenile osteoporosis are important to consider in the discussion about optimal duration of bisphosphonate therapy in growing children.

In conclusion, this case report 1) highlights that AFFs also occur in adolescents treated with bisphosphonates during childhood and pain in weight-bearing bones can point towards this diagnosis 2) supports other reports suggesting that low trauma fractures of other long bones besides the femur may be related to long-term use of bisphosphonates 3) strengthens the concept of an underlying genetic predisposition in some cases, now for the first time reported in X-linked osteoporosis due to a mutation in PLS3 and 4) should be considered in decisions about the duration of bisphosphonate therapy in children with congenital bone disorders.

1. INTRODUCTION

Atypical Femur Fractures (AFFs) are considered a rare but severe adverse effect of bisphosphonate use. AFFs resemble stress fractures and occur at the lateral cortex of the subtrochanteric femur after no or minimal trauma. The incidence rates for an AFF increase with longer duration of bisphosphonate use. According to Dell *et al.* these rates range from 1.78/100,000 persons per year with bisphosphonate exposure under two years to 113.1/100,000 per year amongst patients with long-term bisphosphonate use over eight years.[1] Typically, AFFs have been reported in postmenopausal women on prolonged treatment of bisphosphonates with a median duration of seven years. Also men may be affected. To the best of our knowledge, AFFs have never been documented in children or adolescents. Pediatric patients with fragility fractures, mainly due to Osteogenesis Imperfecta (OI), are extensively treated with bisphosphonates. Although it appears that bisphosphonates improve bone density in children with OI, the evidence on beneficial effects on fracture rates and clinical functional improvement is still inconclusive.[2,3]

There is uncertainty about the optimal duration of bisphosphonate treatment as well as the dose and type of bisphosphonates in children. In adults it is usually advised to reevaluate the necessity of continued use after five years of treatment. For children on bisphosphonates it is unclear at what point these drugs should be discontinued. It has been suggested that termination of antiresorptive drugs in growing children leads to zones of localized bone fragility at the junction of older, denser bone and new bone.[4,5] Based on these findings continued bisphosphonate therapy in younger patients with OI or persistent risk factors for compromised bone health has been suggested until growth is fully or nearly completed.[6,7]

2. CASE DESCRIPTION

A boy born in 1996 has sustained multiple fractures since 2002, usually after mild trauma. In total he has experienced approximately 14 fractures until the age of 16, e.g. fingers, wrist, shoulder and arm. In 2006 a DXA-scan showed severe osteoporosis with Z-score -3.7 SD of L2-L4 and -4.6 SD of total body. His family history was positive for osteoporosis and his brother similarly presented with numerous fractures at a young age. Initially, a presumptive diagnosis of OI was made, but could not be confirmed through mutation analysis of *COL1A1* and *COL1A2* genes. In 2013 he was diagnosed with X-linked osteoporosis due to a mutation in PLS3, a gene coding for Plastin 3, an F-actin bundling protein, described as a novel monogenic cause of familial osteoporosis and by some seen as a novel form of OI.[8,9]

At age nine, in 2006, he was started on pamidronate intravenously 1.0 mg/kg every four and later every six months during three days. In total he received intermittent pamidronate intravenously for seven years with a cumulative dose of 2107 mg.During this time his Bone Mineral Density (BMD) improved. A DXA-scan in 2010 showed a Z-score of L2-L4 +0.7 SD and of total body -1.1 SD. Several small fractures of the fingers and a shoulder fracture occurred after relatively minor traumas during bisphosphonate treatment.

At the age of 16, he broke his tibia and fibula of the left leg after a low-impact fall when he slipped during walking (**Figure 1)**. He had complained of pain in this lower leg for several weeks before. He underwent surgery with placement of intramedullary pins. He was then switched to risedronate orally 35 mg once per week. Two months afterwards, he sustained a spontaneous fracture just above the intramedullary nail of the lower leg after a very soft fall from standing height. When he was 18 years old, he complained of pain in his upper right leg for several weeks. Just before a scheduled visit to the neurologist for this pain he fractured the right femur after a slight fall during walking at a normal

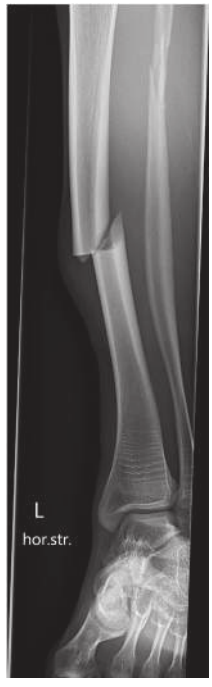

Figure 1*: X-ray showing tibia and fibula fracture after low-energetic trauma. Transverse sclerotic bands are visible in the metaphysis of the distal tibia, induced by intermittent pamidronate infusions. Note the predominantly transverse fracture line of the tibial shaft.*

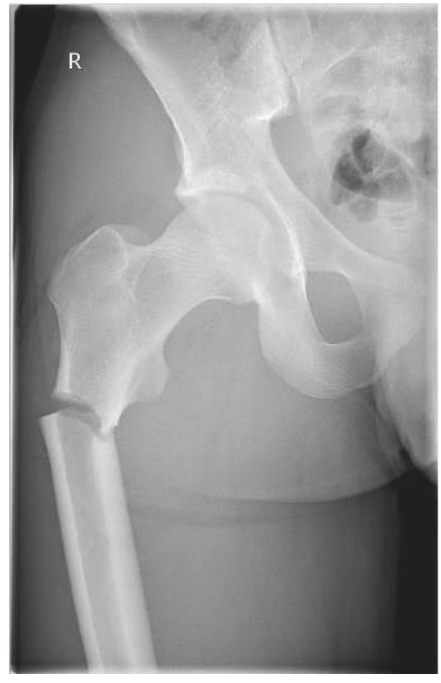

Figure 2: *X-ray showing subtrochanteric non-comminuted femoral fracture with transverse orientation after minimal trauma, fulfilling all diagnostic criteria of an AFF.*

pace (**Figure 2**). Following intramedullary fixation, he was referred to the Bone Centre of our hospital. Under suspicion of an AFF of the right femur, bisphosphonate therapy was discontinued and the patient was started on calcium and vitamin D supplementation. There was no delayed healing of the AFF. His BMD Z-score remained stable. A timeline of this case is shown in **Figure 3**.

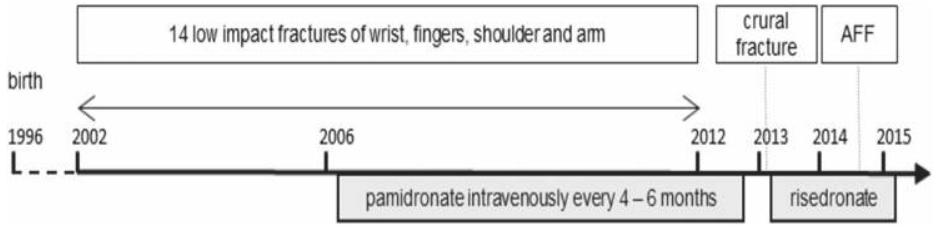

Figure 3: *Horizontal diagram describing the timing of fractures and bisphosphonate use.*

3. DISCUSSION

We present an 18-year-old patient with juvenile osteoporosis based on a PLS3 mutation and a spontaneous fracture of the right femur. AFFs have mostly been described in postmenopausal women but also in men. Our case meets all diagnostic criteria for an AFF. It concerns a 1) subtrochanteric fracture with minimal prior trauma, 2) is transverse in orientation whilst the fracture line becomes more oblique as it progresses, 3) extends through both cortices and 4) is not comminuted. He also presented with prodromal pain.[10]

To our knowledge, this is the first documentation of an AFF in an adolescent and furthermore the first report of such a fracture in a patient with juvenile osteoporosis based on a PLS3 mutation. The fracture of the lower leg preceded by prodromal pain and a trivial trauma, shows a predominantly transverse fracture line of the tibial shaft. We cannot exclude that the tibia fracture in our patient may be considered an atypical fracture as well.

Several previous studies point out the actuality of atypical fractures in patients with OI treated with bisphosphonates. In a retrospective study amongst 176 bisphosphonate-treated patients with OI compared to a historic group without bisphosphonate treatment, an apparent change in pattern of fracture was observed. In the bisphosphonate-treated group proximal, subtrochanteric fractures without any history of trauma were more frequently observed compared to the control group. In case reports, AFFs have been described in adults with OI and bisphosphonate treatment[12,13], where a possible

synergistic relationship between atypical fractures associated with bisphosphonate use and OI was suggested by the authors.[13]

Recently a manuscript was published describing six children with OI who had sustained unusual subtrochanteric femoral fractures located over pre-existing intramedullary rods. Although these fractures do not fit the definition of an AFF because of the presence of the intramedullary nails, the authors proposed the possibility of a pediatric variant of the AFF associated with prolonged bisphosphonate use.[14] Similarly, these children also displayed atypical fractures without prior trauma in other bones, such as the humeri.

A potential underlying genetic susceptibility for AFFs has been advocated occasionally by the manifestation of these fractures in various monogenetic bone disease other than OI with or without prior use of bisphosphonates like hypophosphatemia, hypophosphatasia, osteopetrosis and pycnodysostosis.[15-21] Our finding of AFF in an adolescent boy who was treated long term with bisphosphonates intravenously for X-linked osteoporosis contributes to the hypothesis that these rare fractures may have an underlying genetic predisposition a least in some cases.

Our patient has received a cumulative dose of pamidronate infusions of 2107 mg and 3500mg of risedronate orally. We propose that children and adolescents who are extensively treated with bisphosphonates for conditions such as OI, juvenile familial osteoporosis or secondary osteoporosis are at risk of an AFF. We underline that in previous studies atypical, low-energetic stress fractures appear to occur in other weight-bearing bones as well, such as the tibia fracture in our case.[22,23]

The possibility of the occurrence of AFFs in children treated with bisphosphonates should be considered in decision-making about the duration of therapy. Our case is important to view in light of the current tendency to continue bisphosphonates in children until growth is fully or nearly complete. In conclusion, we report for the first time an adolescent boy with X-linked osteoporosis due to a PLS3 mutation who developed a classical AFF after seven years of intravenous and two years of oral bisphosphonate use. Furthermore, he experienced an unusual fracture of the lower leg after a minor trauma two years earlier. These findings highlight that AFFs also occur in adolescents treated with bisphosphonates during childhood and suggest that similarly low trauma fractures of other bones may be related to long-term use of bisphosphonates. Moreover, this case supports the concept of an underlying genetic predisposition in some cases of AFF. The possibility of AFFs in children with bisphosphonate therapy for OI or other bone disorders at risk for fragility fractures should be taken into account when deciding upon

the continuation of antiresorptive drugs. Pain in weight-bearing bones amongst these patients should prompt investigation for an incomplete AFF.

Acknowledgments

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Patient evaluation: MCZ. Drafting manuscript: DvdL and MCZ. Revising manuscript content: DvdL and MCZ. Approving final version of manuscript: DvdL and MCZ. DvdL and MCZ take responsibility for the integrity of the data analysis.

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7

Genetic risk factors for atypical femoral fractures (AFFs): a systematic review

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ABSTRACT

Atypical femoral fractures (AFFs) are uncommon and have been associated particularly with long-term antiresorptive therapy, including bisphosphonates. Although the pathogenesis of AFFs is unknown, their identification in bisphosphonate-naïve individuals and in monogenetic bone disorders has led to the hypothesis that genetic factors predispose to AFF. Our aim was to review and summarize the evidence for genetic factors in individuals with AFF. We conducted structured literature searches and hand-searching of conference abstracts/reference lists for key words relating to AFF and identified 2566 citations. Two individuals independently reviewed citations for (i) cases of AFF in monogenetic bone diseases and (ii) genetic studies in individuals with AFF. AFFs were reported in 23 individuals with the following seven monogenetic bone disorders (gene): osteogenesis imperfecta *(COL1A1/COL1A2)*, pycnodysostosis *(CTSK)*, hypophosphatasia *(ALPL)*, X-linked osteoporosis *(PLS3)*, osteopetrosis, X-linked hypophosphatemia *(PHEX)*, and osteoporosis pseudoglioma syndrome *(LRP5)*. In eight cases (35%), the monogenetic bone disorder was uncovered after the AFF occurred. Cases of bisphosphonate-naïve AFF were reported in pycnodysostosis, hypophosphatasia, osteopetrosis, X-linked hypophosphatemia, and osteoporosis pseudoglioma syndrome. A pilot study in 13 AFF patients and 268 controls identified a greater number of rare variants in AFF cases using exon array analysis. A whole-exome sequencing study in three sisters with AFFs showed, among 37 shared genetic variants, a p.Asp188Tyr mutation in the *GGPS1* gene in the mevalonate pathway, critical to osteoclast function, which is also inhibited by bisphosphonates. Two studies completed targeted *ALPL* gene sequencing, an *ALPL* heterozygous mutation was found in one case of a cohort of 11 AFFs, whereas the second study comprising 10 AFF cases did not find mutations in *ALPL*. Targeted sequencing of *ALPL, COL1A1, COL1A2*, and *SOX9* genes in five cases of AFF identified a variant in COL1A2 in one case. These findings suggest a genetic susceptibility for AFFs. A large multicenter collaborative study of well-phenotyped AFF cases and controls is needed to understand the role of genetics in this uncommon condition.

INTRODUCTION

There is currently a crisis in the treatment of osteoporosis, with a call to action by multiple international professional societies to aggressively reduce fracture risk in our aging population.[1] Despite the availability of effective antiresorptive osteoporosis drugs, namely bisphosphonates and denosumab, treatment rates after hip fracture—in patients at the highest risk for subsequent fractures—have halved from 40% in 2002 to 21% in 2011.[1] This crisis is driven by in large part by fear of rare complications of antiresorptive drugs, such as atypical femoral fractures (AFFs) (**Figure 1**).[2,3] Despite the significant burden of osteoporosis and fractures globally, since the first clinical reports of bisphosphonate-associated AFFs in 2005[4] and the subsequent FDA safety report in 2010[5], there has been a 50% decline in the use of these effective osteoporosis therapies.[6]

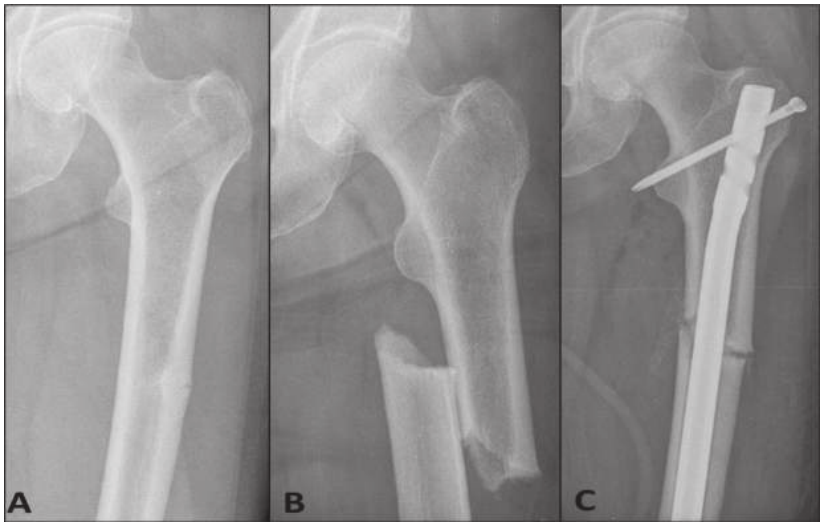

Figure 1. X-ray series of a 61-year-old woman with postmenopausal osteoporosis presenting with prodromal left thigh pain in the setting of 11 years of alendronate therapy. An initial radiograph of the left femur demonstrated a transverse midshaft lateral stress fracture consistent with an incomplete atypical femoral fracture *(A)*. After a minimal trauma fall, the fracture progressed to a complete fracture *(B)*, and this required surgical fixation with an intramedullary nail *(C)*. Reproduced from Nguyen *et al.* Bone Rep. 2017;6:34–7.

Currently, the pathogenesis of AFFs is not known, but AFFs have also been described in individuals with monogenetic bone disorders and can occur in bisphosphonate-naïve individuals, who comprise about 7% of cases.[2,3] As such, it is likely that genetic variants exist that predispose to AFFs. This article reviews and summarizes the evidence for genetic factors in individuals with AFFs after first discussing the epidemiology and clinical problem of this condition.

Osteoporosis and anti-osteoporosis drugs

Osteoporosis is a condition with reduced bone strength due to abnormalities in the material composition and microstructure of bone predisposing to fractures. Hip fractures are catastrophic events resulting in chronic pain, disability, and increased mortality up to 35% within 12 months.[7] Bisphosphonates are well-established drugs for the management of osteoporosis. They are effective at reducing the risk of vertebral fractures by up to 70%[8] and also reduce nonvertebral and hip fractures.[9] They have been approved treatments for osteoporosis for more than two decades.

Bisphosphonates are structural analogs of inorganic pyrophosphate and inhibit bone resorption by binding avidly to bone mineral surfaces, are subsequently internalized by boneresorbing osteoclasts, whereby they disrupt various biochemical processes.[10] In particular, nitrogen-containing bisphosphonates inhibit farnesyl pyrophosphate synthase, a key enzyme in the mevalonate pathway. This pathway is important for the biosynthesis of isoprenoids, molecules essential for multiple cellular processes. Disruption of this pathway affects osteoclast function and viability, ultimately resulting in reduced bone resorption.

The problem of atypical femoral fractures

In 2005, Odvina and colleagues published the first case series of femoral shaft fractures associated with long-term bisphosphonate use and showed evidence of markedly suppressed bone formation on a bone biopsy.[4] A subsequent series of femoral fractures from Singapore in 2007[11] emphasized the unusual location of this fracture in the subtrochanteric region. This region (from just distal to the lesser trochanter to just proximal to the supracondylar flare of the femur) is notably resilient to traumatic injuries.[12] In 2010, the ASBMR convened an international Task Force to commission a report on and to create a case definition of AFF.[2] This case definition was subsequently revised in 2013 (**Table 1**).[3]

Epidemiology and consequences of AFFs

In a population-based Swedish study including 12,777 women aged ≥55 years with femoral fractures, 59 AFFs were identified, of which 46 occurred in bisphosphonate users.[13] Linkage to the Swedish Prescribed Drug Register identified that 46 AFFs occurred in 83,311 women ever prescribed bisphosphonates in the preceding three years, whereas only 13 cases of bisphosphonate- naïve AFFs occurred out of 1,437,820 Swedish women aged ≥55 years who were not prescribed bisphosphonates. The age-adjusted relative risk of AFF with any use of bisphosphonates was 47.3.

Meier and colleagues found that the proportion of patients exposed to bisphosphonates was higher in patients with AFFs than in those with non-atypical fractures (82.1% versus

Table 1. ASBMR task force revised case definition of AFFs

AFF must be located along the femoral diaphysis from just distal to the lesser trochanter to just proximal to the supracondylar flare. At least four of five major features must be present. No minor features are required.
Major features • Minimal or no trauma as in a fall from a standing height or less • The fracture line originates at the lateral cortex and is transverse, although it may become oblique as it progresses medially • Complete fractures extend through both cortices and may be associated with a medial spike; incomplete fractures involve only then lateral cortex • The fracture is non- or minimally comminuted • Localized periosteal or endosteal thickening of the lateral cortex is present at the fracture site ("beaking" or "flaring")
Minor features • Generalized increase in cortical thickness of the femoral diaphyses • Unilateral or bilateral prodromal symptoms such as dull or aching pain in the groin or thigh • Bilateral incomplete or complete femoral diaphysis fractures • Delayed fracture healing

6.4%).[14] The risk of AFF increases with longer duration of bisphosphonate exposure (1.8 per 100,000 cases per year after 0.1 to 1.9 years, rising to 113.1 per 100,000 cases per year for 8.0 to 9.0 years of use).[15] AFFs are also associated with a younger cohort[16], Asian ethnicity[17], a previous stress fracture of the contralateral femur[14] and glucocorticoid use.[2]

Although the incidence of AFF is relatively low, the effect of a painful, spontaneous femoral fracture is devastating to the individual. In addition, up to 40% of AFFs occur in bilateral femora and delayed healing is common, resulting in prolonged immobilization. Prodromal pain occurs in more than 70%.[2]

Pathophysiology: proposed mechanisms

Although the pathogenesis of AFFs remains largely unknown, its epidemiological association with bisphosphonate therapy led to several proposed mechanisms. Bisphosphonates alter collagen maturity and cross-linking, as indicated by the increase in pyridinoline (PYD)/deoxypyridinoline (DPD) ratio, increasing the strength but also stiffness of bone.[18,19] Moreover, reducing bone remodeling also increases pentosidine, which interacts with collagen through oxidative nonenzymatic cross-linkage, leading to advanced glycation end product accumulation, which results in reduced toughness. Both effects increase matrix stiffness and consequently reduce the peak-tolerated strain. The stiffening by increased matrix mineralization and pentosidine cross-linking reduces ductility; the structure becomes more brittle.[20,21]

Also, more homogeneously mineralized bone tissue facilitates fracture crack initiation and propagation.[22,23] Thus, remodeling suppression increases microfracture burden, allowing cracks to lengthen and reducing crack removal.[24,25] Microdamage accumulation may be further compounded by the preferential uptake of bisphosphonates at sites of high bone remodeling, including sites of stress fractures. Therefore, by suppressing remodeling at these local sites, bisphosphonates could potentially affect the intracortical repair of a developing stress fracture such as an AFF, allowing crack progression to a complete fracture.

Iliac crest bone biopsies from AFF cases showed reduced bone turnover in most cases, as would be expected with bisphosphonate treatment, but this has not been a universal finding.[2] In bone biopsies obtained close to the fracture site in AFF cases, both decreased and increased bone remodeling have been described, although the latter may be influenced by the recent fracture and may not be indicative of the underlying pathogenic mechanism of AFFs.

Hypotheses and aims

The rarity of AFFs amongst the millions of bisphosphonate users worldwide is suggestive of an individual susceptibility, which could be an underlying genetic predisposition.

The potential importance of genetic factors is supported by the occurrence of AFFs in bisphosphonate-naïve individuals.[13,26,27] Some of these bisphosphonate-naïve individuals were found to have an underlying monogenetic bone disease, such as hypophosphatasia, pycnodysostosis, osteopetrosis, X-linked hypophosphatemia (XLH), and osteoporosis pseudoglioma syndrome (OPPG), leading to the hypothesis that carriers of pathogenic mutations (very rare variants) or polymorphisms (common variants) in genes related to these monogenetic bone diseases may predispose to AFFs. Mild unrecognized forms of such heritable bone diseases may underlie the etiology of AFFs in some patients. Additionally, AFFs have been reported in patients with osteogenesis imperfecta (OI) and X-linked osteoporosis based on a *PLS3* mutation. Yet these cases may be related to the coexistent antiresorptive treatment rather than the underlying genetic condition, and no analysis has ever been done to investigate whether AFFs occur more frequently in patients with these genetic bone disorders. Even more suggestive of a genetic background of AFFs is the identification of two families with multiple family members with AFF.[28,29]

Furthermore, racial differences in risk and site of AFFs exist, which may be consistent with a genetic background. The age-adjusted relative risk for AFFs in Asians is 6.6 compared with white women corrected for current bisphosphonate use and duration

of bisphosphonate treatment.[17] Schilcher and colleagues showed ethnic differences in location of the AFFs, with fractures mainly occurring in the subtrochanteric region in Singapore, compared with diaphyseal in Sweden.[30] The differences may be related to femoral geometric parameters that are more common in Asian women, such as increased femoral bowing and smaller neck-shaft angles.[31-34] Increased femoral curvature may lead to an altered distribution of loading with more tensile strain on the lateral side and more compression on the medial side of the femur. This imbalance of biomechanical stresses with increased femoral bowing might contribute to spontaneous, transverse femoral fractures such as AFFs.[35]

We speculate that genetic factors may also interact with clinical risk factors for AFFs, including a high number of comorbid conditions, and concomitant medications, like glucocorticoids.[2]

The aim of this systematic review is to gather the data of AFF in relation to genetics and scrutinize the available evidence of genetic risk factors underlying the susceptibility for AFFs and to inform future directions for further research.

METHODS

We conducted a structured literature search of electronic databases, including Embase, Medline, Web of Science, Cochrane Central, and Google Scholar, and hand-searching of conference abstracts/reference lists using the following key words: femur/femoral fracture or subtrochanteric fracture, atypical and drug-induced disease, bisphosphonates, antiresorptives, and denosumab.

We identified 2,566 citations, and authors HHN and DMvdL independently reviewed citations with the following inclusion criteria: (i) cases of AFFs in monogenetic bone diseases, and (ii) genetic studies in individuals with an AFF. Articles were only included if images of the femoral fracture were published and fulfilled the ASBMR case definition (**Table 1**) or if the authors used the recent ASBMR case definition to define the presence of AFFs. Twenty-six citations fulfilled the inclusion criteria and are described below. During preparation of this manuscript, two additional published articles and a conference abstract relevant to this topic were identified and also included in this review, making a total of 29 included studies.

RESULTS AND DISCUSSION

Reports of AFFs occurring in monogenetic bone disorders

Subtrochanteric femoral fractures fulfilling the ASBMR case definition of AFF were identified in seven monogenetic bone disorders. These findings are summarized in **Table 2** and explored further below.

Table 2. List of monogenetic bone disorders associated with AFFs

Monogenetic bone disorder	Genes associated with disorder	Sex	Age (yrs)	Bilateral AFF *n*	BP exposure *n*	Disorder diagnosed following AFF *n*
Hypophosphatasia[36–39]	ALPL	4 F	50–55	4	1	4
XLH[41]	PHEX	1 M	27	0	0	0
Pycnodysostosis[44–49]	CTSK	3 M/4 F	23–55	3	0	2
Osteopetrosis[51–53]	TCIRG1, CLCN7, OSTM1, PLEKHM1, SNZ10, TNFS11, TNFRSF11A, CA11	4 F	21–56	2	0	1
OPPG[57]	LRP5	1 M	38	0	0	1
OI[61–65]	LRP5 COL1A1/1A2, CRTAP, LEPRE1, PPIB, SERPINH1, FKBP10, PLOD2, SP7	4 F/1 M	11–75	2	5	0
X-linked osteoporosis[70]	PLS3	1 M	18	0	1	0

AFF = atypical femoral fracture, F = female, M = male, BP = bisphosphonate, XLH = X-linked hypophosphataemia, OPPG = osteoporosis pseudo-glioma syndrome, OI = osteogenesis imperfect.

The seven bone disorders included primary defects in bone mineralization, bone remodeling, collagen synthesis and structure, and osteocyte function (**Figure 2**). AFFs were also reported in juvenile forms of osteoporosis linked with long-term bisphosphonate use and may reflect a pediatric variant of bisphosphonateassociated AFF. These cases provide insight into the possible pathogenesis of AFFs and indicate potential candidate genes that may encode for variants predisposing to AFF. When dealing with bisphosphonate-naïve patients with AFFs, clinicians may need to carefully consider and exclude these underlying genetic conditions in their diagnostic assessment for secondary causes of skeletal fragility.

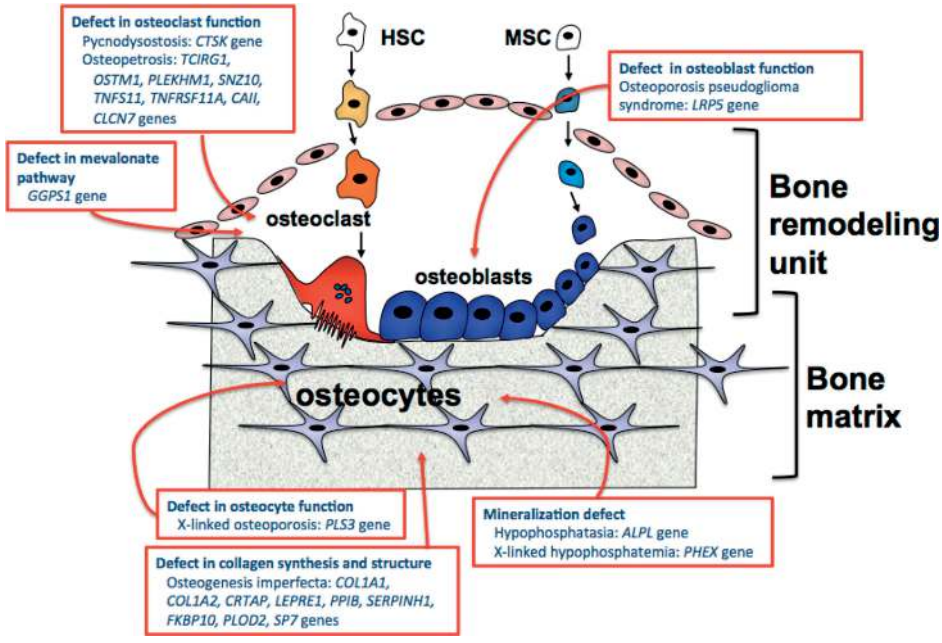

Figure 2. Genes implicated in atypical femoral fractures and their relationship to bone remodeling and bone matrix. HSC = hematopoietic stem cell; MSC = mesenchymal stem cell.

Mineralization defect

Hypophosphatasia: The literature search identified 4 cases of AFFs occurring in adult hypophosphatasia[36–39], in all of whom the genetic condition was unmasked after the femoral fracture. Three cases were bisphosphonate-naïve and in one case, reported by Sutton and colleagues, of bilateral atraumatic AFFs occurred simultaneously in a postmenopausal woman after four years of bisphosphonate therapy for osteoporosis.[3]

Hypophosphatasia is an inborn error of metabolism characterized by low alkaline phosphatase (ALP) levels, due to a loss-of-function mutation in the *ALPL* gene (also known as *TNSALP* gene) that encodes the tissue nonspecific ALP.[40] This defect in enzyme function leads to accumulation of substrates, such as inorganic pyrophosphate, pyridoxal 5' phosphate (active form of vitamin B6), and phosphoethanolamine. Inorganic pyrophosphate is an inhibitor of mineralization, and its accumulation in hypophosphatasia results in skeletal and dental manifestations.

The clinical spectrum of hypophosphatasia is broad and can range from severe lethal forms in infancy to mild forms in adulthood or with only dental complications (odontohypophosphatasia).[40] Skeletal manifestations in adulthood may include osteopenia, poorly healing stress fractures of the metatarsal bones, and pseudo-fractures. The

pseudo-fractures commonly occur on the lateral side of the femoral shaft and can resemble bisphosphonate-associated AFFs.[41]

The similarity in the pseudo-fractures found in hypophosphatasia and the AFFs that occur in patients with osteoporosis treated with bisphosphonates may be explained by the fact that bisphosphonates are synthetic analogs of inorganic pyrophosphate resistant to ALP activity. There has been one case report of pyrophosphate accumulation at a site of a bisphosphonate-associated AFF.[42] Alternatively, the presence of AFFs found in bisphosphonate-naïve patients with hypoposphatasia may suggest that variants in the *ALPL* gene may be implicated in the pathogenesis of AFFs, independent of bisphosphonate exposure. Speculation also exists as to whether bisphosphonate therapy in individuals with mild forms of hypophosphatasia may precipitate AFFs. Targeted genetic testing of the *ALPL* gene in small cohorts of individuals with AFF have been conducted, and results will be described further below.

X-linked hypophosphatemia (XLH): Whyte and colleagues reported that pseudo-fractures in the lateral cortex of the femoral shaft can occur in XLH similar to AFFs.[41] In this report, a 27-year-old bisphosphonate-naïve male with XLH had radiological features of an incomplete AFF.

XLH is the most common form of hereditary rickets and is caused by loss-of-function mutations of the *PHEX* gene.[43] Biochemical findings in this condition are hypophosphatemia with renal phosphate wasting and associated inappropriately low 1,25-hydroxyvitamin D levels and high FGF-23 levels. Low bone mineral density, rickets, and/or osteomalacia with shortening and deformities of the lower limbs are common features of this condition.

Defect in bone remodeling

Pycnodysostosis: Subtrochanteric femoral fractures fulfilling criteria for AFFs have been described in seven adult cases of pycnodysostosis. In three of these cases, pycnodysostosis was unmasked after the femoral fracture.[44-49] Five cases were bisphosphonate-naïve, whereas prior bisphosphonate use was unknown in the remaining two cases. Bilateral AFFs occurred in four cases of pycnodysostosis. Nakase and colleagues[45] reported delayed healing of the femoral shaft fracture of up to three years in two patients with pycnodysostosis.

Pycnodysostosis is a rare, autosomal recessive disorder of osteoclast function, characterized by short stature, osteosclerosis, pathological fractures with poor healing, acro-osteolysis of the terminal phalanges, and craniofacial dysmorphisms.[50] It is caused by

mutations in the *CTSK* gene resulting in deficiency of cathepsin K activity. Cathepsin K is an enzyme that is highly expressed in osteoclasts and is responsible for degradation of bone matrix proteins during osteoclast-mediated bone resorption. The defect in osteoclast activity leads to osteosclerosis, increased bone density, abnormal bone matrix and brittle bones, predisposing to pathological fractures of the long bones. Pycnodysostosis is managed symptomatically, and antiresorptive therapy has no place/is not indicated in this condition. The occurrence of AFFs in the absence of bisphosphonate use in pycnodysostosis, as described in these case reports, raises the possibility that genetic variants of the *CTSK* gene may predispose individuals to AFF.

Osteopetrosis: Our literature search identified 4 cases of AFFs occurring in bisphosphonate-naïve individuals with osteopetrosis, although the underlying mutated gene was not reported in these articles.[51–53] All authors described the surgical difficulties in the repair of the subtrochanteric femoral fractures in sclerotic bone.

Osteopetrosis is a class of rare heterogeneous genetic disorders characterized by high bone mass due to a failure of osteoclast-mediated bone resorption.[54] Mutations in eight genes have been described, including *TCIRG1, OSTM1, PLEKHM1, SNZ10, TNFSF11, TNFRSF11A, CAII,* and *CLCN7*. Despite increased bone density, the sclerotic bone is brittle and fragility fractures occur[55], and antiresorptive therapies are avoided in this condition. Other clinical features include craniofacial deformities, neurological compression from sclerotic bone, bone marrow failure due to reduction of bone marrow space, complications from extramedullary hematopoiesis, osteoarthritis, and dental complications.

Osteoporosis pseudoglioma syndrome (OPPG): OPPG is a rare, autosomal recessive form of juvenile osteoporosis caused by a loss-of-function mutation in the *LRP5* gene.[56] We identified a single case report of an AFF occurring in a 40-year-old bisphosphonate-naïve male with OPPG, who had multiple fragility fractures since childhood and evidence of low bone turnover on bone biopsy.[57] This is the only report of an AFF occurring in a genetic condition with primary osteoblast dysfunction. LRP5 acts through the osteoblastic Wnt/β-catenin canonical signaling pathway to regulate bone formation.[56] Homozygous and compound heterozygous loss-of-function mutations in *LRP5* result in OPPG, whereas gain-of-function mutations in *LRP5* results in high bone mass. Common polymorphisms of *LRP5* can affect bone density in the general population.[58]

Defect in collagen synthesis and structure

Osteogenesis imperfecta (OI): OI is a heterogeneous, heritable connective tissue disorder with prominent skeletal features, including low bone mass, hypermineralized bone matrix, multiple fragility fractures, bone deformities, and short stature.[59] Bisphospho-

nates are widely used therapies in children and adults with this condition. However, in a recent Cochrane Review of bisphosphonate use in OI, the authors concluded that although bisphosphonates may improve bone mass, the evidence for long-term fracture reduction is unclear.[60]

To date, four case reports have been published describing classical AFFs in adults with OI.[61-64] All cases had prior bisphosphonate exposure. Vasanwala and colleagues reported bilateral AFFs in an 11-year-old female with OI after five years of pamidronate therapy[65,] representing the only reported case of classical AFFs occurring in a pediatric patient with OI.

Hegazy and colleagues published a case series of AFF occurring in six pediatric OI patients[66], who all had prior long-term bisphosphonate therapy. However, the stress fractures occurred at periprosthetic sites and would technically be excluded from the ASBMR case definition. The occurrence of bisphosphonate-associated AFF in children raises the question of long-term safety of these drugs in pediatric populations.

A retrospective study by Nicolaou and colleagues demonstrated that a different pattern of femoral shaft fractures occurred in patients with OI and bisphosphonate use compared with a historical cohort of OI patients without bisphosphonate therapy, with more fractures occurring in the proximal third of the femur in children treated with bisphosphonates, whereas mid-diaphyseal femoral fractures were more common in a control group without bisphosphonate exposure.[67]

OI is most often caused by defects in type 1 collagen synthesis (encoded by *COL1A1* and *COL1A2* genes), resulting in aberrant protein posttranslational modification, folding, intracellular transport, and bone matrix incorporation.[68] This either results in collagen protein deficiency or mutant collagen protein synthesis and leads to abnormal composition and organization of bone matrix, which increases bone stiffness and skeletal fragility.

Mice models suggest that skeletal microdamage levels are increased in OI, resulting in higher bone remodeling activity to target microcrack repair.[69] As bisphosphonates suppress bone remodeling and may impede microcrack repair, its use in patients with OI may result in even higher levels of microdamage accumulation and compromised bone toughness, predisposing to stress fracture development and AFF.

Although bisphosphonate treatment has been used to improve bone density in OI, they do not reverse the underlying impaired collagen defect. AFFs have been described in

adult cases with OI and bisphosphonate exposure, whereas the risk of AFFs and the long-term safety of bisphosphonate use in pediatric populations remains unclear.

Defect in osteocyte function

X-linked osteoporosis: Our group has published a case report on an AFF occurring in an 18-year-old male with X-linked osteoporosis who had been treated with bisphosphonates for 9 years.[70] X-linked osteoporosis is a form of juvenile osteoporosis caused by pathogenic variants in *PLS3*, located on the X chromosome, encoding for the protein plastin 3.[71] Mutations in this gene have been associated with skeletal fragility in hemizygous males, whereas the clinical phenotype of heterozygous females may vary, ranging from normal bone mineral density and an absence of fractures to early-onset osteoporosis. Affected individuals can present in childhood with low bone density, vertebral compression fractures, and long bone fractures.[72] Although the exact mechanism through which PLS3 mutations cause skeletal manifestations is unclear, decreased mechanosensing of osteocytes was proposed.[71] This is supported by a recent finding of altered osteocyte protein expression in low-turnover osteoporosis caused by mutations in *WNT1* and *PLS3*.[73] Similar to bisphosphonate use in pediatric cases of OI discussed previously, this case report of a bisphosphonate-associated AFF in an adolescent raises the concern of long-term safety of these agents in children.

Candidate gene studies

Three studies have been conducted to search for variants in selected genes in patients with AFFs. All three studies included *ALPL* mutation analysis, with one study also including *COL1A1, COL1A2*, and *SOX9* genes (**Table 3**).

Because inorganic pyrophosphate is a structural analog of bisphosphonates and femoral fractures with atypical features occur in cases of hypophosphatasia without prior antiresorptive therapy, it has been hypothesized that this condition is a genetic risk factor for AFF. The exact prevalence of mutations in the *ALPL* gene in the general population is unknown, but in the European population, it is estimated that the prevalence of mild forms of hypophosphatasia is 1:6300.[74] Carriers of mutations in the *ALPL* gene with a mild phenotype may be asymptomatic.

At the annual meeting of the ASBMR in 2013, an abstract was presented by Sum and colleagues[75] on prospective *ALPL* analysis in 11 patients with bisphosphonate-associated AFFs. All coding exons and adjacent splice sites were sequenced in these individuals. In one patient, a single mutation was found affecting the donor splice site in exon 6 that is reported in lethal infantile hypophosphatasia when associated with a second missense mutation on the other chromosome.[76] The patient was a 66-year-old woman with ALP

levels between 33 and 40 U/L (range 35 to 129 U/L) while on bisphosphonate therapy. Bone mineral density was in the osteopenic range. Vitamin B6 status was not reported. The conclusion of this finding was that mutations of *ALPL* associated with subclinical hypophosphatasia may rarely result in bisphosphonate-associated AFFs.

In 2016, Bhattacharyya and colleagues[77] published on a retrospective case-control study that investigated hypophosphatasia as a risk factor for AFFs. Controls (*n*=13) without an AFF had used bisphosphonates for at least five years. Patients (*n*=10) had sustained a complete AFF while using bisphosphonates, and three patients were continuing bisphosphonate treatment. In both patients and controls, a standardized history, physical exam, and standing long leg radiographs were performed. Additionally, levels of ALP and pyridoxal 5' phosphate were measured. Participants withheld vitamin supplementation for at least one week before the blood test because this can affect pyridoxal 5' phosphate levels. DNA testing was performed in all patients with AFF (*n*=10). Because of the high costs of genetic testing, analysis of *ALPL* was only performed in the controls whohad low(- normal) serum ALP levels <60 U/L, which was found in nine of 13. Serum ALP <50 U/L was considered abnormal in this study.

Mean ALP levels in AFF patients and controls were 58 U/L (range 37 to 73) and 56 U/L (38 to 74), respectively. Five of 10 AFF cases (50%) had an ALP level <50 U/L versus five of 13 controls (38%). Despite cessation of vitamin supplementation, pyridoxal 5' phosphate levelwas elevated in two controls with low ALP who used multivitamins on a regular basis. No mutations of the *ALPL* gene were found in either the AFF patients or controls. Four different coding variants in the *ALPL* gene were found in patients and controls,which had an allele frequency of 0.1 or higher in a cohort with 4,300 European American samples (Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA, USA). Rare variants are usually defined by a minor allele frequency (MAF) <0.01, whereas variants with a MAF >0.05 are considered common and less common variants have a MAF of 0.01 to 0.05.

The four coding variants were found in equal frequencies in the AFF group versus the control group; rs1780316 (1.00 versus 0.94), rs3200254 (0.20 versus 0.17), rs3200255 (0.2 versus 0.17), and rs34605986 (0.10 versus 0.00). A post hoc analysis revealed that in this study a 40% prevalence of *ALPL* mutations in AFF patients would have been needed to detect a difference with 80% power.

These results suggest that the low ALP levels in this study population are most likely related to antiresorptive treatment rather than an underlying mild form of hypophosphatasia.

In this study, no evidence was found for hypophosphatasia as a risk factor for AFFs. However, considering the small sample size and ensuing lack of power in this study, *ALPL* cannot be ruled out as a potential susceptibility gene based on these results.

In a study by Funck-Brentano and colleagues in 2016[78], the *ALPL, COL1A1, COL1A2*, and *SOX9* genes were sequenced in four females and one male with AFF. Fourteen AFF cases were identified by reviewing radiographs of patients with femoral fractures in three academic hospitals in France between 2007 and 2010, but only five patients gave consent for genetic testing. The rationale for *ALPL* and *COL1A1/1A2* testing in AFFs has been discussed above. *SOX9* plays a role in chondrocyte differentiation and regulation of the anti-Mullerian hormone (AMH). Mutations in this gene are associated with campomelic dysplasia, a syndrome characterized by skeletal malformations and sex reversal.

One patient carried a heterozygous missense mutation in *COL1A2* that was found in the NHLBI GO Exome Sequencing Project (ESP) with a MAF of 0.0008 (rs72658163; c.2123G>A;p. Arg708Gln). Apart from short stature (146 cm), the 78-year-old patient had no specific physical features of OI. Vertebral fracture status in this patient is not reported. She also had a single nucleotide polymorphism (SNP) in the *ALPL* gene that does not alter the protein (rs370212283; MAF 0.0002 in ESP). She had used risedronate for at least five years. The potential pathogenicity of the missense variant in *COL1A2* is unclear. Initially it was regarded as a pathogenic variant in Marfan syndrome or OI. However, because this variant was also found in unaffected or mildly symptomatic family members, it was later considered not clinically relevant or possibly a genetic modifier, having small effects on the expression level of other, disease-causing genes. In cultured dermal fibroblasts of two unrelated heterozygous carriers of this variant, the diameter of the collagen fibrils was approximately 20% of control collagen fibrils.[79] This may imply that this variant affects connective tissue structure and is possibly involved in collagen-related disorders.

In another patient, six common variants of the *ALPL* gene were detected, including a nonprotein-altering variant, present in the Exome Aggregation Consortium (ExAC) with a MAF of 0.17 or higher (rs3200254; rs2275377; rs2275376; rs74063111; rs75829132; rs3200255). Another patient had a different nonprotein-altering variant in the *ALPL* gene (rs3200256; MAF 0.01 in ExAC). In two patients, no genetic variants were found. No variants were found in *COL1A1* and *SOX9*.

Although this was a small cohort study, genetic testing of four genes identified a previously reported mutation in *COL1A2* and common variants in the *ALPL* gene, supporting the hypothesis that AFF populations are enriched with variants in genes associated with monogenetic diseases.

Exon array analysis

In a pilot study, Pérez-Núñez and colleagues[80] conducted an exon array analysis (Affymetrix Axion 2.0 exome array) in 13 women with AFFs and 268 controls that consisted of healthy women (*n*=87) and patients with postmenopausal osteoporosis without AFFs (*n*=181) (**Table 3**). By including the osteoporosis patients in the control group, the investigators intended to avoid the finding of osteoporosis-related variants rather than variants associated with AFFs. The analysis was restricted to variants with a minor allele frequency <0.03 in the overall study population. Twenty-one SNPs in 20 genes were defined as risk variants based on the arbitrary threshold of a *p* value < 0.0025. However, only one variant remained statistically significant after correction for multiple testing, a missense variant in the *PPEF2* gene, which has no known function in bone metabolism. The distribution of these less common variants in cases and controls was statistically significantly different. In 12 of 13 AFF cases, three or more risk variants were present. In 15.7% (*n*=42) of the control group, one risk variant was present, but none of the controls had more than one risk variant. The genes involved are not linked to known bone disorders, although the authors suggested that a possibly damaging missense variant in the *HHAT* gene, belonging to the hedgehog protein family, may be connected to developmental bone defects, while another possibly damaging missense variant in

Table 3. Genetic studies in AFF cohorts and heir indings

Author, year	Cases of AFF, *n*	Genetic analysis	Major findings
Roca-Ayats et al. 2017[28]	6 (3 sisters)	Whole exome sequencing	37 rare mutations in 34 genes were identified, including: GGPS1, CYP1A1, MVD, FN1, SYDE2, NGEF (The remaining 28 genes were not published)
Lau et al. 2017[29]	2 sisters	Whole exome sequencing	Novel rare homozygous variant in CTSK gene (C.784 + 3A > C) was identified.
Perez-Nunez et al. 2015[80]	13	Exon array analysis	Rare variants were more common in AFF cases compared to a control group (n ¼ 268), and several rare variants tended to accumulate in AFF cases. Variants in 20 genes associated with AFF were identified: PPEF2, ACOXL, GGA3, LIPN, DOCK2, CCDC147, OR51T1, PCK2, CRYBB2, CXCR7, EDC3, SF3B3, SLC15A5, SLC2A6, FOXK2, CNGB1, NAT8B, HHAT, OR2L13, SYTL2
Funck-Brentano et al. 2016[78]	5	ALPL, COL1A1, COL1A2, SOX9 gene sequencing	Heterozygous mutation in COL1A2 (c.213G > A; p.Arg708GIn) in 1 case
Bhattacharyya et al. 2016[77]	10	ALPL gene sequencing	No mutations found
Sum et al. 2013[75]	11	ALPL gene sequencing	Heterozygous mutation in ALPL gene (c.648 + 1G > A) in 1 case

the *CXCR7* gene (also known as *ACKR3*) modulates the activity of precursor osteoblasts. These findings indicate that several variants combined may be associated with (a higher risk of) AFF. Based on these results, the authors concluded that AFFs have a polygenic background. However, this study is also limited by a small sample size and lack of functional studies to understand the potential mechanisms leading to AFF.

Whole-exome sequencing

To date, only one study has been published on whole-exome sequencing in patients with bisphosphonate-associated AFFs[28] (**Table 3**). Exome sequencing may lead to the discovery of yet unknown genetic variants related to the risk of AFFs, although potential susceptibility variants for AFFs in noncoding regions and regulatory areas of the genome may still be missed.

In a recent letter to the editor by Roca-Ayats and colleagues[28], results were presented of a whole-exome sequencing study in three sisters with bisphosphonate-associated AFFs and in three unrelated patients with AFF after long-term bisphosphonate treatment. Rare, protein-altering mutations shared only by the three sisters were considered in this analysis. A dominant model was assumed by the authors. In total, 37 rare mutations were detected in 34 genes including a novel missense variant (p.Asp188Tyr) in geranylgeranyl diphosphate synthase 1 (*GGPS1*), a gene encoding for the enzyme geranylgeranyl pyrophosphate synthase (GGPPS). GGPPS catalyzes the formation of geranylgeranyl pyrophosphate in the mevalonate pathway. This novel variant is expected to severely impair GGPPS enzyme activity, potentially impairing osteoclast function (**Figure 2**). However, when the mutation in *GGPS1* is believed to decrease osteoclast function, it might also be expected that the siblings have a high bone mineral density and an osteopetrosis-like phenotype, which is not evident from the case description in this letter.

In addition, the authors describe a mutation of the gene encoding *CYP1A1* in the three sisters and in one unrelated patient with AFF. Also, in one unrelated patient with an AFF, they identified a mutation encoding mevalonate diphosphate decarboxylase (*MVD*). Pathway analysis of the mutated genes showed enrichment of the isoprenoid biosynthesis, which proceeds through the mevalonate pathway in humans, including *GGPS1, CYP1A1*, and *MVD*.

According to Roca-Ayats and colleagues, missense changes in the *FN1, SYDE2*, and *NGEF* genes might also be relevant variants. However, the authors do not discuss the potential mechanism of action of these variants with regard to the pathophysiology of AFFs. An overview of all found rare variants is not presented in this letter. Replication of the novel *GGPS1* mutation and the other 36 genetic variants in other cases of AFFs could provide

evidence that one or more of these variants are potential susceptibility genes for AFFs. Otherwise, several variants may only be a result of shared DNA amongst the three siblings.

It is also possible that the *GGPS1* is a private mutation in this family and related to the underlying bone disease, not necessarily to the AFFs. However, it is plausible that the mutation is related to AFFs because the mevalonate pathway is believed to be inhibited by bisphosphonates containing a nitrogen side-chain, such as alendronate, risedronate, and zoledronate. The novel *GGPS1* variant would possibly disrupt a binding site for magnesium of the GGPPS enzyme so that binding of farnesyl pyrophosphate and catalysis are disturbed. Blocking the farnesyl diphosphate synthase in this pathway induces the apoptosis of osteoclasts, decreasing bone resorption. In theory, this mutation could lead to a further accumulation of the mevalonate pathway substrate, isopentenyl pyrophosphate. In the literature, this substrate may indirectly activate T lymphocytes and is considered the cause of bisphosphonate-induced acute phase reaction in patients on intravenous treatment.[81,82] It has been suggested that this mechanism may result in chronic immune stimulation and compromised immunity in patients on long-term bisphosphonate therapy, which may contribute to another bisphosphonate- associated adverse event, such as osteonecrosis of the jaw.[81,82]

Whole-exome sequencing in another family of AFFs was presented in an abstract at the Australian New Zealand Bone & Mineral Society Annual Meeting in 2017(**Table 3**).[29] Lau and colleagues described a consanguineous family in whom three siblings sustained bilateral AFFs without a history of bisphosphonate exposure. Whole-exome sequencing of two siblings revealed a novel homozygous variant in the splice site of exon 6 of the cathepsin K gene (*CTSK*) (c.784+3A>C), with a variant frequency of 0.0000577. Mutations in *CTSK* are associated with pycnodysostosis, and although the authors reported that the proband had short stature and high bone mass (*T*-scores of +2.02 at the femoral neck and +2.75 at the lumbar spine), the proband had no other dysmorphic, clinical, or radiographic features to suggest this condition. This finding supports our hypothesis that individuals who sustain AFFs may carry a rare variant associated with a monogenetic bone disorder.

CONCLUSION

Although AFFs are rare fractures associated with antiresorptive therapy, fear of this complication has been linked to the poor uptake of this effective treatment for osteoporosis. The pathogenesis of AFFs has not yet been elucidated, and the future challenge lies in improving our understanding of the association with antiresorptive therapy and the predisposing risk factors, including genetic factors, in order to prevent these fractures from occurring.

Here we summarize the evidence for genetic factors in AFFs. These fractures can occur in patients with monogenetic bone diseases, even without prior bisphosphonate exposure and in some cases unmasking the underlying condition. Targeted sequencing of some of these genes in AFF populations have identified variants in *CTSK, COL1A2*, and *ALPL* genes, and we propose that mild, unrecognized forms of such monogenetic bone diseases may underlie the etiology of AFFs. Further, whole-exome sequencing and exon array analysis of AFF cohorts have identified novel genes that may predispose to AFFs, including genes related to the mevalonate pathway. These findings provide new insights into the pathogenesis of AFF. It is important that these initial findings can be replicated in future studies, in order to determine the exact genetic architecture of this rare complication. Consequently, a simple genetic test can be developed with all potential susceptibility variants involved. This test could be used to screen patients before prescribing (long-term) treatment with bisphosphonates or denosumab.

Identification of susceptibility genes predisposing to AFFs may provide a solution in detecting patients at greatest risk of AFFs by genetic testing, for whom alternative anabolic treatment should be recommended. To date, genetic studies in AFF cases have comprised small cohorts. An international, multicentered collaborative study of well-phenotyped AFF cases and controls is needed to detect rare variants associated with AFFs, as well as common variants in multiple genes. This would enable future targeting of antiresorptive therapy to those with low AFF risk.

DISCLOSURES

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Whole exome sequencing in two Asian families with atypical femur fractures

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ABSTRACT

Background Atypical femur fractures (AFF) are rare associations of anti-resorptive therapy. Devastating to the affected individual, they pose a public health concern because of reduced uptake of an effective treatment for osteoporosis due to patient concern. The risk of AFF is increased six to seven-fold in patients of Asian ethnicity compared with Caucasians. Genetic factors may underlie the AFF trait, with recent interest in *GGPS1* and *CYP1A1* genes. Identifying genetic associations with AFF is important to facilitate precision medicine in osteoporosis treatment. Given the rarity of AFFs, studying familial AFF cases is valuable in providing insights into their genetic predisposition.

Methods We present two Singaporean families, one of which comprised a mother (I-1) and a daughter (I-2), and the other comprised two sisters (II-1 and II-2). All 4 cases presented with bisphosphonate-associated AFF. Whole exome sequencing was performed on I-2, II-1 and II-2. DNA for I-1 was not available. Variants were then examined using a candidate gene approach comprising a list of genes previously associated with AFF in the literature. Sanger sequencing was used to confirm variants of interest.

Findings Using a candidate gene approach, rare variants shared between all three cases were not identified. Heterozygous variants of interest were identified in *CYP1A1*, *PLOD2*, and *TMEM25*. One variant in *CYP1A1,* shared by the 3 patients with AFF from both families, is common, and even twice as frequent in East Asians. The second variant in *CYP1A1* has an overall low frequency (0.01043) and was shared by the two sisters. This variant is rare in the South Asian population, but common in East Asians. A rare variant in *TMEM25,* also shared by the two sisters with AFF, was identified. A rare heterozygous *PLOD2* variant was present in the daughter case with AFF (I-2) but not the sisters. Furthermore, variants of interest in *GGPS1* were not identified.

Conclusion Although the findings from this genetic analysis are inconclusive, the existence of AFFs in families is suggestive of a genetic component in AFF pathogenesis. We further discuss heterozygous variants of interest identified in three genes, *CYP1A1*, *PLOD2* and *TMEM25,* and their potential role in AFF development.

1. INTRODUCTION

Despite the effective and low-cost antiresorptive amino-terminal bisphosphonates to reduce fragility fractures, fear of a rare association with atypical femur fractures (AFFs) has reduced their uptake.[1] These unusual stress fractures of the subtrochanteric and the lateral femoral diaphyseal regions occur at sites usually resilient to traumatic fracture.[2] Although rare, with an estimated incidence of 0.3-113 per 100,000 person years[3], AFFs can be devastating to the affected individual, as well as posing a public health concern. Proposed pathophysiological mechanisms for AFFs include adverse femoral geometric parameters and unfavourable bone microarchitecture. Prolonged antiresorptive therapy may progressively alter the material properties of bone such that with increasing toughness, bones are stiffer and less resilient against mechanical loading when weightbearing – particularly at the lateral femoral diaphyseal cortex.[4] The lowered peak tolerated strain leads to microcrack development, which accumulates as healing of micro-damage is impaired by antiresorptive therapy, thus precipitating femoral stress fractures such as AFFs. However, it is notable that bisphosphonate-naïve individuals can also sustain AFFs, described in up to 22% of AFF cohorts[5], suggesting that other individual factors contribute to AFF risk.

Ethnic variation in AFF risk has also been described. Early AFF case reports arose in Asia[6], whilst Asian ethnicity comprise up to half of AFF cohorts in North America.[3,7] Lo *et al*. described a hazard ratio for AFF of 6.6 in Asian compared with Caucasian bisphosphonate users.[8] Similarly, we identified an AFF incidence rate in Asians 3.4-fold higher than other ethnic groups in an Australian cohort study.[9] Further, ethnic variation in anatomic AFF location has also been described, being predominantly subtrochanteric in Singapore compared with diaphyseal in Sweden.[10] The mechanism underlying the increased AFF risk in Asians is not known, but an unexplored possibility is that genetic factors predisposing to AFFs are more prevalent in Asian populations.

Genetic factors have been associated with AFFs, and this literature is summarized in our recent systematic review.[11] In support of a genetic predisposition is the rarity of AFFs, occurrence in bisphosphonate naïve individuals, familial cases of AFFs, and case reports of AFF occurring in those with underlying monogenetic bone disorders **(Table 1a)**, at times unmasking the genetic disease. It is possible that mild phenotypes of such heritable bone disease may underlie the aetiology of AFFs in some patients.

Few genetic studies, albeit with small sample sizes, have been conducted in bisphosphonate associated AFF cohorts. In a whole exome sequencing study of three sisters with bisphosphonate associated AFFs, Roca-ayats *et al.* identified 37 rare variants in

34 genes, including two genes of interest, *GGPS1* and *CYP1A1*. *GGPS1* interacts with the mevalonate pathway, which is important in the production of cholesterols and steroidal hormones, and, critically, is targeted by the amino-terminal bisphosphonates to reduce osteoclast action.[12,13] *CYP1A1* is involved in steroid metabolism, specifically in the oxidative metabolism of estrogens. Polymorphisms in this gene have been studied for a possible association with the risk for osteoporosis and low bone mineral density (BMD) in Caucasian and Mexican postmenopausal women[14,15], but results are not consistent. Although rare variants in *CYP1A1* have been identified in two unrelated patients with AFF, rare variants in *GGPS1* have not been identified in other AFF cases outside this described family.[13,16] Other studies have reported rare variants in *CTSK, COL1A2,* and *ALPL*.[17,18] **Table 1b** presents a list of genes in which low frequency variants were found by WES analysis and shared by three sisters with AFF in one report[13] or used in candidate gene studies for AFF.[16-18] These genes listed in **Table 1** have not been replicated or confirmed at this moment to be causal for AFF.

Table 1: Genes implicated in AFFs

1A: Monogenetic bone disorders in which AFFs have occurred[11]	
Monogenetic disorder	*Associated genes*
Hypophosphatasia	ALPL
Osteogenesis imperfecta*	COL1A1, COL1A2, CRTAP, LEPRE1, PPIB, SERPINH1, FKBP10, PLOD2, SP7
Pycnodysostosis	CTSK
X-linked hypophosphatemia	PHEX
Osteopetrosis*	TCIRG1, CLCN7, OSTM1, PLEKHM1, SNX10, TNFSF11, TNFRSF11A, CA2
Osteoporosis pseudoglioma syndrome	LRP5
X-linked osteoporosis	PLS3
1B: Genes with low frequency variants identified in AFF cases	
Gene	*Reference*
ALPK1	[13]
ALPL	[16, 18]
ATP6AP1	[13]
BRAT1	[13]
CD37	[13]
CHERP	[13]
COG4	[13]
COL1A2	[17]
CTSK	[19]
CUL9	[13]
CYP1A1	[13, 16]
EML1	[13]
ERCC6L2	[13]

Table 1: Genes implicated in AFFs (*continued*)

1B: Genes with low frequency variants identified in AFF cases	
FN1	[13]
GGPS1	[13]
GPR20	[13]
HEPHL1	[13]
IQCF6	[13]
KDM4C	[13]
LFNG	[13]
LRRC1	[13]
LURAP1L	[13]
MEX3D	[13]
MGA	[13]
MVD	[13]
NGEF	[13]
NKAP	[13]
NTPCR	[13]
NVL	[13]
PGRMC1	[13]
POLI	[13]
SHC4	[13]
SMS	[13]
SNAPC4	[13]
SYDE2	[13]
TMEM25	[13]
TUSC2	[13]
XAB2	[13]

** Osteogenesis imperfecta and Osteopetrosis are associated with a number of genes. Although there have been case reports of AFFs occurring in these two conditions, the specific gene involved was not provided. As such, all genes associated with the two disorders are listed in the table*

Despite a recognised increase in risk in Asians, genetic studies of Asian familial AFF cases have not yet been described. In this case report we present two small Singaporean families in each of which two members have sustained bisphosphonate-associated AFFs. We conducted whole exome sequencing on DNA of three cases, performed candidate gene analyses using genes implicated in AFFs, and describe the potential variants of interest.

2. DESCRIPTION OF AFF CASES

We studied two Singaporean families of Chinese origin (**Figure 1**). Family 1 comprised a mother (I-1) and daughter (I-2), who both sustained AFFs whilst on alendronate treat-

ment. Genetic data was only available from the proband daughter, as the mother had died. However, AFF was confirmed on X-ray for both mother and daughter (HTS, data not shown). I-2 is a postmenopausal woman who sustained bilateral AFFs at age 66 years following a fall from standing height requiring bilateral surgical repair. This occurred on a background of four years of alendronate therapy for osteopenia, without a preceding fragility fracture. Her other comorbidity included hypothyroidism, treated with levothyroxine, and being an ex-smoker. She had no significant alcohol history.

Family 2 included two postmenopausal sisters (II-1 and II-2) who sustained AFFs through falls from a standing height at the age of 55 and 66 years respectively. They were treated with alendronate for five and nine years, respectively, for osteoporosis diagnosed by DXA criteria, without a history of minimal trauma fractures. Neither had a significant smoking or alcohol history. The sister aged 66 years had prior menopausal hormone therapy (duration unknown), and also had received topical cortisone treatment for eczema.

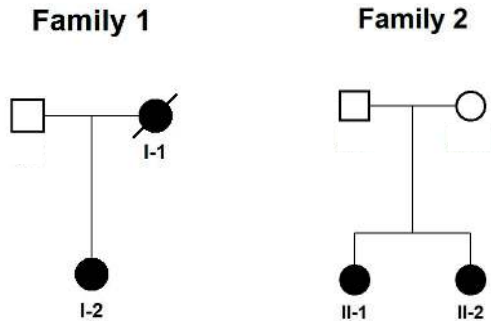

Figure 1: Pedigrees of two Singaporean families of Chinese origin. Black symbols represent individuals with AFF. Open symbols represent unaffected individuals.

3. MATERIALS AND METHODS

3.1. Data collection and adjudication of AFFs

The three living patients (I-2, II-1 and II-2) consented to providing blood for DNA analysis. Clinical history was obtained via structured interviews, and AFFs were confirmed radiologically to fulfil ASBMR case definition (HTS, data not shown).[1] The study was approved by Monash Health HREC (approval number 15550X).

3.2 DNA isolation

Genomic DNA was isolated from blood samples using the Promega Reliaprep DNA isolation kit (Leiden, Netherland) in combination with the Tecan robot.

3.3 Whole exome sequencing

DNA was processed using the KAPA library preparation (Roche Diagnostics, Inc, Pleasanton, CA, USA), followed by exome capture using the Nimblegen SeqCap EZ MedExome Capture kit (Roche Nimblegen, Inc, Madison, WI, USA). Paired-end 2x150 bp sequencing was performed on the Illumina NovaSeq 6000 platform.

Reads were demultiplexed and aligned to the human reference genome hg19 (UCSC) using the Burrows-Wheeler alignment tool (BWA version 0.7.3a). After indel realignment and base quality score recalibration using the Genome Analysis ToolKit (GATK version 3.8) and masking of duplicates (Picard Tools version 2.18.4), gvcf files were generated using HaplotypeCaller (GATK v3.8) and genotyped using GenotypeGVCFs (GATK 3.8). Raw genotype data was QC-ed and filtered using the VQSR methodology of GATK. All detected variants were annotated based on RefSeq annotation (NCBI Reference Sequence Database) using ANNOVAR (version 2019-10-24). Allele frequencies from the gnomAD Exome and Whole Genome dataset version 21120190318 were used in addition to the 1000 Genomes (version p3v5). Additionally, predictions on damaging properties of each variant were determined using CADD[20], which also includes the scores for programs like SIFT and PolyPhen and a series of conservation programs. Average WES coverage for the three samples were 62.63 (I.2), 124.09 (II.1) and 113.78 (II.2).

3.4. Data analysis

Variants were identified in a candidate gene-based approach using the list of genes implicated in AFFs (**Table 1a and 1b**). Variants are filtered when: 1) present in the designated candidate genes (**Table 1a and 1b**); 2) UTR, exonic, splicing, stopgain, stoploss, nonsynonymous or exonic indels; 3) with a frequency < 0.005 or not present in the gnomAD or 1000 genomes database; 4) present in either I-1 and/or in both sisters II-1 and II-2.

Given recent interest in *GGPS1* and *CYP1A1*, we additionally undertook a full inventory of the variants in these genes to identify shared variants across cases, regardless of population frequency.

3.5 Genetic variant validation

Selected variants were confirmed with Sanger sequencing, see **Supplemental figure 1.**

Polymerase chain reaction (PCR) was carried out to amplify the fragments containing the variants. Primers were designed with Primer-Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primer sequences are listed in **Table S1** in **Supplemental figure 1**. Amplification was carried out at an annealing temperature of 59 °C and 40 cycles.

Sanger sequencing of both strands was performed at Eurofins GATC Biotech (https://www.eurofinsgenomics.eu/de/custom-dna-sequencing/gatc-services/).

4. RESULTS

Whole exome sequencing was performed on DNA from three female individuals of Asian origin with atypical femoral fractures from two families (I-2, II-1, II-2), **Figure 1**.

4.1 Candidate gene analysis

Using the list of candidate genes **(Table 1a and 1b)**, we investigated for potential interesting variants, irrespective of type of inheritance. Filtering according to the selection criteria indicated in Material and Methods resulted in two rare variants (**Table 2**), both present in a heterozygous state in either I-1 or both sisters II-1 and II-2.

Table 2: Analysis flowchart of Candidate Genes with freq < 0.005

Total number of variants	53,299
All variants in Candidate Genes from Table 1	203
Selecting UTR, exonic nonsynonymous + splice variants (exluding intronic + exonic synonymous)	67
Variants with gnomAD WES and WGS freq < 0.005	8
Additional filtering with 1000 genomes freq < 0.005	6
Filtering out variants only carried by one of the affected sisters II-1 or II-2	2

In the mother-daughter AFF family, a rare variant was present in the known bone disease-related gene *PLOD2* in individual I-2, but not in the sisters from the other family (II-1 and II-2)**(Table 3)**. This variant (rs776654051) has a very low overall frequency in the gnomAD database (0.000003988). Only one allele (in 250756 alleles) was found in this database and this was present in an East Asian individual. It was predicted to be tolerated by SIFT and possibly damaging by PolyPhen. The predicted CADD score of this variant was 10.78.

In the sisters with AFF, a rare variant (rs782188288) in *TMEM25*, with an overall frequency of 0.0001125, was shared by the two sisters II-1 and II-2 but was not identified in I-2 **(Table 3)**.

Given the interest in literature in *GGPS1* and *CYP1A1* as possibly AFF related genes, an inventory of variants in these genes in our three cases, regardless of population frequency, was conducted. We did not identify any exonic variants of interest in *GGPS1*. Two *CYP1A1* variants were present, one shared by the two sisters II-1 and II-2., and one by all three patients **(Table 4).**

Table 3: Details of rare variants identified through filtering of Candidate Genes with frequency < 0.005

Gene	Variant	Chr	Start	Ref	Alt	genomic funtion	exonic function	transcript information	dbSNP150	gnomAD WGS_ALL	gnomad WGS_SAS	gnomad WGS_EAS	gnomAD_ WES_ALL	gnomAD_EAS*	gnomaD_SAS*	1000G _ALL	SIFT score	SIFT _pred	Polyphen2_H VAR_score	Polyphen2 _HVAR_pred	CADD	GERP++	I-2	II-1	II-2
PLOD2	SNV	chr3	145799628	T	A	exonic	nonsynonymous SN\	NM_182943:exon12:c.A1255T:p.T419S	rs776654051	0	0	0	3.988E-06	0.00005441 (1/1	0	0	0.88	T	0.515	P	10.8	5.53	0/1	0/0	0/0
TMEM25	SNV	chr11	118402939	G	A	exonic	nonsynonymous SN\	NM_032780:exon3:c.G145A:p.A49T	rs782188288	0	0	0	0.0001125	0.001306(24/28(	0.0001313(4/28(	0	0.55	T	0.209	B	15.5	3.26	0/0	0/1	0/1

chromosome positions are given for build GRCh37

* numbers between brackets indicate the frequency of that alle within the east or south Asian populations

SNV=single nucleotide variant

indel=insertion/deletion

Sift score and prediction: 0 - 0.05 damaging (D); >0.05 tolerated (T)

Polyphen HVAR score and prediction: 0-0.446 benign(B); 0.446-0.908 possibly damaging(P); 0.908-1.0 probably damaging(D)

CADD = Combined Annotation Dependent Depletion tool, higher values indicate a higher chance of being damaging (max 60)

GERP++ = Conservation score based on the likelihood of substitutions and the deviation thereof. Higher score indicates more conservation at the site (max 6)

Table 4: CYP1A1 variants identified regardless of population frequency

Gene	Variant	Chr	Start	Ref	Alt	genomic funtion	exonic function	transcript information	dbSNP150	gnomAD WGS_ALL	gnomad WGS_SAS	gnomad WGS_EAS	gnomAD_ WES_ALL	gnomAD_EAS*	gnomaD_SAS*	1000G_ ALL	SIFT score	SIFT_ pred	Polyphen2_H VAR_score	Polyphen2_H VAR_pred	CADD	GERP++	I-2	II-1	II-2
CYP1A1	SNV	chr15	75015305	C	T	exonic	nonsynonymous SNV	CYP1A1:NM_000499:exon2:c.G134A:p.G45D	rs4646422	0.0077	0	0.1535	0.0108	0.1427 (2845/2948)	0.001992 (61/2948)	0.0024	0.08	T	0.72	P	14.86	4.7	0/0	0/1	0/1
CYP1A1	SNV	chr15	75012985	T	C	exonic	nonsynonymous SNV	CYP1A1:NM_000499:exon7:c.A1384G:p.I462V	rs1048943	0.05	0	0.2529	0.1081	0.2463 (4911/28739)	0.1187 (3633/28735)	0.13	0.01	D	0.219	B	14.09	4.46	0/1	0/1	0/1

chromosome positions are given for build GRCh37

* numbers between brackets indicate the frequency of that alle within the east or south Asian populations

SNV=single nucleotide variant

indel=insertion/deletion

Sift score and prediction: 0 - 0.05 damaging (D); >0.05 tolerated (T)

Polyphen HVAR score and prediction: 0-0.446 benign(B); 0.446-0.908 possibly damaging(P); 0.908-1.0 probably damaging(D)

CADD = Combined Annotation Dependent Depletion tool, higher values indicate a higher chance of being damaging (max 60)

GERP++ = Conservation score based on the likelihood of substitutions and the deviation thereof. Higher score indicates more conservation at the site (max 6)

The first variant in *CYP1A1* (rs4646442), shared by the two sisters II-1 and II-2, has a low frequency of 0.01043 in the overall population in the gnomAD database, however, the frequency is higher in the East Asian (0.1427), and lower in the South Asian (0.001992) subpopulation. It involves the substitution of a negatively charged amino acid (Gly) by a neutral amino acid (Asp) (p.G45D). The risk of the variant was predicted to be tolerant by SIFT and possibly damaging by PolyPhen, while the predicted CADD score was 14.86. The second variant in *CYP1A1* (rs1048943) was shared by all three patients and is common in gnomAD (0.1016) with similar frequencies in all Asian subpopulations.

The four variants (rs776654051, rs782188288, rs4646442, rs1048943) in *PLOD2, TMEM25* and *CYP1A1* were confirmed by Sanger Sequencing with result plots shown in the **Supplemental figure 1**.

5. DISCUSSION

In this report, we describe two families with two related bisphosphonate-associated AFF cases in each. This is the first study using whole exome sequencing to describe genetic findings from familial bisphosphonate-associated AFF cases of Asian ethnicity. Using the candidate gene approach, comprising a list of genes linked to AFF in the literature, we identified four heterozygous variants of interest in *PLOD2*, *TMEM25*, and *CYP1A1* genes, and discuss their potential links to a bone phenotype below. Rare variants shared between all three cases were not identified. We did not identify any variants of interest in the *GGPS1* gene previously reported as a potential AFF-related candidate gene.

PLOD2

As AFFs have been associated with a number of monogenetic bone disorders, we screened for genes associated with these heritable disorders (**Table 1a**). A rare heterozygous variant (rs776654051) in *PLOD2* was identified in the single patient I-2 of the mother-daughter AFF family. DNA was not available from the mother (I-1) to confirm whether this variant is shared.

Homozygous mutations in *PLOD2* cause Bruck syndrome 2 (MIM609220)[23], a rare form of osteogenesis imperfecta, and includes clinical features of short stature, bone abnormalities, osteopenia and bone fragility. *PLOD2* codes for telopeptide lysyl hydroxylase, a protein important for hydroxylysine aldehyde crosslinking of bone collagen.[24] While bisphosphonates are associated with increased non-enzymatic cross-linking, which decreases bone strength[25], the added effects of reduced hydroxylysine cross-linking might contribute to collagen deformation and thus to AFF.

Bisphosphonate-associated AFFs have been reported in individuals with osteogenesis imperfecta, however the specific gene implicated has not always been provided. A direct link between *PLOD2* gene and AFF has not been previously reported, however, a tibial diaphyseal fracture with radiological features similar to AFFs has been described in a *PLOD2*-related osteogenesis imperfecta case due to a homozygous variant p.Trp588Cys.[23] Given this background, it is interesting to find a rare *PLOD2* variant in a familial AFF case. Whether heterozygosity of a (pathogenic) variant in *PLOD2* could predispose to atypical fractures such as AFFs requires further investigations.

TMEM25

Another rare variant was identified in the gene *TMEM25* (rs782188288) and shared by both sisters (II-1, II-2), but not by case I-2. Roca-Ayats *et al*. also describe a variant in this gene which was shared by their three studied sisters (a deletion of one amino acid: p.V239del not reported in gnomAD).[13] A link between this gene and AFF, or a bone phenotype, has not been reported in the literature and different (clinical) databases, but it is interesting that both familial AFF studies report a rare variant in the same gene.

CYP1A1

CYP1A1 has been described as a potential AFF candidate gene in the literature due to identification of rare *CYP1A1* variants in three sisters and two unrelated cases with AFFs.[13,16] *CYP1A1* encodes an enzyme that belongs to the Cytochrome P450 pathway and has a number of endogenous substrates related to bone fragility, including steroid hormones such as 17β-estradiol and vitamin D.[26] Although CYP1A1 is known to metabolise a number of drugs, a link with antiresorptive therapies has not been described.

In our study, two variants in the *CYP1A1* gene were identified. The first variant is shared by the two sisters II-1 and II-2 (rs4646422; gnomAD 0.01043) and the second variant is shared by all three AFF cases (rs1048943: gnomAD 0.1016). Although the first variant (rs4646422) has a low frequency in the general population, the variant frequency differs depending on ethnic population. In the South Asian population, rs4646422 has a frequency below 0.005, but a higher frequency of 0.14 in the East Asian population is seen (**Table 4**). It is possible that genetic polymorphisms in AFF candidate genes more prevalent in East Asians may underlie the AFF trait. Genetic polymorphisms within drug metabolising genes are known to cause inter-individual variability in drug response and are reported to vary between different ethnic groups.[27]

The potential functional implication of the rs4646422 polymorphism is less well described. Functional characterization of this variant by Lee *et al*.[28] using heterologous expression in E. coli and mammalian cells suggest this variant alters folding of the en-

zyme at the heme insertion site, and that this disruption of the protein structure reduces *CYP1A1* catalytic activity.

The common nonsynonymous rs1048943 polymorphism has been described by *in vitro* studies to increase CYP1A1 enzyme activity by two-fold, leading to higher estrogen 2-hydroxylase activity and decreased hormonal activity of estrogen.[26] However, whether this leads to increased susceptibility to either osteoporosis or AFF is unclear. Two polymorphisms of *CYP1A1*, rs1799814 and rs1048943, leading to an Ile462Val amino acid change and Thr461Asn respectively, have been investigated in two previous small studies in relation to low BMD in Caucasian and Mexican postmenopausal women and possible genetic risk for osteoporosis or fractures, but results were inconsistent.[14,15]

Given that *CYP1A1* variants have been described in familial AFF cases, further functional studies are required to elucidate the potential role of *CYP1A1* in AFF pathogenesis, and whether polymorphisms in this gene interact with bisphosphonates.

Limitations

We acknowledge that there are several limitations to our analysis, such as the small sample size, lack of genetic data from appropriate control groups, as well as the inability to obtain genetic data from I-1. Unfortunately, large families segregating with AFF are rare, as are large cohorts of AFF cases with available genetic data. Therefore, studying small families with AFF is necessary and provides insight into associated genetic components, even though these findings have not yet been supported by functional data.

6. CONCLUSION

Bisphosphonate use in osteoporosis leading to AFF is rare but could be conferred by genetic susceptibility. Our candidate gene approach did not identify rare variants in *GGPS1*, and we report four heterozygous variants in three potential candidate genes of interest, *CYP1A1, TMEM25*, and *PLOD2*. Further work is required to replicate these findings in larger AFF cohorts, as well as investigate their functional role in AFF development. Although our findings are inconclusive, the presence of AFFs in families lends support to the hypothesis that genetic factors contribute to AFF risk and provides motivation for future genetic studies in larger cohorts of familial and unrelated AFF cases, taking into account potential differences related to ethnic background.

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DISCLOSURES

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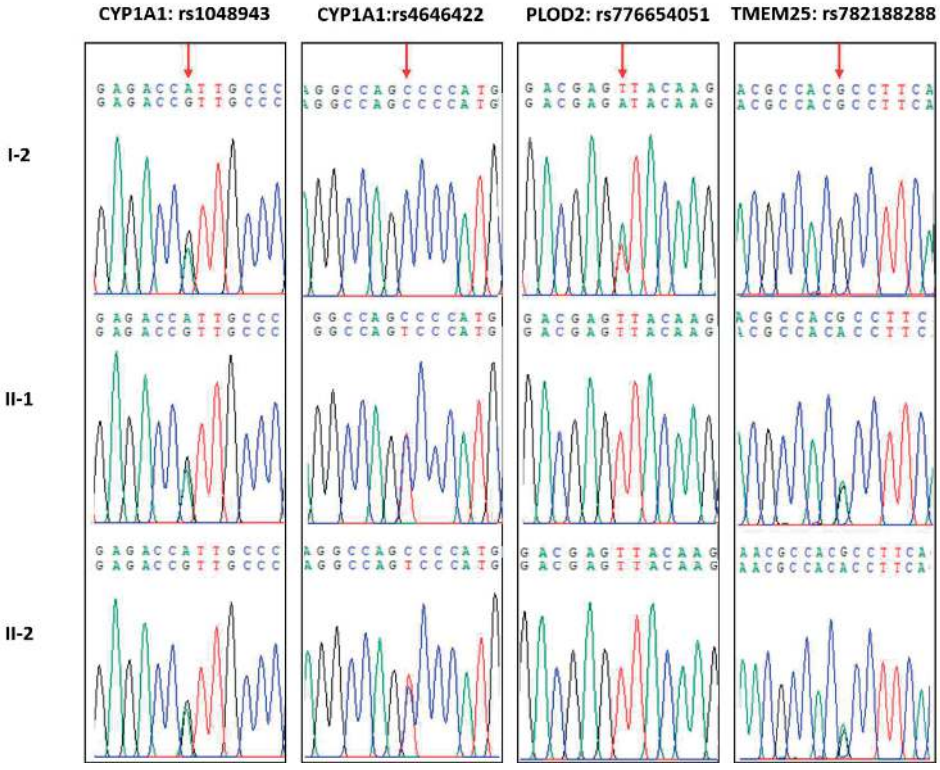

Supplementary Figure 1: Sanger sequencing validation for the different identified variants.
Samples I-2, II-1 and II-2 show a heterozygous variant for rs1048943 in *CYP1A1* (column1), samples II-1 and II-2 show a heterozygous variant for rs4646422 in *CYP1A1* (column2) and for rs782188288 in *TMEM25* (column4), sample I-2 shows a heterozygous variant for rs776654051 in *PLOD2* (column3). The other samples are reference sequence.
Green indicates A, Black indicates G, Red indicates T, Blue indicates C.

Table M&M2: primers used for variants verified by Sanger Sequencing

Gene	Variant	Forward primer (5' to 3')	Reverse primer (5' to 3')
PLOD2	rs776654051	GCAATGAGCTTGTTCCTTTGA	CTACAGGTTTGTTGAATGAGC
CYP1A1	rs4646422	TGGGTCAGAGGCAATGGAGAA	GCTTTTCTCATCCCCCAATCT
CYP1A1	rs1048943	ATTGCATTGATCCTCCTGTCC	CCAGATCAGTGTCTATGAGTT
TMEM25	rs782188288	TCTCTCCCCTGTCTGCACTTC	TGCACATTAAGGATGACAGAG

9

Whole-exome sequencing in a family with atypical femur fractures and osteoporosis

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ABSTRACT

Atypical femur fractures (AFFs) are rare adverse events associated with bisphosphonate use, but the pathophysiology is unclear. AFFs have been reported to cluster in families and have occurred in patients with and without monogenetic bone diseases, in some cases without exposure to bisphosphonates. These observations suggest that an underlying genetic bone disease can be an independent risk factor for AFF. The aim of this study was to find a genetic cause for AFF in a Caucasian family with three siblings with bisphosphonate-associated AFFs. A fourth sibling had a femoral fracture that might belong to the spectrum of AFFs. In total, seven family members had osteoporosis. By whole-exome sequencing one potentially interesting risk variant for AFF was revealed in a gene involved in bone metabolism, while this variant did not segregate with osteoporosis in this family. We discuss steps for further research and potential clinical implications.

INTRODUCTION

AFFs are non-traumatic fractures of the thigh bone of unknown etiology. They are considered to be rare adverse events associated with the use of bisphosphonates in the treatment of osteoporosis. A genetic predisposition might also play a role in the pathophysiology. This notion is supported by the observation of clustering of AFFs in some families and the occurrence of AFFs in patients with a monogenetic bone disease, with and without prior bisphosphonate use.[1,2]

Previously, two families with multiple AFF cases have been reported.[3,4] Lau *et al*. described a novel, rare homozygous variant in the Cathepsin K gene (*CTSK*) using whole-exome sequencing (WES) in a consanguineous family[3], potentially leading to a very mild form of pycnodysostosis. No detailed information is available on this family because it concerns a meeting abstract. Roca-Ayats *et al.* performed a WES study in a Spanish family with three sisters who sustained an AFF and found 37 shared rare, exonic variants.[4] The authors highlighted a variant found in geranylgeranyl diphosphate synthase 1 gene (*GGPS1*) as a potential susceptibility gene for AFF. *GGPS1* encodes for geranylgeranyl pyrophosphate synthase (GGPPS), an enzyme that is involved in the mevalonate pathway, a target of nitrogen-containing bisphosphonates. It is thought that bisphosphonates lead to osteoclast apoptosis through the mevalonate pathway, but this process is not completely understood.[5] RNAi knockdown of *GGPS1* displayed a reduced mineralization capacity in osteoblasts and a lower resorption activity in osteoclasts compared to the wild type.[6] The variant identified in this family of three sisters with AFF leads to an amino acid substitution, p.Asp188Tyr, which creates a bulky side chain on the protein. This interferes with substrate binding on the enzyme and thus decreases the enzyme activity.[7] The disrupted enzyme activity is further reduced by zoledronic acid, a potent bisphosphonate.[7]

It is possible that the altered GGPPS protein in the Spanish family, combined with the effect of bisphosphonate exposure, resulted in osteoclast dysfunction to the extent that the femur could not sustain normal loading.[7] However, the causality of the variant in *GGPS1* to AFFs has not been determined.

AFFs have been documented in patients with a variety of underlying monogenetic bone diseases such as osteogenesis imperfecta or hypophosphatasia.[1] Interestingly, the majority of these patients were bisphosphonate-naïve and some were only diagnosed after the occurrence of AFF. Hence, a mild and unrecognized form of a monogenetic bone disease could be an independent risk factor for the development of AFF.[1] Candidate gene studies in modestly sized cohorts of AFF patients have found pathogenic variants only

in a minority of patients.[8-11] In these targeted studies, however, the research was limited to a small number of candidate genes. WES offers a genome–wide and hypothesis-free approach and may reveal potentially causal mutations in genes hitherto not known to be involved in bone biology, and therefore identify novel pathways involved in AFF.

In this study, a Caucasian family with three siblings with bisphosphonate-associated AFFs is clinically evaluated and analyzed by WES. Awaiting the final outcome of our investigations, the preliminary WES results are anonymously presented. In addition, we propose several steps for further analysis and elaborate on the potential methods to validate our findings.

Identifying the responsible genetic factors in an unresolved family like this can help to understand the underlying biology of AFF and will also –eventually- lead to development of genetic tests that can be used in a clinical setting. Consequently, treatment for osteoporosis could be personalized based on the genetic signature indicating risk of AFF.

MATERIALS AND METHODS

Clinical evaluation

Information on comorbidities, fracture history, medication use and results from DXA scanning and X-rays were obtained from all family members who signed informed consent.

Whole-exome sequencing

WES was performed in DNA samples from blood (II.2, II.3, II.5, II.6, II.7, III.1, III.14) or saliva (II.4, III.9) of nine individuals from two generations (See **Figure 1**) who agreed to participate in the genetic study.

Laboratory procedure

DNA was isolated by the Promega Reliaprep DNA isolation kit (Leiden, Netherland) in combination with the Tecan robot and DNA concentrations normalized to 50ng/uL.

For WES a sequencing library was constructed using the KAPA library preparation kit (Roche Diagnostics, Inc, Pleasanton, CA, USA). Exonic regions were captured with the Nimblegen SeqCap EZ MedExome Capture kit (Roche Nimblegen, Inc, Madison, WI, USA), followed by paired-end 2x150 bp sequencing on the Illumina NovaSeq 6000 platform.

Sequence data analysis

The reads were demultiplexed and mapped to the human reference genome (UCSC hg19) using the Burrows-Wheeler alignment tool (BWA version 0.7.3a). Duplicate reads were marked by Picard Tools (version 2.18.4). Indel re-alignment and base quality score recalibration were conducted subsequently using the Genome Analysis ToolKit (GATK v3.8). Per-sample gVCF files were generated using HaplotypeCaller (GATK v3.8) and jointly genotyped using GenotypeGVCFs (GATK v3.8). Average sequencing reads coverage was 69.6 (II.2), 74.5 (II.3), 107.23 (II.4), 75.0 (II.5), 74.0 (II.6), 75.7 (II.7), 63.4 (III.1), 124.0 (III.9) and 76.9 (III.14). Variant Quality Score Recalibration (VQSR) methodology of GATK was applied for variant quality control and filtering. The tranche sensitivity threshold was 99.8% for filtering single-nucleotide variants (SNVs) and 80.0% for filtering insertion/deletions (INDELs).The observed and called variants were annotated using ANNOVAR (version 2019-10-24) with RefSeq hg19 gene definitions (NCBI Reference Sequence Database).

Bioinformatics analysis

The predicted pathogenicity of variants was determined by *in silico* algorithms using CADD[12], including several scores such as SIFT[13], PolyPhen2[14] and the evolutionary conservation score GERP++.[15]

Candidate gene approach

Based on the family studies described by Roca-Ayats *et al.* and Lau *et al.*[3,4], the genetic analysis was separately focused on *GGPS1, CYP1A1* and *CTSK* as genes potentially associated with AFF in this family.

Additionally the WES results were examined for 22 genes with a function in bone biology or association with monogenetic bone disease (*COL1A1, COL1A2, LRP5, LRP6, IFITM5, WNT1, LGR4, PLS3, CRTAP, LEPTRE1, PPIB, SERPINH1, BMP1, PLOD2, FKBP10, SERPINF1, SP7, TMEM38B, ATF4, SMAD3, SEC24D, DOK7*).

Hypothesis free approach

We filtered for rare, exonic SNVs, indels, stop-gains, stop-losses and splicing variants and variants in UTRs, shared by the three siblings with AFF (II.2, II.4, II.7). Synonymous variants were excluded. The incidence of AFF is reported to be one in 1000 long-term bisphosphonate users[16], although the frequency of carriers of a risk variant for AFF might be higher in the common population. We therefore used a cutoff value of 0.005 for the minor allele frequency to define rare variants, obtained from gnomAD (version 2.1.1) and 1000 Genomes (version p3v5) using the overall population and the (non-Finnish) European population.

RESULTS

Clinical description of the family

The family pedigree is shown in **Figure 1.**

A detailed description of the individual family members can be found in **Appendix I**.

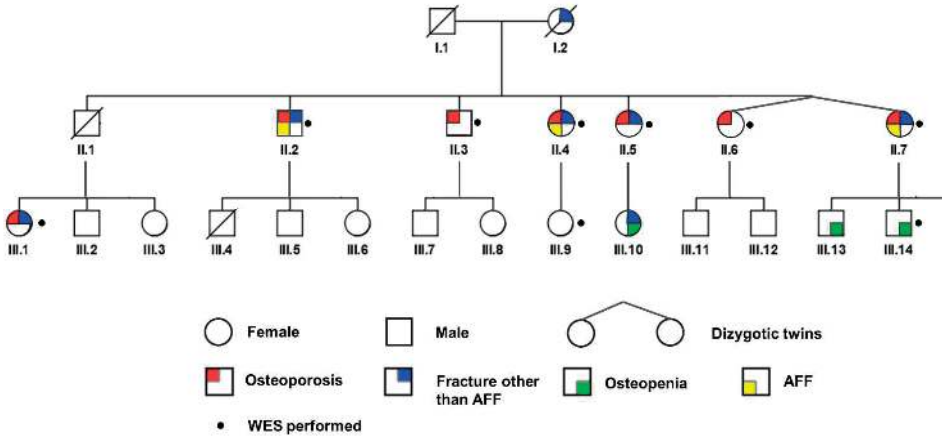


Figure 1. Family pedigree.

This family comprises three siblings in the second generation, who sustained an AFF after use of bisphosphonates for osteoporosis (II.2, II.4, II.7). Their father (I.1) died at a high age (exact age unknown) and had never sustained any fractures, but their mother (I.2) experienced loss of height with aging and she had a hip fracture when she was 90 years old.

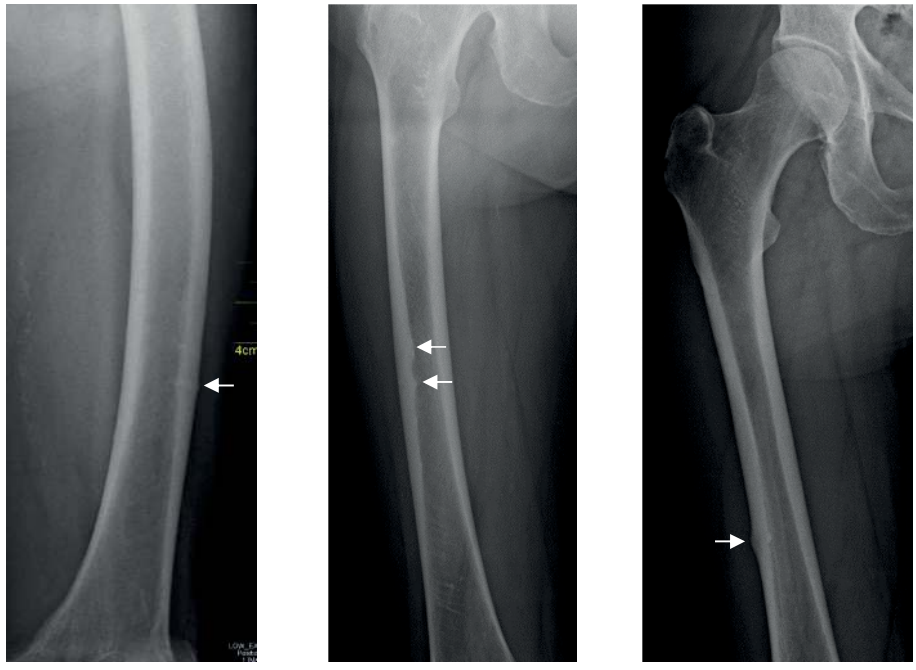
The AFF in the three individuals II.2, II.4 and II.7 all occurred spontaneously after five years or more of oral bisphosphonate use and met the radiologic criteria formulated by the ASBMR Task Force.[17] All three patients suffered from incomplete forms of AFF (**Figure 2A-B-C**) and one of these siblings (II.4) also had a complete AFF on the other side. Two had bilateral fractures (II.4, II.7). Additionally, the three AFF cases had osteoporosis with vertebral fractures (II.2, II.4, II.7) and/or non-vertebral fractures (II.2, II.7).

The three other living siblings from this generation (II.3, II.5, II.6) all had osteoporosis based on DXA scanning, but did not have AFF. This included one sister (II.5) who had briefly used alendronate between 2006 and 2008 and sustained a low-trauma distal femur fracture that did not meet the diagnostic criteria for AFF because it was a comminuted fracture with intra-articular involvement. The remaining two siblings (II.3,

II.6) had never experienced any fractures nor used bisphosphonates and were only diagnosed with osteoporosis after DXA scanning as part of the family investigation.

In the third generation, clinical information was obtained for six individuals (III.1, III.9, III.10, III.13, III.14, III.15) and DNA of three individuals (III.1, III.9, III.14). One woman had already been diagnosed with osteoporosis with multiple spine fractures at age 56 (III.1). Additional DXA scanning in five individuals (III.9, III.10, III.13, III.14, III.15) showed osteopenia in one woman (III.10) who also had fractures in the past and two men (III.13, III.14) without fractures, all below the age of 48 years. The remaining nine individuals of the third generation never sustained any fractures (III.2 - III.8, III.11, III.12).

One or more risk factors for osteoporosis or fractures were present in all included members of the second generation (**Table 1**) and three members of the third generation (III.10, III.13, III.14).



2A. **2B.** **2C.**

Figure 2: incomplete AFF on X-ray in II.2 (2A), II.4 (2B) and II.7 (2C).

Table 1. Risk factors for osteoporosis in family members.

	Fractures	COPD	Diabetes mellitus	Smoking (PY[1])	Early menopause	Use anti-epileptics	Other comorbidities
II.2	AFF Radius Foot	Yes	Yes	Yes (34)	NA[2]	No	Hypogonadism Hyperthyroidism
II.3	No	Yes	Yes	Yes (37)	NA	No	No
II.4	AFF Vertebral	No	No	No	Yes	No	No
II.5	Vertebral	No	No	Yes (40)	No	No	No
II.6	No	No	Yes	Yes (40)	No	No	No
II.7	AFF Radius	No	No	No	No	Yes	RA[3] SLE[4]
III.1	No	No	No	No	No	Yes	No
III.10	No	No	No	Yes (34)	Yes	No	No
III.13	No	No	No	No	NA	No	PMR[5]
III.14	No	No	No	Yes (8)	NA	No	No

[1] PY = packyears of smoking; [2] NA = not applicable for men; [3] RA = rheumatoid arthritis; [4] SLE = systemic lupus erythematosus; [5] PMR = polymyalgia rheumatica for which III.13 had used oral corticosteroids

Segregation of phenotypes

In the clinical assessment of this family, it showed that seven family members have osteoporosis and three have osteopenia. Low bone mass and AFF may be independent phenotypes or could both be manifestations of one genetic disease. A dominant autosomal mode of inheritance of AFF and/or osteoporosis seems plausible given the involvement of multiple family members of both sexes. Assuming a dominant model and shared genetic cause for osteoporosis and AFF, then family members with osteoporosis but without AFF could be considered as heterozygous for a risk genetic variant for AFF. However, this approach is complicated by the fact that at least five out of seven family members (II.2, II.3, II.4, II.6, II.7) with osteoporosis have other reasons for low BMD due to comorbidities and lifestyle factors (**Table 1**). It cannot be distinguished with certainty whether these individuals have a genetic form of osteoporosis rather than non-genetic secondary osteoporosis. We decided to regard the remaining two individuals, II.5 and III.1 as affected for genetic osteoporosis, since smoking in II.5 and anti-epileptic use in III.1 appear to be unsatisfactory explanations for the severity of the osteoporosis and/or fragility fractures in these women. The only family member with a normal BMD, III.9, could be used as a normal control (unaffected homozygous reference).

Following the hypothesis that AFF is induced by a combination of bisphosphonate use and a genetic predisposition for AFF, those family members without AFF cannot be used

as reference in a segregation analysis because they might have developed AFF with (longer) use of bisphosphonates.

Since II.5 had a low-trauma femoral fracture after bisphosphonate exposure, it could be argued to consider her a carrier of the genetic causal factor(s) of AFF even though this fracturedid not completely fulfill the diagnostic radiological criteria. Based on the scenario that AFF and this non-atypical femoral fracture share a common genetic cause with the osteoporosis in this family, then five family members are affected (II.2, II.4, II.5, II.7, III.1) and one can be used as a homozygous reference (III.9).

Although probably a rare occurrence, it should also be kept in mind that different genetic causes for osteoporosis might exist within different pedigree branches of one family. The individual III.1 who presented with severe vertebral fractures had a father without fractures. Thus, she might have a *de novo* mutation or a maternally inherited mutation for osteoporosis.

Preliminary results of WES

No DNA was available from the individuals of the first generation, but six DNA samples were obtained from the living siblings of the second generation (II.2, II.3, II.4, II.5, II.6, II.7).

Candidate gene approach

In the candidate gene approach, no pathogenic rare variants associated with monogenetic bone diseases were found when screening 22 genes of interest, nor were any of the variants in the previously reported AFF families detected in *CYP1A1, GGPS1* or *CTSK.*[3,4,6] One common intronic indel was found in *GGPS1* only carried by II.3 and III.9. Analysis of *CYP1A1* and *CTSK* did not yield any results.

Hypothesis free approach

The three siblings with AFF shared 78 nonsynonymous, exonic, heterozygous variants in 75 genes, regardless of the genotype of other family members.

An initial assessment of the protein function, using the summary of the gene function in National Center for Biotechnology Information, of the 75 genes of interest, showed one gene involved in bone metabolism that has not yet been associated with human bone disease. The variant in question was present in the three AFF cases and the sister with a low trauma femoral fracture (II.5), but not in the cousin with severe osteoporosis (III.1). The CADD score is 35 and the allele frequency in the gnomAD database is 0.0001.

Using the segregation pattern of a common genetic cause for AFF, the non-atypical femoral fracture and osteoporosis in this family resulted in eight nonsynonymous variants in seven genes.

A separate analysis in the cousin with severe osteoporosis (III.1) for a genetic cause of osteoporosis yielded three common variants in *COL1A1*, *COL1A2* and *LRP6* and one rare nonsynonymous variant in a gene involved in osteoblast differentiation, but never associated with human bone disease.

DISCUSSION

We clinically assessed a family with three cases of AFF after bisphosphonate use and present preliminary findings of whole-exome sequencing data analysis in nine family members, in search of a genetic cause of AFF. One family member displayed a femoral fracture that did not fully meet the diagnostic criteria for AFF, but might belong to the spectrum of AFF. Seven family members had osteoporosis, but five of these family members had comorbidities, medication use or life style factors that could lead to low bone mass. In our initial genetic analysis, we found a potential susceptibility variant for AFF in a gene involved in bone metabolism, shared by the three siblings with AFF and the fourth sibling with a non-atypical femoral fracture. The variant of interest in this gene does not segregate osteoporosis in this family, since the variant was absent in a cousin with severe osteoporosis. Based on this observation, AFF and osteoporosis may be distinct phenotypes with different genetic backgrounds in this family. However, 77 other variants of interest shared by the three AFF cases were found in 74 genes that cannot be dismissed without further research.

Our study is limited by this high number of variants found in the hypothesis-free exome-wide approach combined with the lack of sufficient informative meiosis within this family to filter out the non-co-segregating ones. In this initial analysis, we applied a more stringent filtering based on the known function of the gene in bone biology, which led us to a main variant of interest. Yet, this biased approach had the disadvantage that we may have overlooked the true genetic cause of AFF in this family, arising from a gene with hitherto unknown function in skeletal pathophysiology. Using WES, we also neglected the role of noncoding variants from regulatory regions of the genome, the role of large structural variations such as copy number variations, and a potential role of epigenetics.

Strengths of our approach for a family-based WES were the availability of detailed clinical information for in-depth phenotyping of the subjects, and a filtering approach

considering different scenarios for the genetic architecture of AFF and osteoporosis in this family.
We propose the following steps for further research of the 75 genes found in our hypothesis-free approach. Data from genome-wide association studies (GWAS) on bone mineral density or fracture may show hits near the genes of interest, indicating a potential role in bone biology. Similarly, the presence of the gene in RNA expression datasets of bone tissue could serve as a rough reference, although at this point expression data for bone tissues in public databases are lacking. In addition, the predicted *in silico* pathogenicity such as the CADD score, based on the predicted effect on the protein and conservation across species, can be a factor to take into account.[12,18] A cutoff value of 15 can be used in Mendelian disease to define benign and pathogenic variants.[19] Arguably, a high pathogenicity score may not be warranted in AFF, since this adverse event might be the result of a risk variant(s) with a small effect(s) over a long period of time in patients using bisphosphonates for years. Lastly, bone phenotyping information from knock-out mouse models in publicly available datasets may be useful to assess the potential function of the gene in humans.

Once a selection of variants of interest from this family has been made, these variants can be studied in other human study populations including other families with AFF or a cohort of unrelated patients with AFFs.[20] We need to take into account that a causal genetic variant in this family could be a private mutation only or that may not necessarily be disease-causing in other families or individuals.

The occurrence of the variant of interest in a population-based study cohort such as the Rotterdam Study could also be informative.[21] The latter is a Dutch cohort of elderly people including 2,604 subjects with WES data and longitudinal information available on BMD and fractures. An association between rare variants in the gene of interest and osteoporosis or fractures in this population may establish a link with human bone disease. Furthermore, common polymorphisms in such a candidate gene – using GWAS data - could be associated with low BMD or fracture in the Rotterdam Study, revealing a novel susceptibility gene for bone disease.

To study the potentially pathogenic effects of a variant of interest in this family, skin punch biopsies could be obtained to generate osteoblasts from fibroblasts that serve as a proxy for bone tissue. Based on the known protein function from the gene of interest, we might observe decreased gene expression, defective protein function and reduced quality of osteoblast differentiation, extracellular matrix composition and mineralization in patient's samples in comparison to healthy controls. Alternatively, animal models could be considered for functional analysis, since novel genetic causes for osteoporosis

have been successfully investigated with the use of knockout animals such as for LRP5 and PLS3 in skeletal disease.[22-25] On the other hand, models with a knockout gene do not establish the specific role of the variant of interest. For this purpose, site-directed mutagenesis (SDM) or CRISPR-directed gene editing may be used to create the exact variant. A cell or mouse model for AFF would require wildtype and knockout animals with and without bisphosphonate use to clarify if any differences can be attributed to the genetic makeup or the use of antiresorptive drugs. Mice treated with bisphosphonates have been used to create mouse models for osteonecrosis of the jaw[26,27] and to study fracture healing.[28,29]AFF is a spontaneous fracture that may not be simulated in an animal model, especially since the mechanical strain on the femur of rodents differs from humans and the administration of bisphosphonates may be challenged by a proper timing, dosage and duration. Yet, several analyses could be used as derived parameters for the risk of AFF, including microCT-scanning of the femur to provide information on cortical and trabecular bone mass and architecture, X-rays of the femur to establish bone mineral content and three point bending of the femur to test bone strength and stiffness.

The aforementioned steps for further analyses are based on the assumption that AFF is an autosomal dominant trait. Alternatively, an X-linked or recessive mode of inheritance could be considered. Another option is that a combination of multiple genetic factors each with small effects are involved in the pathogenesis of AFF, for which array data in this family would be needed to calculate a polygenic risk score.
In conclusion, this genetic study in a family with unexplained fractures such as AFFs is important to unravel the pathophysiology of bone disease, provide genetic counseling for relatives and hopefully in the future contribute to personalized medicine in the treatment of osteoporosis. Whole-exome sequencing generated many potential susceptibility variants within this family, but we aim to find the genetic cause of AFF by prioritization of variants based on predicted pathogenicity, allele frequency and a potential link with bone biology and will eventually validate our findings by replication and functional analysis.

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APPENDIX I: DESCRIPTION OF INDIVIDUAL FAMILY MEMBERS

First generation

I.1 reportedly never sustained any fractures and died at an old age (> 80 years).

I.2 reportedly sustained a hip fracture at the age of 90 years and had evident height loss. She deceased when she was 95 years old.

Second generation

II.1 was a man who deceased at the age of 70 years due to a tumor. Reportedly he had never had any fractures nor loss of height. His height was 172cm.

II.2 is a 74-year-old man when he was diagnosed with a unilateral, incomplete AFF in 2013 after 16 years of treatment with alendronate because of vertebral fractures and osteoporosis diagnosed at the age of 57 years. Prior to the diagnosis of AFF, he had had prodromal pain during five years. His fracture history further included a radius fracture in 2007 and a foot fracture.

In 2014 a DXA scan showed osteopenia of the femoral neck (T-score -1.6 SD) and the lumbar spine (L2-L4 T-score -1.1 SD), although the latter was unreliable due to vertebral fractures and degenerative changes. His medical history included asthma, cardiovascular disease, insulin-dependent diabetes mellitus, hypogonadism and hyperthyroidism and past smoking. His height was 162cm and his maximum height was 164cm. His weight was 77g (BMI 29.3kg/m^2). He did not drink alcohol. He quit smoking around 2000 after 34 packyears.

II.3 is a 70-year-old man who was diagnosed with osteoporosis of the lumbar spine (L2-L4 T-score -2.7 SD) and osteopenia of the femoral neck (T-score -2.1 SD) on a DXA scan performed as part of the family history in 2014. He did not have fractures in the past and an X-ray of the thoracolumbar spine did not show vertebral fractures. He had never used antiresorptive drugs. An X-ray of the femora and the pelvis showed no abnormalities. Risk factors for osteoporosis included COPD, use of inhalation steroids and former smoking (37 packyears). Furthermore his medical history included insulin-independent diabetes mellitus type II and coronary artery disease. His height was 168 cm and his weight was 84 kg (BMI 29.8 kg/m^2).

II.4 is a 62-year-old woman who sustained a complete AFF of the left femur in March 2008 after six years of risedronate use. She reported prodromal pain since two years. In January 2012, an incomplete AFF of the right femur was seen with a pending complete

fracture. She was initially diagnosed with osteoporosis with multiple vertebral fractures in 2003 at the age of 58 years. She had been treated with risedronate from 2003 until 2009 and strontium ranelate during 1.5 years until 2012. Her medical history recorded hypertension and did not include corticosteroid use nor use of proton pump inhibitors. She did not use alcohol nor smoked. She had an early menopause around the age of 36 years without subsequent hormone replacement therapy. Her height was now 150cm after 7cm height loss and her weight was 62kg (BMI 27.5kg/m^2). In 2014 a DXA scan showed osteopenia of the femoral neck (T-score -2.2 SD) and a normal BMD of the lumbar spine (L2-L4 T-score -0.3 SD). Upon the diagnosis of the incomplete AFF in 2012, she was prescribed daily teriparatide injections.

II.5 is a 59-year-old woman who was diagnosed with osteoporosis in 2006 after multiple, spontaneous vertebral fractures. She was subsequently treated with alendronate which she discontinued after one or two years on her own initiative. In May 2011 she sustained a complex intra-articular fracture of the distal femur after a soft fall from standing height. In 2015 her DXA scan showed osteopenia of the lumbar spine (L2-L4 T-score -2.3 SD) and osteoporosis of the femoral neck (T-score -3.1 SD). She had no other comorbidities.

Her height was 152cm after a height loss of 10cm and her weight was 59kg. She had an early menopause at the age of 38 years and she used oral contraception until she was 48 years old. She was an active smoker with over 40 packyears.

The general practitioner prescribed denosumab injections of 60mg subcutaneously every six months in 2015.

II.6 is a 66-year-old woman when diagnosed with osteoporosis of the femoral neck (T-score -3.3 SD) on a DXA scan in 2014. She had a normal BMD of the lumbar spine (L1-L4 T-score +0.3 SD). X-rays of the spine, pelvis and femora were normal. She had never had any fractures. Her medical history included insulin-dependent diabetes mellitus. She quit smoking in 2012 after more than 40 packyears. She never used alcohol. Her height was 158cm after 2cm height loss and her weight was 55kg (BMI 22.0 kg/m^2). Her last menstruation was at the age of 50 years. Based on the results of the DXA screening, the general practitioner started vitamin D and denosumab 60mg subcutaneously every six months in 2015.

II.7 is a dizygotic twin sister of II.6. She was diagnosed with bilateral incomplete AFFs in March 2013. A SPECT-CT scan showed a hotspot of the lateral cortex with localized cortical thickening of the left femur and a similar abnormality was also very subtly visible on the right femur. An MRI scan of the femur revealed diffuse bone marrow edema

bilaterally in the proximal femora without a fracture line. She had been treated with alendronate since at least six years. Her fracture history included a radius fracture in 2010. Osteoporosis was previously diagnosed in 2002 because of a positive family history and corticosteroid use for seropositive rheumatoid arthritis and SLE (systemic lupus erythematosus). A DXA scan in 2013 showed osteoporosis of the femoral neck (T-score -2.6 SD) and osteopenia of the lumbar spine (L2-L4 T-score -1.2 SD).

In 1999, at the age of 51 years, she had had a cerebrovascular event that was interpreted as a complication of the SLE. This was followed by a severe epileptic insult and depression. Her medication use included vitamin D, calcium, solumedrol since 1999, ranitidine, seroxat, diphantoin, vitamin B12, medrol, plaquenil and an anticoagulant. She had received gold injections in the past for arthritis. She had used oral contraception until the age of 51 years. She did not use alcohol nor smoked. Her height was 159cm with height loss of 1 cm and her weight was 56kg (BMI 22.4 kg/m^2).

Third generation

III.2 – III.8, III.11 and **III.12** did not undergo DXA scanning nor contributed DNA samples, but none of them were reported to have experienced any fractures.

III.1 is a 64-year-old woman diagnosed with osteoporosis and eight spontaneous vertebral fractures, two unilateral wrist fractures, rib fracture and an elbow fracture after inadequate traumas. The first spine fracture occurred at the age of 56 years and she underwent multiple spinal surgeries for kyphoplasty. She had no femoral fractures. She wore a brace and had an infusion pump for management of chronic back pain. She was diagnosed with fibromyalgia and had used crutches since 20 years because of chronic pain when walking. Furthermore she was treated for hypertension and an epileptic syndrome of unknown etiology. Medication use included perindopril, depakine, pantoprazole, gabapentin, pravastatin and rivotril. Her height was 153cm and her maximum height was 157cm. Her weight was 100kg (BMI 42.7kg/m^2).

Her last menstruation was at the age of 45 years. She never smoked and did not use alcohol. She was previously treated with alendronate for an unknown duration and she had used denosumab 60mg subcutaneously every six months, started in 2015.

III.9 is a premenopausal, 50-year-old woman. She never experienced any fractures. She had hypertension and hypercholesterolemia for which she used a statin. She visited the physical therapist for chronic stiffness in the hips, spine and upper legs. Her height was 168cm and her weight was 66kg (BMI 23.4 kg/m^2). DXA scanning showed a normal BMD

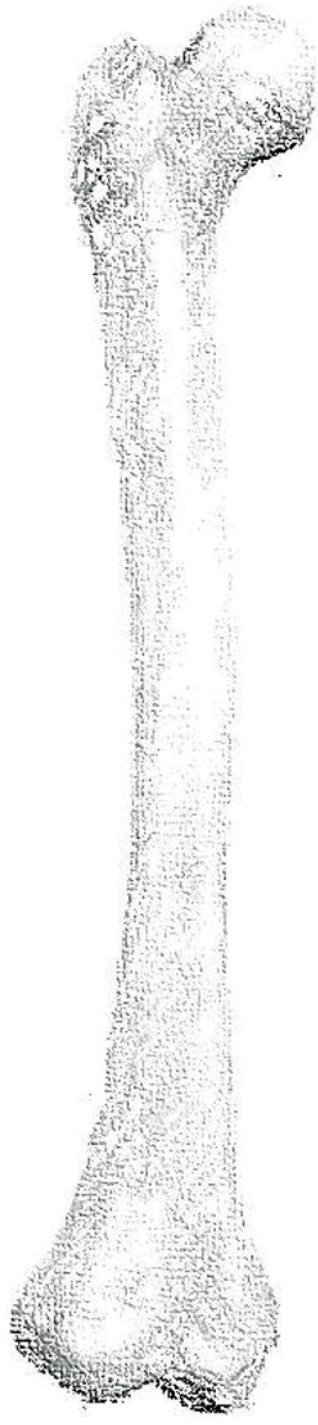
of the lumbar spine (L2-L4 T-score +0.8 SD) and femoral neck (T-score -0.8 SD). Vertebral fracture assessment showed no vertebral height loss.

III.10 is a perimenopausal 45-year-old woman who fractured her nose at the age of 28 years and she had a wrist fracture after a fall from standing height when she was 30 years old. She was recently diagnosed with a thrombotic disorder for which she needed treatment during long flights and apart from vitamin B12 injections, she received no other medication. She was an active smoker (34 packyears) and used one alcoholic beverage per month. Her height was 171cm and her weight 64kg (BMI 21.9kg/m^2). DXA scanning revealed osteopenia of the lumbar spine (L2-L4 T-score -1.7 SD) and a normal BMD of the femoral neck (T-score -0.3 SD) without vertebral fractures on vertebral fracture assessment.

III.13 is a 47-year-old man who never sustained any fractures and had no height loss. At the age of 45 years he had started alendronate because of low bone mass which was diagnosed after corticosteroid use for polymyalgia rheumatica. However, the corticosteroids had already been stopped for three years at time of baseline BMD measurement. DXA scanning now showed osteopenia of the lumbar spine (L2-L4 T-score -1.9 SD) and femoral neck (T-score -2.2 SD). Vertebral fracture assessment showed no abnormalities. He complained of pain in the upper legs, possibly related to heavy physical labor being a construction worker. X-rays of the femora showed no signs of stress fractures.

III.14 is a 44-year-old man who never sustained any fractures. DXA scanning revealed osteopenia of the lumbar spine (L2-L4 T-score -2.1 SD) and femoral neck (T-score -2.1 SD) and a normal vertebral fracture assessment. His height was 174cm and his weight 62kg (BMI 20.5 kg/m^2). He used no more than two alcoholic units per day and he was an active smoker (8 packyears).

III.15 is a 43-year-old man who sustained fractures of the clavicula in childhood, foot fracture in adolescence and wrist fractures as an adult after high-energetic traumas. He had a normal BMD of the lumbar spine (L2-L4 T-score -1.0 SD) and femoral neck (T-sore -0.8 SD) and no vertebral fractures.



Part 3

Medical treatment of atypical femur fractures

10

Medical management of patients after atypical femur fractures: a systematic review and recommendations from The European Calcified Tissue Society

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ABSTRACT

Context. Atypical femur fractures (AFFs) are serious adverse events associated with bisphosphonates and often show poor healing.

Evidence acquisition. We performed a systematic review to evaluate effects of teriparatide, raloxifene and denosumab on healing and occurrence of AFF.

Evidence synthesis. We retrieved 910 references and reviewed 67 papers, including 31 case reports, nine retrospective and three prospective studies on teriparatide. There were no randomized controlled trials. We pooled data on fracture union (n=98 AFFs on teriparatide) and found that radiological healing occurred within six months of teriparatide in 13 of 30 conservatively managed incomplete AFFs (43%), nine of 10 incomplete AFFs with surgical intervention (90%) and 44 of 58 complete AFFs (75%). In nine of 30 non-operated incomplete AFFs (30%) no union was achieved after 12 months and four fractures (13%) became complete on teriparatide. Eight patients had new AFFs during or after teriparatide. AFF on denosumab was reported in 22 patients, including 11 patients treated for bone metastases and eight without bisphosphonate exposure. Denosumab after AFF was associated with recurrent incomplete AFFs in one patient and two cases of contralateral complete AFF. Eight patients had used raloxifene before AFF occurred, including one bisphosphonate-naïve patient.

Conclusions. There is no evidence-based indication in patients with AFF for teriparatide apart from reducing the risk of typical fragility fractures, although observational data suggest that teriparatide might result in faster healing of surgically treated AFFs. Awaiting further evidence, we formulate recommendations for treatment after an AFF based on expert opinion.

INTRODUCTION

Antiresorptive drugs such as bisphosphonates are widely used for the treatment of osteoporosis. Although effective for prevention of osteoporotic fractures, use of bisphosphonates is associated with rare but serious adverse events such as osteonecrosis of the jaw and atypical femur fractures (AFFs). An AFF is a spontaneous or low-trauma, subtrochanteric or femur shaft fracture often complicated by delayed or non-union (26%-39%) and bilateral occurrence.[1,2]

The age-adjusted incidence rate of AFF has been estimated to be 1.8 per 100,0000 person-years in patients on bisphosphonate use under two years, increasing to 113 per 100,000 person-years with over eight years' duration.[3] It is thought that decreased bone resorption in bisphosphonate users results in suppressed bone turnover with accumulation of microcracks and homogeneously mineralized bone, making the bone more brittle and allowing the development of a spontaneous femur fracture. However, it is uncertain if bisphosphonates are causally related to AFF and incidentally AFFs do occur in bisphosphonate-naïve individuals.[4] Usually, bisphosphonates are discontinued after AFF is diagnosed. It has been shown that the risk of AFF decreases with 70% per year since the last use of antiresorptive drugs[5], although it is not certain that this risk reduction is also seen in patients who have already sustained an AFF.

It is unclear if alternative osteoporosis drugs, particularly anabolic drugs, can promote AFF healing. Moreover, there is no guideline on how patients should be treated after an AFF where the risk of causing new atypical fractures should be weighed against the risk of fragility fractures when not treating osteoporosis. It has been proposed that teriparatide, an analog of parathyroid hormone (PTH 1-34) , is a safe option for treatment of osteoporosis in AFF patients, especially since it may also have a beneficial effect on the healing of AFF itself.[6] Teriparatide is the only anabolic osteoporosis drug that is currently globally available. It directly stimulates osteoblasts that might enable the formation of new, heterogeneously mineralized, bone at the fracture site of AFF. Besides teriparatide, antiresorptive drugs other than bisphosphonates, such as raloxifene and denosumab, may be considered for osteoporosis treatment in AFF patients. Denosumab is a human monoclonal antibody to RANKL and a potent inhibitor of bone resorption. Although AFFs have been reported in patients exposed to denosumab in case reports, it has not been clearly established in epidemiological studies how often denosumab, with or without preceding bisphosphonate use, is associated with AFF. The radiological healing or deterioration of AFF whilst on denosumab treatment is also not known. Raloxifene is a selective estrogen receptor modulator (SERM) that acts as an estrogen agonist in bone, with an antiresorptive effect that is milder than that of bisphosphonates and denosumab. The

relationship between raloxifene and the occurrence of AFF has not been investigated. To our knowledge, this is the first review that explored denosumab and raloxifene in addition to teriparatide for medical management of osteoporosis in patients with AFF. Further, we investigated whether AFF occurs as an adverse event in clinical trials with two novel drugs for osteoporosis, romosozumab and abaloparatide. Romosozumab, an antibody to sclerostin with both anabolic and antiresorptive effects, was recently approved in Europe, Japan and the U.S. for the treatment of (severe) osteoporosis. Abaloparatide is a synthetic analog of parathyroid hormone related protein. Strontium ranelate was not included in this review, since this drug is no longer available in most countries.

We performed a systematic literature review to assess both the occurrence and the radiological healing of AFFs in patients who had used or were using teriparatide, denosumab or raloxifene. We formulate recommendations for healing of the AFF itself and for osteoporosis management in patients who have sustained an AFF and are at high risk of fragility fractures.

METHODS

We performed a search using key words related to atypical femur fractures and teriparatide, denosumab and/or raloxifene in Embase, Medline Epub (Ovid), Web of Science and Cochrane Central on 28th of May 2018. We separately searched for AFF as an adverse event in clinical trials with romosozumab or abaloparatide. Reviews and articles written in a language other than English were excluded. Conference abstracts and original research articles were included. Articles were reviewed when AFF was diagnosed during or after the use of teriparatide, denosumab and raloxifene or when the radiological healing of AFF in a specified amount of time was reported using these drugs.

A complete AFF was defined as a non-comminuted subtrochanteric or femur shaft fracture with a predominantly transverse fracture line that may become oblique as it progresses medially, after no or minimal trauma. An incomplete form of AFF was defined as a localized endosteal or periosteal thickening of the lateral cortex of the subtrochanteric femur with or without the presence of a lucent line. When the authors did not describe whether a fracture line was visible, we assessed medical imaging in the article to review the presence of a fracture line.

We extracted data on sex, median age, ethnicity, use of bisphosphonates, surgical interventions and clinical or functional outcome after the AFF as far as this information was available.

We assessed the occurrence of newly diagnosed AFF during or after the use of teriparatide, denosumab or raloxifene. Newly diagnosed AFF could either be the first clinical presentation of AFF, a second AFF of the contralateral femur, or recurrent AFF at the ipsilateral femur.

For the assessment of radiological healing, the results were categorized for each type of drug according to study design (case report, retrospective cohort and prospective studies) and fracture type (complete AFF, incomplete AFF with or without surgical treatment) (**Figure 1**).

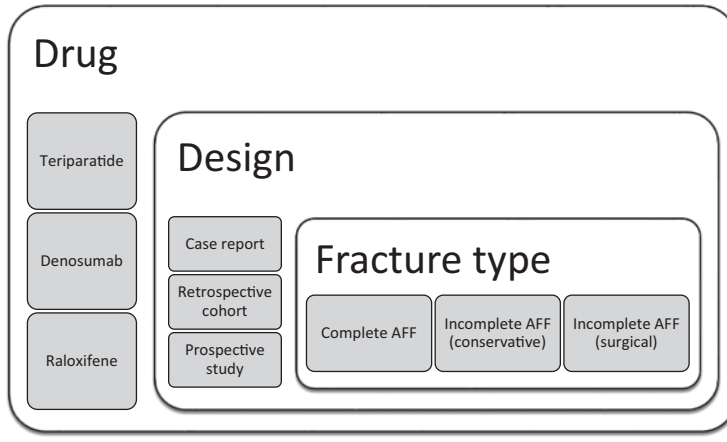

Figure 1. The results for each type of drug were categorized according to study design and fracture type.

We assessed the total number of AFFs described in the literature with complete radiological healing at six months and 12 months after medical management. The number of conservatively treated incomplete AFFs that developed a lucent line or progressed to complete AFF was also noted. We pooled these data on healing from all article types to provide better insight into the effectiveness of the drugs for the healing of AFF.

Radiological healing in complete AFFs and surgically treated incomplete AFFs was defined as adequate callus bridging. Radiological healing of an incomplete AFF on conservative management was defined by disappearance of a visible fracture line. Radiological healing of incomplete AFFs without a lucent line included flattening of cortical thickening, disappearance of bone marrow edema on MRI-scan, or fading of hotspots on bone scintigraphy. Incomplete AFFs with localized cortical thickening only, without abnormalities on MRI-scan or bone scintigraphy were excluded from assessment of radiological healing, because focal cortical thickening can remain unchanged for more than five years after diagnosis of incomplete AFF.[7]

We give our recommendations for teriparatide, denosumab and raloxifene in the medical treatment of patients with AFF. In order to address the decision-making in individual cases, we have formulated treatment advice for patients with a new diagnosis of AFF and patients with AFF who have completed a two-year course of teriparatide. These considerations are based on the findings in this review and our expert opinion.

RESULTS: SYSTEMATIC REVIEW

Our search retrieved 910 references. We selected two conference abstracts and 130 articles after screening of title and abstract. We replaced one conference abstract with the article that was published shortly after our search date.[8,9] After full-text reading, 67 articles were included for this review. Sections on teriparatide, denosumab and raloxifene have overlapping references, because some case descriptions report on a combination of these treatments in AFF patients.

Teriparatide

We found 31 case reports, nine retrospective cohort studies and three prospective studies that have reported the effect of teriparatide on the radiological healing of AFF or occurrence of AFF. There were no published randomized controlled trials (RCTs). Detailed study descriptions of case reports, retrospective cohorts and prospective studies on teriparatide use in AFF patients can be found in **Supplement 1**. The demographic characteristics of the patients with AFF on teriparatide in case reports are stated in **Table 1.** Clinical variables and main findings from retrospective cohorts and prospective studies are summarized in **Table 2** and **Table 3**, respectively. The pooled data on radiological healing of AFF with teriparatide treatment are shown in **Table 4**.

Teriparatide use and occurrence of AFF

New AFF cases during or after teriparatide use were reported in eight patients and always occurred in patients with previous bisphosphonate exposure. The new AFFs occurred after 4, 11, 18 and 24 months of teriparatide treatment in four patients.[10-13] The remaining four patients were described in a conference abstract which did not report the duration of teriparatide at time of diagnosis, but all developed new incomplete AFFs during teriparatide therapy in the same femur in which the first incomplete AFF was diagnosed.[14]

Six of the eight patients had been diagnosed with another AFF before, but in two patients the AFFs during teriparatide were the first AFFs.[11,12] One patient was diagnosed with a complete and contralateral incomplete AFF two years after stopping teriparatide

Table 1. Demographic characteristics of case reports on teriparatide use in AFF patients.

Reference	n = patients[1]	n= in-complete AFF[2]	fracture line visible	n= complete AFF[2]	Sex	Mean age	Background[3]	Antiresorptives[4]	Condition	Mean duration treatment in years (range)[5]
Al Azzani, 2015	1	1	yes	0	M	54	Caucasian	alendronate (5), ibandronate (5)	cystic fibrosis	10
Carvalho, 2011	1	0	-	1	F	77	Caucasian	alendronate	postmenopausal osteoporosis	4
Cerveró, 2015	1	1	yes	1	F	71	(Spain)	alendronate	postmenopausal osteoporosis	5
Chiu, 2013	1	2	yes	0	F	63	(Taiwan)	alendronate	postmenopausal osteoporosis	7
Chew, 2013	1	1	yes	0	F	70	(Malaysia)	alendronate	back pain	6
Etxebarria-Foronda, 2015	1	0	-	1	M	21	(Spain)	pamidronate iv (3), alendronate (5)	osteogenesis imperfecta	8
Fukuda, 2014	1	1	yes	1	F	74	(Japan)	alendronate	postmenopausal osteoporosis	6
Giannotti, 2013	1	1	no	1	F	65	Caucasian	“bisphosphonates”	NS	6
Gomberg, 2011	1	2	no (2)	0	F	63	Caucasian	alendronate	glucocorticoid-induced osteoporosis	13
Holm, 2014[6]	1	0	-	1	NS	NS	(Norway)	“bisphosphonates”	osteogenesis imperfecta	9
Huang, 2012	1	1	yes	0	F	63	Asian	alendronate	vertebral fractures	3
Iwata, 2014	1	0	-	2	F	56	Asian	incadronic iv 10mg two-weekly (3), pamidronate iv 90 mg monthly (1), zoledronate iv 4mg monthly (5)	metastatic bone disease	9
Jain, 2011	1	1	NS	1	F	75	(India)	alendronate	osteopenia	6
Kaur, 2016	1	1	no	0	F	70	Guyanese	alendronate	postmenopausal osteoporosis	10
Lampropoulou-Adamidou, 2013	1	0	-	1	F	84	Caucasian	alendronate (12), ibandronate (1)	postmenopausal osteoporosis	13
Mastaglia, 2016	1	1	no	1	F	57	Caucasian	alendronate	osteopenia	7
Nguyen, 2017	1	0	-	1	F	65	(Australia)	alendronate	postmenopausal osteoporosis	11
Ramchand, 2016	1	2	no (2)	0	F	82	(Australia)	alendronate (6), risedronate (1)	rib fracture osteoporosis	7

Table 1. Demographic characteristics of case reports on teriparatide use in AFF patients. (*continued*)

Reference	n = patients[1]	n= in-complete AFF[2]	fracture line visible	n= complete AFF[2]	Sex	Mean age	Background[3]	Antiresorptives[4]	Condition	Mean duration treatment in years (range)[5]
Reddy, 2012	1	0	-	1	M	70	Asian	zoledronate iv 4mg monthly	androgen deprivation therapy	2
Righetti, 2018	1	2	yes (1)	0	F	67	Armenian	alendronate	hypophosphatasia	10
Román, 2015	1	0	-	2	M	72	(Spain)	alendronate	glucocorticoid-induced osteoporosis	11
Schilcher, 2015	1	0	-	1	F	84	(Sweden)	"bisphosphonates"	rheumatoid arthritis/Wegener granulomatosis	16
Selga, 2016	1	0	-	2	F	62	(Spain)	alendronate (10), risedronate (2), ibandronate (3), denosumab (2)	osteoporosis	17
Spyridonidis, 2014	1	1	yes	1	F	78	(Greece)	alendronate	osteoporosis	8
Stathopoulos, 2011	1	0	-	1	F	76	Caucasian	zoledronate iv 4mg yearly	osteoporosis	6
Tan, 2017	1	1	yes	0	M	63	(Singapore)	alendronate (7), etidronate (2)	osteogenesis imperfecta	9
Tarazona-Santabalbina, 2013	1	1	yes	1	F	73	(Spain)	alendronate	osteoporosis	13
Tsuchie, 2015	2	3	yes (3)	0	F	78	(Japan)	alendronate	osteoporosis	5 (4-6)
Uppin, 2016	1	0	-	2	F	56	(India)	alendronate	rheumatoid arthritis	4
Vaishya, 2013	1	2	yes (2)	0	F	63	(India)	alendronate	osteoporosis	3
Visekruna, 2008	2	0	-	3	F	69	Caucasian	alendronate, raloxifene	steroid-dependent rheumatoid arthritis	13 (10-16)

NS = not stated, F = female, M = male

[1] From case series, only patients in whom the effect of teriparatide could be assessed on healing or occurrence of AFFs were included in this table.

[2] The number of AFFs included (contralateral) AFFs that had already healed by the time teriparatide was started. This means that the total number of AFFs in this table is higher than the total number of AFFs that was treated with teriparatide.

[3] The country of the affiliation is given, when ethnicity of the AFF case was not specified in the article.

[4] The types of bisphosphonates prior to the occurrence of the first AFF. When a patient had used several antiresorptive drugs, the total number of years the patient had used this specific drug is indicated in brackets. In some cases, type of bisphosphonates was unknown ("bisphosphonates"). For intravenous bisphosphonates the dosage and treatment interval are given in the table. Alendronate dosages included 70mg weekly or 10mg daily. Etidronate was given 400mg two-weekly, ibandronate 150mg monthly, risedronate 35mg weekly and raloxifene 60mg daily.

[5] *The total duration of antiresorptive drugs use prior to the first diagnosis of AFF is given in years, not including drug holidays.*

[6] No access to full-text article.

Table 2. Summary of retrospective cohorts of AFF patients and use of teriparatide.

Reference	Total cohort, *n*	Patients on TPT, *n*	Controls without TPT, *n*	Fracture type of TPT users*	Female, *n* (%)	Mean age (years)	Country	AR use	Mean duration AR, years (range)	Main outcome
Cheung, 2013	22	22	0	Incomplete *surgical = 3* *conservative = 19*	22 (100%)	66	Canada	Yes (100%)	12 (3.4-28.7)	Of 19 incomplete AFFs without surgery, 2 healed, 5 were healing, 12 were stable after 2 years of TPT, but 4 patients developed new incomplete AFFs
Lee, 2013	51	19	32	Incomplete *surgical = 12* *conservative = 7*	50 (98%)	70.4	South Korea	Yes (77%)	4.5	7 patients on TPT and 19 patients without TPT required surgery; Use of teriparatide did not significantly reduce the need for surgery (p = 0.210)
Lee, 2017	44	14	30	Complete *n = 14 AFFs*	44 (100%)	70.1	South Korea	Yes (100%)	5.1	Time to healing was 4.9 months in TPT-group,6.6 months in non-TPT-group and 7.1 months in those continued on bisphosphonates
Miyakoshi, 2015	34	NS (21 AFFs)	NS (24 AFFs)	Incomplete *surgical = 5 AFFs* *conservative = 5* Complete *n=11 AFFs*	34 (100%)	78.5	Japan	Alendronate or risedronate (100%)	4.4 (1-11.7)	Time to healing was significantly shorter for all surgically treated AFF in TPT-group (5.4 vs. 8.6 months)
Petraszko, 2016	7	6	1	Incomplete *conservative = 8 AFFs* *surgical = 1 AFF*	7 (100%)	70.7	USA	Yes (100%)	10.6 (7-15)	Fracture line disappeared in 2 of 6 AFFs with a visible line within one year of TPT
Saleh, 2012	10	9	1	Incomplete *conservative = 13 AFFs*	10 (100%)	66.8	USA	Alendronate or risedronate (100%)	10 (4-17)	5 AFFs without line all healed, 7 of 8 AFFs with fracture line had surgery after 3 months of TPT

Table 2. Summary of retrospective cohorts of AFF patients and use of teriparatide. (*continued*)

Reference	Total cohort, *n*	Patients on TPT, *n*	Controls without TPT, *n*	Fracture type of TPT users*	Female, *n* (%)	Mean age (years)	Country	AR use	Mean duration AR, years (range)	Main outcome
Sato, 2017	12	6	6	Incomplete *conservative = 6*	12 (100%)	55.6	Japan	Alendronate (100%)	5.9 (3.1-9.3)	All AFFs on continued bisphosphonates deteriorated; 1 AFF progressed to complete fracture after 8 months of TPT
Takakubo, 2017	8	4	4	NS *surgical = 5 AFFs*	8 (100%)	54.9	Japan	Alendronate, risedronate, minodronate (100%)	4.3 (2-10)	Time to healing was 11.5 months in 5 AFFs on TPT and 13.3 months in 6 AFFs without TPT, but 1 AFF was not healed after 1 year and lost to followup in the TPT-group
Yeh, 2017	13	NS (8 AFFs)	NS (8 AFFs)	Complete *n = 8 AFFs*	13 (100%)	70.2	Taiwan	Alendronate (100%)	4.0 (2.5-6)	Time to healing was 4.4 months in the TPT-group versus 6.2 months in the non-TPT group

TPT = teriparatide, AR = antiresorptive, NS = not stated.

Percentage of women, mean age, antiresorptive use and mean duration of antiresorptive treatment were based on the whole cohort, including controls.

**When the number of AFFs is not stated in the article, the number of patients is given.*

Table 3. Summary of prospective studies on AFF patients and use of teriparatide.

Reference	Total cohort, *n*	Patients on TPT, *n*	Controls without TPT, *n*	Fracture type of TPT users*	Female, *n* (%)	Mean age, (years)	Country	AR use	Mean duration AR, years (range)	Main outcome
Chiang, 2013	14	5	9	Incomplete *n = 4* Complete *n = 1*	13 (93%)	76	Australia	Alendronate, risedronate, pamidronate, zoledronate (100%)	7 (4-10)	TPT users: 2 healed, 3 had partial healing Controls: 3 prophylactic surgery, 1 contralateral AFF, 6 with non-union
Greenspan, 2018	13	13 - 7 immediate post-surgery - 6 on teriparatide 6 months postoperatively	0	Incomplete *surgical = 1* Complete *n = 12*	13 (100%)	74	USA	Risedronate, ibandronate, alendronate (100%)	NS	Higher bone healing scores in immediate TPT group, but not statistically significant
Watts, 2017	14	14	0	Complete *n = 9* Incomplete *surgical = 1* *conservative = 4*	14 (100%)	68	USA	Alendronate, ibandronate, zoledronate, risedronate (100%)	8.8 (3-14.5)	Complete AFFs: 4 healed, 5 partial healing, 1 nonunion. Incomplete AFF: 4 partial healing, 3 unchanged. 2 contralateral complete AFFs

TPT = teriparatide, AR = antiresorptive, NS = not stated

Percentage of women, mean age, antiresorptive use and mean duration of antiresorptive treatment were based on the whole cohort, including controls.

** The number of patients is given.*

without any antiresorptive use in the meantime, but the patient had been treated for eight years with antiresorptives in the past.[11]

Teriparatide use after AFF

Descriptive data of case reports, retrospective and prospective studies

In 33 patients, a total of 24 incomplete AFFs and 27 complete AFFs were reported at the time of starting teriparatide treatment in 31 case reports. In 13 incomplete AFFs (54%) a fracture line was described or visible on the images in the publication, whilst the other cases of incomplete AFFs only showed focal cortical thickening on X-ray. The majority of cases were women (n=27, 82%). The mean age of all AFF patients was 67 years, ranging from 21 to 84 years. Only a minority of studies (39%) reported ethnicity in 13 patients of whom nine were Caucasian. All cases of AFF were associated with the use of bisphosphonates. A total of 27 patients (82%) were previously exposed to alendronate therapy. The mean treatment duration with antiresorptive drugs was 8.3 years, with a minimum duration of two years and a maximum exposure of 17 years. Three patients were diagnosed before the AFF with osteogenesis imperfecta[15-17] and one patient was genetically tested after the occurrence of bilateral incomplete AFFs which revealed hypophosphatasia.[18]

Nine retrospective cohorts that comprised a total of 201 AFF patients reported the effect of teriparatide use on radiological healing. Five cohorts involved incomplete forms only [14,19,22], three cohorts described complete fractures only[23-25] and one cohort was mixed.[26] Six cohorts consisted of entirely Asian populations. In eight cohorts, all AFF cases were exposed to antiresorptive therapy and one cohort had 23% bisphosphonate-naïve patients.

Three prospective studies comprised a total of 31 women and one man with a mean age of 73 years who were treated for bisphosphonate-associated AFFs with teriparatide. Only one of these studies had controls (n=9 patients) without teriparatide treatment.[27] All three studies had a mix of complete and incomplete AFFs. Teriparatide was started immediately after surgery in one study and compared to delayed commencement of teriparatide six months postoperatively[28], whilst in the other two studies teriparatide was started between seven weeks to just over one year after the diagnosis of AFF.[27,29] The study by Greenspan *et al.* included four individuals with periprosthetic fractures [28], which strictly does not adhere to the diagnostic criteria for AFF as formulated by the American Society for Bone and Mineral Research (ASBMR).[2]

Radiological healing of AFF after teriparatide: pooled data

We pooled findings on fracture union and teriparatide use in case reports and retrospective studies. Apart from deterioration of incomplete AFFs to complete fractures in two patients[29], no data on radiological healing from the three prospective studies could be used for this analysis, because either the fracture type[27] or time to healing[28,29]could not be established from these publications.

Data on fracture healing of 165 AFFs in 140 patients were pooled in **Table 4**, of which 96% were women.[10,13,15-17,20-26,29-48] Teriparatide treatment was given for 98 AFFs (59%) while 67 AFFs from control groups in the cohort studies (all complete AFFs) did not receive teriparatide. The number of incomplete non-operated AFFs without teriparatide was too small for comparison (n=4) and there were no controls for surgically managed incomplete AFF. Healing of the fracture was achieved within six months of starting teriparatide in 13 (43%) incomplete non-operated AFFs, nine (90%) surgically treated incomplete AFFs and 44 (76%) complete AFFs. In the non-teriparatide treated group, 34 complete AFFs (51%) healed within six months. Complete AFFs appeared to heal faster with teriparatide compared to controls without teriparatide, but in both groups non-healing occurred at 12 months postoperatively in a small portion of patients: five AFFs (9%) in the teriparatide users; and four AFFs (6%) in those without teriparatide. Teriparatide was started in 11 patients because of signs of delayed healing or nonunion, ranging from two months to two years after the initial diagnosis of AFF (n=2 incomplete conservatively managed AFFs, n=9 complete AFFs).[13,16,17,25,30,33,35,38,40,41,43] Sixteen patients with 18 fractures had not

Table 4. Radiological healing of AFF after teriparatide: pooled data.

Fracture healing and teriparatide use *n*=140 patients	Incomplete AFF (conservative)	Incomplete AFF (surgical)	Complete AFF	
	TPT	TPT	TPT	*No TPT*
Number of AFFs (total 165)	*30*	*10*	*58*	*67*
Healing ≤ 6 months of TPT	13 (43%)	9 (90%)	44 (76%)	*34 (51%)*
Healing 6 < or ≥ 12 months of TPT	4 (13%)	1 (10%)	9 (16%)	*29 (43%)*
No union achieved at 12 months	9 (30%)	-	5 (9%)	*4 (6%)*
Progression to complete AFF	4 (13%)	NA	NA	*NA*

NA = not applicable, TPT = teriparatide

Five AFFs that underwent surgical procedures from Takakubo et al. were categorized as complete fractures. In the study by Miyakoshi et al., one non-operated incomplete AFF and one surgically treated incomplete AFF on teriparatide and eight complete AFFs without teriparatide were labeled as healed by the authors between six and 24 months. These fractures were categorized as "healing at 12 months". From the study by Sato et al., only progression to complete AFF in one patient on teriparatide and one without teriparatide could be established, whilst for the other 19 incomplete AFFs the fracture healing was not specified.

Included articles:[10, 13, 15-17, 20-26, 29-38, 40-49]

Excluded: Patients (n=7) without fracture consolidation after ≤ six months of teriparatide use (18, 50, 51) (n=3), (20) (n=3 with surgery after three months), (48) (n=1, case no. 3), fracture healing could not be assessed with certainty (52, 53), duration of fracture healing or fracture type were not reported[14,19,27,28].

discontinued bisphosphonates immediately after the diagnosis of AFF, ranging from three weeks up to one year, including four AFFs in four patients in the teriparatide-treated group (n=2 incomplete conservatively managed AFFs, n=2 complete AFFs) and 12 controls with 14 complete AFFs.[23,24,29,30,44] Progression from incomplete to complete AFFs occurred in four patients after initiation of teriparatide at varying intervals: nine days, two months, eight months, and 21 months.[22,29,47]

Denosumab

Denosumab use and occurrence of AFF

A total of 31 AFFs in 22 patients were reported after the use of denosumab in 14 case reports and two clinical trials. The characteristics of these patients are summarized in **Table 5**. Ethnicity was stated only in three reports, with subjects of a Caucasian (n=1) or Japanese (n=4) background.[49-51] Eleven patients with 15 AFFs were treated for osteoporosis with denosumab 60mg half-yearly[42,51-59], while 16 AFFs in 11 patients have been reported after denosumab treatment with a high dose of 120mg monthly for metastatic bone disease.[49,50,60-63]

Table 5. Occurrence of AFF during or after the use of denosumab.

	Osteoporosis (*n*=11)	Bone metastases (*n*=11)	Overall (*n*=22)
No. of AFFs	15	16	31
Mean age (min-max)	70.7 (59-81)	54.7 (50-86)	62.7 (50-86)
Female (%)	10 (91%)	10 (91%)	20 (91%)
Complete AFFs (%)	11 (73%)	6 (38%)	17 (77%)
Incomplete AFFs (%)	4 (27%)	10 (62%)	14 (64%)
BP use	7 (64%)	7 (64%)	14 (64%)
BP-naive	4 (36%)	4 (36%)	8 (36%)
Mean duration of BP, years (range)	9.0 (5 weeks-15 years)	7.8 (6-11.3)	8.4 (5 weeks – 15 years)
Number of denosumab doses, mean (range)	3.2 of 60mg half-yearly (1-14)	30 of 120mg monthly (18-48)	-
Accumulative dose, mg/year	120	1440	-
Number of denosumab doses in BP-naïve patients, mean (range)	5.8 (1-14)	29 (21-42)	-

BP = bisphosphonate. Parameters are based on the time of the first AFF. Mean duration of bisphosphonates was calculated in bisphosphonate-users only. Incomplete fractures with progression to complete fractures were excluded from the number of incomplete AFFs. Denosumab was dosed 120mg monthly in oncological patients and 60mg six-monthly in osteoporosis patients. Missing data: age (n=2)[63], mean duration of bisphosphonates (n=3)[55], median no. of denosumab doses (n=3) [55]. Included articles: [8, 42, 54-68]

AFF occurred in eight patients without prior bisphosphonate use[8,51,58-60,62,63] of which four were in patients treated in an oncology setting[60,62,63], meaning that only four cases were documented of AFF after use of denosumab for management of osteoporosis.[8,51,58,59]

Two bisphosphonate-naïve individuals developed an AFF following the sixth and the fourteenth dose of denosumab in the FREEDOM-trial, a phase III clinical trial with denosumab in 4,550 women with osteoporosis.[58,59] The first patient stopped denosumab and achieved fracture healing within six months, whilst the latter continued denosumab but no data on the healing of AFF are available in this case (personal communication by Amgen). One 60-year-old male who had been on glucocorticoids for asthma for over 30 years developed an AFF without any previous bisphosphonate use, two months after the second dose of denosumab that was given in a randomized controlled trial of denosumab in patients with glucocorticoid-induced osteoporosis.[8] The fourth case without bisphosphonate-exposure concerns an incomplete, medially located AFF after only one injection of denosumab without abnormalities on X-ray but with periosteal reaction on the MRI scan.[51] Although stress fractures resembling AFF located on the medial instead of the lateral cortex have been described[64], this case does not meet the diagnostic criteria of AFF according to the ASBMR Task Force.[2] The four bisphosphonate-naïve AFF cases treated for metastatic bone disease occurred after 21, 24 or 42 doses of 120 mg denosumab monthly.[60,62,63]

In two other cases, the influence of bisphosphonates on the risk of AFF cannot be excluded, but AFF was preceded by very short bisphosphonate treatment before starting denosumab.[52,54] These two cases are very similar, since both patients had used alendronate for just a few weeks before switching to strontium ranelate because of side effects, which was subsequently replaced by denosumab, again because of intolerance to the drug. Both patients developed an AFF after three doses of denosumab.[52,54]

These reports of AFF after denosumab with minimal or no previous bisphosphonate use are suggestive of a role for denosumab in the development of AFF but the numbers are small and AFFs have also been reported rarely in patients never treated for osteoporosis.[4,65,66] In another report, the AFF appeared to be triggered by one dose of denosumab in December 2012, after five years of alendronate use between 1994-1999[56] followed by a subsequent drug holiday for 13 years.

Denosumab use after AFF

We found seven papers that report on the use of denosumab after an AFF in 10 patients.[17,44,57,67-70]

Bisphosphonates switched to denosumab treatment

Seven patients switched from bisphosphonates to denosumab just before or after the first AFF. One patient with an incomplete AFF after four years of risedronate who underwent preventive placement of an intramedullary gamma-nail, was switched to denosumab and had delayed healing after six and 12 months.[67]

In a case series of complete AFFs associated with alendronate use[68], four patients started denosumab after the first AFF. There were four different outcomes. One patient had delayed fracture healing at 12 months but with minimal pain and almost the same activity level. One patient had a second complete AFF on the contralateral side one year after switching to denosumab; this contralateral AFF showed bridging callus formation at nine months' follow-up. One patient had bridging callus formation at 12 months and was pain-free. One patient had resumed normal daily activities at 18 months of follow-up and radiographs showed bone healing.[68]

In a case report one patient, who sustained a first complete AFF after one dose of denosumab and eight years of alendronate[57], continued denosumab treatment but sustained a second complete AFF after three more doses of denosumab. The authors describe healing of both AFFs within five months postoperatively.

Another case is described of denosumab started postoperatively for complete AFF with full weight-bearing after three months and no adverse events at 18 months of follow-up; complete bony union was achieved at one year postoperatively.[69]

Teriparatide switched to denosumab treatment

Three cases are reported of denosumab therapy following teriparatide. One case involved bilateral incomplete AFFs without visible fracture lines after seven years of oral bisphosphonates who was treated with teriparatide for 18 months and a subsequent drug holiday of 12 months.[70] The cortical thickening had almost completely flattened on X-rays when denosumab was prescribed as treatment for low bone mineral density (BMD). The authors report that the patient had increasing thigh pain in both upper legs six months after the first dose of denosumab and that X-rays and bone scintigraphy showed recurrent incomplete bilateral AFFs with presence of a lucent line after which the surgeon decided to perform bilateral internal fixation.[70] Two case reports (one with incomplete AFF and one with complete AFF) mention that the initiation of denosumab therapy had a good outcome in the short term (< one year).[17,44]

Raloxifene

Raloxifene use and occurrence of AFF

Six papers[28,48,71-74] stated the use of raloxifene prior to the diagnosis of AFF in eight patients, although in four patients it was unclear whether this was preceded by bisphosphonate treatment.[73,74] Two patients had simultaneous use of raloxifene and bisphosphonates during six months and six years, respectively.[48,71] One had had prior bisphosphonate use.[28] In a case series of surgically treated AFFs from Japan[72], a patient treated with raloxifene only was reported. This concerned a 77-year-old woman who had taken raloxifene and vitamin K2 for only one year when she sustained an AFF after a fall from standing height. Because delayed union was suspected, she received low-intensity pulsed ultrasonography three months postoperatively and partial fracture healing was seen nine months after the surgery.[72]

Raloxifene use after AFF

We found reports of two patients treated with raloxifene after AFF, in both cases after teriparatide treatment.[36,45] One 63-year-old Asian woman received ten months of teriparatide after incomplete AFF with a visible fracture line, which was subsequently replaced by raloxifene. The fracture line had already diminished after three months of teriparatide and was invisible 15 months after the diagnosis, which was five months after starting raloxifene.[36] One 78-year-old woman with incomplete AFF with a lucent line received teriparatide; the fracture line had almost disappeared three months postoperatively. After 12 months of teriparatide, she switched to a SERM, most likely raloxifene, and had an event-free follow-up three years after the diagnosis.[45]

Romosozumab

Twelve studies have been performed with romosozumab. Two studies reported three cases of AFF. One case of AFF occurred 3.5 months after the first monthly dose in a phase III clinical trial[75], but the association between romosozumab and the AFF is questionable given that the participant had complained of prodromal pain prior to the first romosozumab administration. Two cases of AFFs that occurred during open-label alendronate treatment after one year of monthly romosozumab in another trial.[76]

Abaloparatide

A a total of nine clinical trials with abaloparatide were published. No cases of AFF were reported in patients who used or had used abaloparatide.

DISCUSSION

In clinical practice there is great uncertainty of how to treat patients after they have sustained an AFF. This relates both to potential (positive or negative) effects of bone agents on the healing of the fracture and to the safety of osteoporosis drugs in those patients, who are still at high risk of fragility fracture after an AFF. Bisphosphonates are usually stopped, because patients are considered at risk of an AFF of the other femur since bilaterality is commonly reported, varying from 28% up to 44%.[1,6]

In this systematic literature review, we aimed to assess the effects of teriparatide, denosumab, raloxifene, romosozumab and abaloparatide on both the occurrence and healing of AFF in order to give recommendations for medical management. It is difficult to draw firm conclusions, because there are no reported RCTs of treatment in AFF patients with any of these drugs.

Based on descriptions of 165 AFFs treated with teriparatide in observational studies, we made a crude estimate of effects of teriparatide on radiological healing of AFF after six and 12 months. The majority of surgically treated incomplete (n=9, 90%) and complete AFFs (n=44, 76%) healed within six months of teriparatide treatment, in contrast non-operated incomplete fractures treated with teriparatide (n=13, 43%) and complete AFFs that were not treated with teriparatide (n=34, 51%). The reported data are insufficient for an evidence-based recommendation of the use of teriparatide to accelerate healing of AFF. Yet, keeping in mind the flawed study designs and heterogeneity between studies, the observational data might suggest that teriparatide could have a beneficial effect on the healing time of surgically treated AFF, although non-union after one year can still occur. There is no evidence of improved fracture healing for conservatively managed incomplete AFFs based on these observational data. Our findings clearly show that even during and after teriparatide treatment a new AFF can occur, either as a first presentation of AFF or as a second AFF of the contralateral femur, but only in patients previously treated with bisphosphonates.

The role of teriparatide for healing of any type of fracture is debated. One meta-analysis of five RCTs in patients with osteoporotic fractures found a significantly shorter healing time in the teriparatide-treated group[77], whilst another analysis including also non-osteoporotic fractures did not demonstrate any effectiveness for teriparatide with regard to faster union.[78] Two RCTs involved subjects with femoral fractures. In one trial with postmenopausal women and low-trauma femoral neck fractures, teriparatide did not improve radiological fracture healing, but the sample size was too small to detect any differences.[79] The other RCT involved premenopausal women with acute stress fractures

of the lower extremities and showed a tendency towards improved healing on MRI in the teriparatide group (83.3%) in comparison to controls (57.1%), but not statistically significant (p=0.18).[80]

There are no documented cases of AFF with the use of abaloparatide. This drug might have equivalent effects on AFF as teriparatide given the biological similarity. The results from the literature search were insufficient to assess the effects on AFF healing by denosumab and raloxifene. Despite the lack of epidemiological studies, our analysis of the literature suggests that the absolute risk of AFF when using denosumab or raloxifene for osteoporosis is very low given the limited reports of AFF cases using these drugs, eleven and eight patients respectively, and they also mostly occurred after previous use of bisphosphonates. However, this risk may be increased in patients who have already had an AFF suggested by the reports of two patients with a second complete AFF[57,68] on denosumab and in another patient with bilateral recurrent incomplete AFFs on denosumab even after use of teriparatide.[70] These cases suggest curtailing use of denosumab treatment after an initial unilateral AFF. Romosozumab is linked to three AFF cases in clinical trials, but it remains to be seen if more cases of AFF will develop in patients treated with romosozumab with or without bisphosphonate exposure.

Based on our findings we conclude that there is a clear need for randomized controlled clinical trials to evaluate whether teriparatide and/or abaloparatide enhances fracture union of (any type of) AFF, since this is the only drug that is not associated with the development of AFF without prior use of bisphosphonates. The observational studies in this review are biased and lack information on confounding factors such as time between diagnosis and starting medical treatment, surgical fixation techniques, smoking, body mass index, fracture localization, use of concomitant medication and postoperative weight-bearing protocols. Currently, one clinical trial is ongoing for patients with incomplete AFF who are randomized to receive either placebo injections or teriparatide. Changes in pain score and physical function using the WOMAC scale and the proportion of participants requiring surgery after 12 months serve as primary outcomes.[81] There are no trials registered investigating teriparatide for complete AFF, non-healing AFF or electively operated incomplete AFF. Also no trials are currently evaluating the risks and benefits of antiresorptive therapy compared with placebo in AFF patients after stopping teriparatide or in AFF patients managed conservatively or surgically. It is difficult to set up an adequately powered study because of the low incidence of AFF. Therefore, an international registry of AFF cases could be very useful to gain insight into the safety and efficacy of osteoporosis drugs in relation to fracture healing, bone mineral density and bone turnover and development of new AFFs in these patients, but this is only possible when AFF patients are referred to specialized centers.

Recommendations for clinical practice based on expert opinion

Based on the results in this review and our expert opinion we advise on medical treatment for patients with AFF. Our recommendations for medical treatment are summarized in a decision tree (**Figure 2)**, encompassing the occurrence of AFF when using bisphosphonates or denosumab and what to do after a patient with AFF has completed a two-year course of teriparatide. In any case, extensive monitoring with imaging of both upper legs is advised during the first one or two years after the diagnosis of AFF, because non-healing of AFF and contralateral AFF may still occur, even on teriparatide.

When AFF is diagnosed during the use of bisphosphonates or denosumab, it is recommended to stop this treatment, since continuation may lead to worsening of the AFF or a new contralateral AFF. To prevent a rebound effect, discontinuation of denosumab could be followed by a short course of bisphosphonates or SERMs in patients with surgically treated AFFs. In patients at low fracture risk without prevalent vertebral fractures who have only had one or two half-yearly injections, consider stopping denosumab treatment without subsequent therapy. After healing of bilateral, surgically managed AFFs, bisphosphonates or denosumab may be continued. It should be kept in mind that discontinuation after three or more years of bisphosphonate treatment may result in increased risk of hip fractures and clinical vertebral fractures as shown by some studies[82,83], although this was not found in another recent retrospective analysis of a population-based cohort.[84] Continuation of bisphosphonates might lead to a risk of atypical fractures at skeletal sites other than the femur. Anecdotally, spontaneous fractures of other long bones e.g. ulna, forearm and tibia have been reported in relation to bisphosphonate use[85-92], but no association has been established and the potential risk of such atypical fractures does not appear to weigh against the risk of typical osteoporotic fractures.

Teriparatide might be started for surgically treated AFFs, although strong evidence for improved fracture union is lacking. Further, teriparatide, SERMs, romosozumab or abaloparatide may alternatively be considered in patients at high risk of fragility fractures. SERMs are preferably prescribed in relatively young postmenopausal women who are at low risk of hip fractures and deep vein thrombosis.[93] Hormone replacement therapy or tibolone might be considered when SERMs are not tolerated, preferably in younger women (< 65 years) who do not have an increased risk of venous thromboembolism, without a history of myocardial infarction or stroke and also keeping in mind the increased breast cancer risk.[93] If the patient is not eligible for any of the aforementioned drugs, calcitonin can be prescribed as in accordance with the recent guideline of the Endocrine Society on pharmacological management of osteoporosis.[93] The definition of high risk of fragility fractures varies across countries, but is often defined by a hip BMD

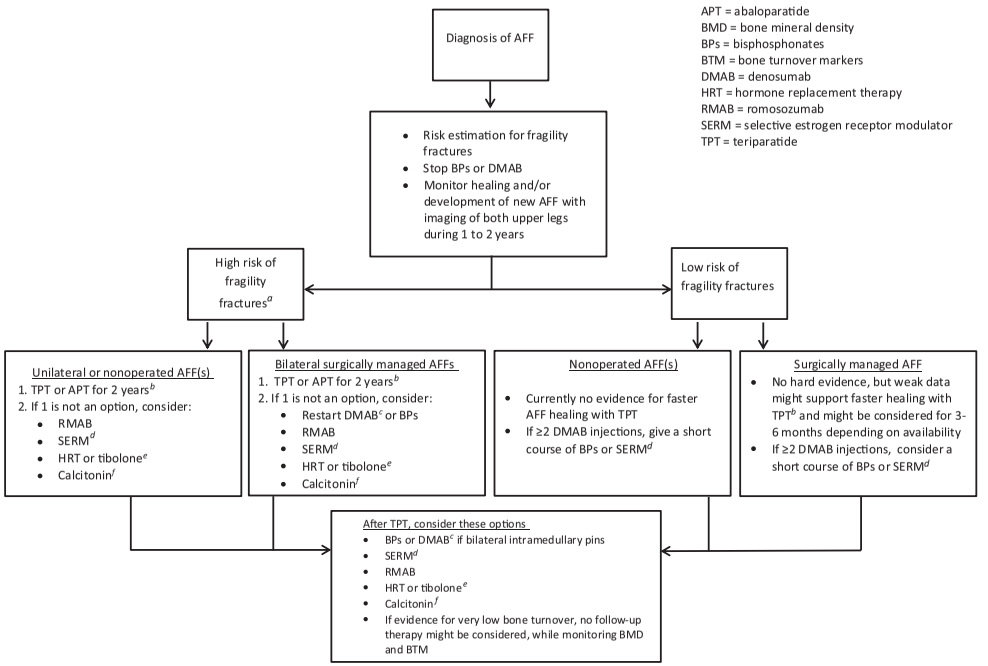

Figure 2. Decision tree with considerations for medical management after AFF.

^ Definition may vary across countries, e.g. a hip BMD T-score ≤ -2.5 SD, older age (70-75 years), a recent fragility fracture, other strong risk factors for fracture or a FRAX fracture risk score that is above country specific thresholds[94].

**Raloxifene or bazedoxifene are preferably prescribed in relatively young postmenopausal women who are at low risk of hip fractures and deep vein thrombosis[93], or in women in whom the use of teriparatide is contra-indicated.*

#In case of intolerance to SERMs, hormone replacement therapy or tibolone could be considered in women with a low risk of deep vein thrombosis and breast cancer, without a history of myocardial infarction or stroke[93].

+ Switching denosumab to teriparatide may result in progressive BMD loss.

0 Be aware that antiresorptive therapy may be needed after stopping denosumab.

^A Calcitonin can be prescribed in patients who are not eligible for bisphosphonates, SERMs, hormone replacement therapy, tibolone, abaloparatide or teriparatide.

T-score ≤ -2.5 SD, older age (70-75 years), a recent fragility fracture, other strong risk factors for fracture or a FRAX fracture risk score that is above country specific thresholds.[94]

After two years of teriparatide, subsequent therapy may be given with raloxifene (or hormone replacement therapy) in women and – in those with bilateral surgical fixation of AFF – denosumab or bisphosphonates. In patients at the end of a (short) course of teriparatide who have low bone turnover markers after teriparatide or who are deemed to be at low risk of osteoporotic fractures teriparatide may be discontinued without further antiresorptive treatment, but close monitoring of BMD and bone turnover markers is recommended.

The considerations for each individual drug are given in more detail below.

Teriparatide

There is no evidence-based indication for teriparatide to enhance healing of AFF, but a tendency towards faster healing with teriparatide for surgically managed AFFs is seen in the limited, observational data. Hence teriparatide 20ug daily, when reimbursed, might be considered for surgically-treated AFF, both incomplete AFF and complete AFF. Even during the use of teriparatide, non-unions do still occur in surgically managed AFF. The limited data on conservatively managed incomplete forms of AFF and use of teriparatide, do not demonstrate improved fracture healing, but should be interpreted with caution, pending the result of an RCT that is awaiting results. When teriparatide is given for the sole purpose to enhance fracture healing of AFF, a short treatment duration of three to six months may suffice.

Teriparatide is a reasonable treatment option for patients who have had an AFF and are still at high risk for fragility fractures. A big clinical dilemma is what to do after a full two-year course of teriparatide treatment. Normally, antiresorptive therapy is advised after two years of teriparatide, because the positive effects on bone mass and strength will in time disappear, as with any drug without skeletal retention. Some patients with AFF may have inherent low bone turnover, for example due to an underlying monogenetic disease[95] or due to previous long-term use of bisphosphonates. It can be speculated that accelerated bone loss after cessation of teriparatide may not occur in these cases. A few studies describe the effect of teriparatide on bone turnover in AFF patients, but the results are inconclusive. Administration of teriparatide during six months has been associated with a significant increase in bone turnover markers in patients with AFF[27,29] and values returned almost to baseline level after two years of teriparatide[29], but pre-treatment values varied widely[29,96] and bone turnover markers did not correlate with

histomorphometric findings from bone biopsies before and after teriparatide treatment in AFF patients.[96]

We suggest monitoring bone turnover markers on a regular basis in patients with AFF before, during and after teriparatide treatment and considering antiresorptive drugs when levels start to increase or when BMD starts to decrease in patients at high risk of fractures. In this situation, we suggest either a SERM, romosozumab, calcitonin, tibolone, estrogens, denosumab or bisphosphonates, based on sex and on bilaterality of surgical intervention (see below).

Denosumab

When a patient sustains an AFF during the use of denosumab, the risk of a rebound effect with rapid loss of BMD and potential risk of multiple vertebral fractures following cessation of denosumab[97] must be weighed against the potentially increased risk of a contralateral AFF when continuing denosumab. Patients who have already had vertebral fractures appear to be at greatest risk of developing multiple vertebral fractures after denosumab discontinuation.

In general, a course of bisphosphonates is recommended after stopping denosumab.[97] This is not advisable for a conservatively managed incomplete AFF, but a short course of a SERM or bisphosphonates may be considered in patients with bilateral surgically treated AFFs or a unilateral surgically treated AFF without any radiological signs of incomplete AFF of the contralateral femur. Denosumab could be stopped without follow-up therapy in patients at low risk of fragility fractures without prevalent vertebral fractures, especially in those who have only had one or two half-yearly injections of 60 mg subcutaneously.

For patients at high risk of fragility fractures, a switch to teriparatide or a SERM could be considered. However, the rebound effect after stopping denosumab might still occur since teriparatide increases bone turnover. One should also be aware of a decrease in BMD especially at cortical sites, as was seen in osteoporotic women who transitioned to teriparatide after two years of denosumab in the DATA-switch study.[98] Alternatively, hormone replacement therapy or tibolone can be considered in women in absence of contra-indications such as a high risk of breast cancer of deep veen thrombosis, history of stroke or myocardial infarction. Calcitonin is an option if the patient does not tolerate any of the aforementioned drugs.[93]

Denosumab could be continued or initiated when the patient has bilateral surgically treated AFFs and a persistently high risk of fragility fractures, including those who have

completed two years of teriparatide. Denosumab therapy for up to 10 years has been associated with increasing BMD and low fracture incidence.[58] Long-term use of denosumab could especially be considered in elderly patients with a life expectancy of less than 10 years, for whom this may serve as life-long osteoporosis treatment.

Raloxifene

Raloxifene could be considered as follow-up therapy after teriparatide when bone turnover markers are high in postmenopausal women who do not have a history of venous thrombo-embolic events. Preferably it is given to women who are relatively young and are at lower risk of hip fractures. As mentioned above, it could also be considered in patients who have to stop denosumab because they are at risk of another AFF and to potentially prevent the rebound in bone turnover and risk of multiple vertebral fractures, especially when they have already had vertebral fractures. However, no studies have been performed using SERMs to prevent rebound after stopping denosumab. Because it has a weaker antiresorptive effect than bisphosphonates or denosumab and few cases of AFF have been reported on raloxifene, this may be a preferred option after teriparatide.[99,100] Yet it should be kept in mind that raloxifene is not regularly prescribed for osteoporosis, hence a low number of AFF associated with raloxifene does not guarantee a lower risk of AFF compared to other antiresorptive drugs.

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SUPPLEMENT 1

Detailed study descriptions of case reports, retrospective cohort studies and prospective studies on teriparatide use and AFF.

IA. Case reports: incomplete AFF and use of teriparatide

In 13 patients with incomplete AFFs the course of radiological healing was described after or during teriparatide use.

Radiological healing within one year of teriparatide use was reported of nine patients with 11 incomplete AFFs. These included four patients with five incomplete AFFs who underwent prophylactic surgery[1-3] and five patients with six incomplete AFFs that were treated conservatively with teriparatide only.[4-8]

The surgically treated cases compromised three patients who started immediately after the prophylactic surgery[1,2] and one patient who started teriparatide five months postoperatively because of delayed healing of a contralateral complete AFF.[3] The latter case by Fukuda *et al.* showed complete union of both AFFs after three months of teriparatide use. Tsuchie *et al.* reported two patients who were pain-free within two to three weeks following the surgery and a fracture line that was almost invisible within three to six months postoperatively in three incomplete AFFs.[1] Chew *et al.* described a case with good callus formation in a patient who was pain-free two months postoperatively.[2]

In three of six incomplete AFFs treated conservatively a fracture line was visible. In one case, the lucent line had completely disappeared within six months.[5] In two cases, the fracture line and pain diminished after starting teriparatide, but the lucent line was only completely closed after 12 months of teriparatide use.[7,8] This included a case of a 63-year-old man with osteogenesis imperfecta who refused any medical or surgical treatment during two years before commencing teriparatide.[8]

The remaining three incomplete AFFs without a fracture line concerned one patient with bilateral focal thickening that had almost completely flattened after 18 months of teriparatide (although the incomplete AFFs recurred on bone scan and X-rays after denosumab 1.5 years later)[4], whilst in another patient focal cortical thickening was reduced according to the authors[6] from 12.2mm to 11.3mm after two years of teriparatide. However, it is unclear in both cases whether this flattening is a significant decrease, or if this could also be explained by variation in positioning and measurement on the X-ray, or if it could simply reflect the natural healing of incomplete AFF over time rather than a healing effect of teriparatide itself.

One patient with bilateral incomplete AFFs with radiolucent lines was electively operated because of persistent hotspots on bone scintigraphy and a waddling gait despite six months of teriparatide treatment.[9] After one year callus formation was visible on X-rays. In this 67-year-old Armenian woman genetic testing revealed hypophosphatasia, after she had been treated with alendronate for 10 years.

Persistent non-union after more than one year was found in two patients with conservatively managed AFFs and a visible fracture line.[10,11] One case reported that it took 15 months before the fracture line completely disappeared even though teriparatide was started one month after the diagnosis, but it is noteworthy that the pain did improve within the first months of teriparatide and the patient was able to walk after 10 months.[10] In another case of bilateral incomplete AFFs, full healing was seen only 22 months after the diagnosis on MRI scan despite the use of teriparatide 20ug daily during the past 19 months.[11]

Progression to complete AFF was reported in one patient with bilateral incomplete AFFs with radiolucent lines whilst on teriparatide for two months and the subsequent fracture healing of the operated femur was delayed up to 18 months.[12]

IB. Case reports: complete AFF and teriparatide use

In 17 patients with 20 complete AFFs the radiological healing was reported after or during the use of teriparatide.

Fifteen patients with 18 complete AFFs reached fracture healing within one year of teriparatide. In 13 patients with 15 complete AFFs full fracture union was achieved within six months[3,5,13-23], and full consolidation was reported after one year in two patients with three complete AFFs.[24,25] Duration of teriparatide use varied from two months [16] up to two years, whilst in other cases the duration was not specified. In nine patients teriparatide was started within two months after the first surgery, whilst six patients were prescribed teriparatide because of slow fracture healing[3,17-20], starting 12 months postoperatively at the latest. This includes one case[17] in which bisphosphonate treatment was continued during the first year after the occurrence of AFF before switching to teriparatide. There was one report of teriparatide use in a patient with metastatic bone disease.[21] The first complete AFF was presumed to be pathological fracture and bisphosphonates were continued until she sustained a contralateral complete AFF two year later, after which teriparatide was then started with successful healing within five months and a bone biopsy from the fracture site did not show malignancy.[21]
Three patients with unilateral AFF did not achieve fracture healing whilst on teriparatide.[12,26,27] One patient had non-union regardless of 24 months teriparatide. Consolida-

tion did occur after replacement of the intramedullary nail and switching to strontium ranelate.[26] The second case was prescribed teriparatide seven months postoperatively because of delayed healing, but still had non-union after a full treatment course of 24 months.[27] The AFF only healed after re-operation to replace the plate and sliding screw with an intramedullary gamma-nail. The third case concerned an incomplete AFF that had progressed to a complete fracture whilst using teriparatide for two months and postoperatively the healing process was delayed up to 18 months.[12]

Three other patients with unilateral AFF were reported with signs of abnormal fracture healing; however, follow-up was limited to four or five months of teriparatide and thus the status of fracture healing at six months could not be determined.[24,28,29] These included a patient who had insufficient callus bridging six months after the diagnosis following five months of teriparatide.[28] A second patient with a history of osteonecrosis of the jaw, started using teriparatide eight months postoperatively because of delayed healing of AFF, without any effect after five months of teriparatide.[29] In the third patient, no effect was seen of four months teriparatide which was initiated 18 months postoperatively due to delayed healing.[24]

IC. Case reports: occurrence of new AFF during or after teriparatide

Four patients have been newly diagnosed with five AFFs after teriparatide use.[22,23,30,31]

One patient with a complete AFF who initially had a normal femoral cortex of the contralateral femur on X-ray, was newly diagnosed with a contralateral incomplete AFF after 18 months of postoperative teriparatide treatment and four months of strontium ranelate.[22] In the second case, a contralateral incomplete AFF was diagnosed 22 months after the first complete AFF and during the eleventh month of teriparatide use, which was prescribed due to high fracture risk.[23] Thirdly, a patient was reported with a newly diagnosed incomplete AFF that required immediate surgery whilst on teriparatide treatment since four months, which was initiated because of a non-healing metatarsal fracture.[30] This patient had been treated with bisphosphonates for over a decade and (short-term) teriparatide use did not seem to prevent a pending complete fracture in this case.

The fourth case was a first presentation of simultaneous complete AFF and contralateral incomplete AFF two years after discontinuation of teriparatide without any antiresorptive use in the meantime; although this patient had prior exposure to antiresorptives for eight years in the past.[31] This indicates that even anabolic drug agents and a drug holiday of several years from bisphosphonates does not protect from developing a new AFF.

II. Retrospective cohort studies on AFF and use of teriparatide

Nine retrospective cohorts described use of teriparatide in patients with AFF. Five cohorts involved incomplete AFFs only[32-36], three cohorts described complete AFFs only[37-39] and one cohort included both incomplete and complete AFFs.[40]

Lee *et al.* (2013) published a retrospective cohort of 51 patients with 65 incomplete AFFs from four hospitals in South Korea with a minimum follow-up period of 12 months.[32] After the diagnosis, limited weight-bearing was advised, but surgery was performed in those with intractable pain or when complete fracture occurred. In 26 patients with 31 incomplete AFFs (47.6%), surgery was required at the mean of 9.4 months (range 1-26 months). Surgery was required in 17 AFFs for intractable pain and in 14 AFFs for completion of the fracture. Teriparatide was used by 19 patients with 22 incomplete AFFs for a mean of 4.6 months, ranging from one to 10 months. Teriparatide was given in seven patients that required surgery and in 12 patients that did not undergo surgery. Use of teriparatide did not significantly reduce the need for surgery ($p = 0.210$), although the subtrochanteric fracture location was associated with requirement for fixation rather than diaphyseal localization ($p=0.018$). This study does not report on duration of fracture healing and could therefore not be included in the pooled data shown in **Table 4**. The number of patients on teriparatide with elective surgery and those with surgery after completion of AFF and the presence of radiolucent lines in incomplete AFFs are not mentioned in the article.

Saleh *et al.* reported 10 women with 14 incomplete AFFs.[33]

Five AFFs in four patients did not show a fracture line but did have periosteal and bone marrow edema visible on MRI scan; these AFFs were conservatively treated with partial weight bearing and teriparatide for two years. None had progression to a complete AFF and all patients reported improvement in pain and function. The focal cortical thickening on the X-ray remained unchanged, but the MRI scans showed complete resolution of edema after three months in all five cases.

Nine AFFs in the remaining six patients showed a radiolucent line. These patients were given a trial of teriparatide for three months, except for one AFF that was treated with miacalcin (salmon calcitonin) instead because this patient had received radiation on the skeleton in the past. Two AFFs in two patients had healed radiographically and clinically within three months of teriparatide use, but preventive surgery was performed in the remaining seven AFFs because of persistent pain and a radiolucent line at three-month follow-up. For the summary in **Table 4**, it was extracted from this paper that seven

non-operated incomplete AFFs in six patients healed within six months on teriparatide treatment.

Petraszko *et al.* presented a retrospective imaging study comparing tomosynthesis and X-ray to detect fracture lines in eight patients with localized cortical thickening of 10 femora.[34] A fracture line was visible using one or both techniques in all patients except for one 61-year-old-man who turned out to have sustained a trauma of the upper leg and had never used bisphosphonates. The authors stated that the focal cortical thickening was due to a chronic ossification of a subperiosteal hematoma in this case. The remaining seven patients were all women on prolonged bisphosphonate use over seven years, of whom six started on teriparatide and one underwent prophylactic surgery without teriparatide administration. Three women had prodromal thigh pain. During a follow-up period of three to almost five years, none of the patients treated conservatively had completion of the fracture. The fracture line disappeared in two patients with teriparatide after six and 12 months.

Sato *et al.* reported 12 Japanese women with focal thickening of 20 femora without visible fracture lines.[35] Incomplete AFF was defined as development of a fracture line penetrating from the tip of the localized bone reaction on X-ray, without displacement of the femur. The authors retrospectively compared the shape of the focal cortical thickening in a group of seven patients that immediately discontinued bisphosphonates after the diagnosis to five patients who continued on bisphosphonate therapy for two years. All five patients on continued use of bisphosphonates deteriorated: one had a complete AFF after 18 months, one patient developed a visible fracture line, two patients had de novo contralateral beaking and one had increased height of the focal cortical thickening. When bisphosphonates were discontinued two years later, two of five patients were prescribed teriparatide treatment, whilst in seven patients who discontinued immediately, four were switched to teriparatide treatment. Of these six patients on teriparatide, one patient who was discontinued on bisphosphonates immediately after focal cortical thickening was established, had progression to a complete AFF following eight months of teriparatide. In all other teriparatide users the focal cortical thickening improved or remained unchanged. The decision to use teriparatide depended on the attending physician. For the pooled data in **Table 4**, only progression to complete AFF in one patient on teriparatide could be established, whilst for the other incomplete AFFs the fracture healing was not specified.

In a conference abstract by Cheung *et al.* a cohort was reported of 22 postmenopausal women with 27 incomplete AFFs treated with teriparatide.[36] Four patients underwent prophylactic surgery. Of the remaining 19 patients on conservative management, fol-

low-up imaging was available for 15 patients with 19 incomplete AFFs. Every six months for up to two years, the depth of the fracture line through the cortex was measured on conventional radiographs and CT scans during teriparatide therapy. Of these fractures, two healed during teriparatide, five were consolidating, 12 remained unchanged and none had progressed to a complete fracture at last follow-up. However, four patients developed new fracture lines in the same femur of the original incomplete AFF despite teriparatide treatment. It is not stated at what time-points the healing occurred and this cohort is therefore not included in the summary of data in **Table 4**.

Miyakoshi *et al.* reported a cohort of 45 AFFs in 34 Japanese women.[40] Teriparatide was given in 11 complete AFFs and 10 incomplete AFFs, whilst 21 complete AFFs and 3 incomplete AFFs were not treated with teriparatide. Eight incomplete AFFs (18%) did not require surgery; in all other AFFs surgical intervention was performed. All patients were followed up for an interval of one to three months until fracture union was observed. Fracture union following incomplete AFFs was defined as the disappearance of the fracture line. Normal union was defined as fracture healing within six months. Delayed union was defined as fracture healing after more than six months but within two years. Non-union was defined as a fracture that did not achieve union or showed pseudo-joint during final follow-up visit or after more than two years.

In the teriparatide group, 11 complete AFFs (100%) and eight incomplete AFFs (80%) of whom half underwent surgery, healed within six months; two incomplete AFFs showed delayed healing including one case with surgical management. In the non-teriparatide group considering the complete AFFs only, 12 healed within six months, eight showed delayed healing and one case had nonunion. Regarding the incomplete AFFs in the non-teriparatide group, two achieved union within six months and one had delayed fracture healing, all on conservative management. Taking into account surgically managed AFFs only (21 in the non-teriparatide versus 11 in the teriparatide group), time to fracture healing was significantly shorter in the teriparatide-treated group (5.4 months vs. 8.6 months; p=0.012) and the frequency of delayed healing or non-union was significantly lower in teriparatide-treated group (p = 0.014). Sub-analysis for the conservatively treated AFFs showed no significant differences between groups, but these compromised only three AFFs in the non-teriparatide group and five in the teriparatide-group. We categorized ten AFFs (n=8 complete AFF without teriparatide, n=1 non-operated incomplete AFF with teriparatide and n=1 surgically treated incomplete AFF with teriparatide) that healed between six to 24 months as “healed at 12 months” in **Table 4**.

Takakubo *et al.* reported 11 AFFs in eight women with rheumatic disease who all underwent surgical procedures and had a postoperative follow-up of at least one year.[37]

The authors do not specificy whether these AFFs were complete or incomplete. For the combined data in **Table 4**, these AFFs were categorized as complete AFFs.

Five AFFs were treated with a combination of teriparatide and low-intensity pulsed ultrasonography (LIPUS), three AFFs with LIPUS only, and three contralateral AFFs without administration of teriparatide or LIPUS. The mean duration of healing of five AFFs on teriparatide was 11.5 months and 13.3 months in the six AFFs without teriparatide. Of the teriparatide-treated group, one AFF healed at six months, two at one year, one at 16 months, and one did not achieve fracture healing at one year but was then lost to follow-up. In the LIPUS-only group, fracture healing of three AFFs was achieved at 12, 13 and 24 months, respectively (mean 16.3 months). In the AFF without teriparatide and without LIPUS, healing was achieved at nine, 10 and 12 months, respectively (10.3 months).

In four AFFs, bisphosphonates were not immediately discontinued after the occurrence of AFF, one in the teriparatide-treated group, one in the LIPUS group, and two of those with no additional therapy after the surgery. These four AFFs did heal within 10 to 13 months.

Yeh *et al.* retrospectively reviewed 13 Taiwanese women with 16 complete AFFs of which eight were treated with teriparatide for at least six months.[39] They all underwent internal fixation with intramedullary osteosynthesis. One patient had initially refused teriparatide but did start treatment soon after a contralateral AFF and delayed union of the first AFF. Six teriparatide-treated AFFs were completely healed at six months follow-up, versus four non-teriparatide treated AFFs. The remaining AFFs concerned five AFFs with slower fracture healing but good consolidation at nine months' follow-up and one case of implant failure in the non-teriparatide group, a 61-year-old female who needed two revision surgeries thereafter. The average time to union was 4.4 months in the teriparatide-treated group versus 6.2 months in the non-teriparatide group (p=0.116). The authors reported a significantly better functional outcome and lower pain scores at six months postoperatively in the teriparatide-group, but at three and 12 months there were no significant differences between groups.

Lee *et al.* (2017) described a cohort from seven hospitals in South Korea of 44 women with 46 complete AFFs with a minimum follow-up period of six months or until fracture union was achieved.[38] All patients underwent intramedullary nailing. Fracture union was defined as callus bridging of three or four cortices. When no radiographic evidence of union was seen within six months, this was labeled as delayed healing. Fixation failure was defined as absence of complete bony union one year following surgery or implant failure during the follow-up period. Three groups were distinguished: 20 patients who

discontinued on bisphosphonates (21 AFFs), 10 patients who continued bisphosphonates (11 AFFs) and 14 patients who started teriparatide (14 AFFs). In the teriparatide-treated group 11 AFFs healed within six months (78.5%), compared to 13 in the discontinued group (61.9%), and 5 (45.5%) in the group that continued on bisphosphonates. Eventually, in 44 of 46 AFFs complete bony union was achieved without further surgical intervention within one year. Two AFFs needed revision surgery due to non-healing, but it is not stated whether these patients had discontinued bisphosphonates or had used teriparatide. There were no significant differences in time to union among these three groups (p=0.08), although a more favorable trend was seen in the teriparatide group (19.7 weeks), compared to the groups that discontinued bisphosphonates (26.5 weeks) and those on continued use of bisphosphonates (28.4 weeks).

III. Prospective studies on AFF and use of teriparatide

Chiang *et al.* published a prospective study in 13 women and one man with a median age of 76 years, including six cases of complete AFF and eight cases of incomplete AFF.[41] Incomplete AFF was diagnosed when a fracture line was visible or abnormalities on MRI or technetium scans were suggestive of a stress fracture in the lateral cortex. Five patients started on teriparatide 20ug daily for six months, including four patients with incomplete AFFs with ongoing pain and a persistent fracture line since eight to 12 months after the diagnosis and one patient following completion of the AFF. The remaining nine patients (five with incomplete AFFs and four with complete AFFs) were not prescribed teriparatide due to medical contra-indications or refusal to self-inject.

In the teriparatide-treated group, two patients had complete healing and three had partial healing. Two patients were pain-free and three had improved pain scores. In the non-teriparatide group, three patients needed prophylactic surgery of whom one sustained a contralateral AFF, one had fracture union and was pain-free one year post-operatively and a third had poor healing and ongoing pain. The remaining four patients with complete AFFs and two patients with incomplete AFFs without teriparatide all had non-union and persistent pain one year post-fracture. Based on the available data it was not possible to distinguish the outcome in between complete AFF and incomplete AFF cases separately, and the follow-up time is not explicitly stated in this article; therefore, this article is not included in **Table 4**. This anecdoctical evidence suggests a favorable effect of teriparatide in four incomplete AFFs and one complete AFF, but the healing duration for incomplete AFF and complete AFFs separately are not explicitly stated in this article.

Greenspan *et al.* published a randomized pilot trial in 13 patients comparing teriparatide immediately after surgical intervention (n=7) versus delayed teriparatide six months fol-

lowing the surgery (n=6).[42] All patients were female with a median age of 74 years. All but one patient had sustained a complete AFF and four individuals had periprosthetic fractures. Conventional X-rays were done at baseline and six and 12 months after the acute fracture. Additionally, the subjects on delayed teriparatide had an X-ray at 18 months. The fracture healing was assessed on continuity of the bone, disappearance of lucencies, callus formation, and alignment of proximal and distal fracture fragments. These four parameters were combined in a composite score with a maximum of 16 points, a higher score indicating better bone healing. At six and 12 months, no statistically significant differences were found in composite scores between the immediate and delayed group (12.6 vs 11.2, p = 0.3820; 15.4 vs 13.2, p = 0.1456), with a more favorable trend in the immediate group towards fracture healing. It is not stated in this article how many AFFs or patients had achieved full bone union at six or 12 months of teriparatide; therefore, it is not eligible for the pooled data in **Table 4**. The authors do report that one implant failure occurred in the delayed group after 12 months of teriparatide and that there were no cases of non-union in either of the two groups at any time point. No occurrence of contralateral AFF was mentioned. The authors concluded that immediate start of teriparatide appeared to result in improved healing.

Watts *et al.* prospectively followed 14 women with a median age of 68 years, starting with teriparatide after complete AFF or incomplete AFF.[43] The commencement of teriparatide varied from 52 days to 410 days after the AFF. There was radiographic follow-up of the fracture status at month 12 and month 24, but not at six months which made the assessment of healing time of AFFs not suitable for the pooling of the datain **Table 4** apart from two cases of progression from incomplete to complete AFFs. The study population was compromised of 10 complete AFFs in nine patients and seven incomplete AFFs in five patients. Four patients with incomplete AFFs were managed conservatively, whilst one patient had surgical intervention prior to starting teriparatide. Four complete AFFs showed full fracture union after one year in three patients, one had nonunion and the other five had partial healing after one year of teriparatide. Four incomplete AFFs had partially healed including the surgically treated incomplete AFF and the other three incomplete AFFs remained unchanged after one year of teriparatide. Two patients with complete AFF sustained a complete contralateral AFF whilst on teriparatide, after nine days and 21 months, respectively. Focal thickening was visible of the involved femur prior to starting teriparatide in both of these patients. To establish the effect of teriparatide, it is important to know about the fracture healing status prior to using teriparatide. However, the authors do not state if complete AFFs had malunion before starting teriparatide that could explain why seven patients started anabolic therapy more than four months after the diagnosis. Also, it is not reported how many incomplete AFFs had

radiolucent lines and how partial healing was defined in cases without visible fracture lines. Another limitation is the lack of a control group.

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11

General discussion

DISCUSSION

Part 1: Diagnostic imaging of atypical femur fractures

Early diagnosis of incomplete AFF is crucial to prevent the necessity of surgical management of a (pending) complete fracture. Surgical treatment of AFF is associated with major functional limitations[1,2] and an increased risk of reoperation in comparison to typical femoral fractures.[3-5]

The diagnosis of AFF is based on specific radiological characteristics as seen on X-rays, such as a non- or minimally comminuted transverse fracture line and localized periosteal or endosteal thickening of the lateral femoral cortex. In contrast to X-rays that are only performed on clinical indication, DXA scanning is routinely used for follow-up of patients on bisphosphonate treatment.

This raises the question if DXA scanning could serve as an alternative imaging technique that provides insight in etiology and early detection of AFF.

In part 1 of this thesis, DXA scanning was evaluated as a screening tool to detect patients at risk of AFF by using trabecular bone score (TBS) and hip structural analysis (HSA), or identify early stages of AFF on full-length femur scans. In addition, we question the requirement of a lateral localization of the fracture as a strict criterion for the diagnosis of AFF.

Chapter 2 describes that TBS, calculated by a special software based on pixel gray-level variations on lumbar spine DXA scans, did not differ significantly between 30 patients with AFF and 141 controls matched for age and sex. In this study, patients with AFF had higher bone mineral density (BMD) at the femur neck compared to the control population. Geometric variables using HSA on DXA scans of the hip showed no differences between cases and controls at the cross-section of the femoral shaft, nor differences in neck shaft. However, AFF patients unexpectedly did have a lower buckling ratio and a higher centroid position at the narrow neck. These outcomes may correspond to a lower risk of typical hip fractures.

TBS is an indirect measure of trabecular connectivity and thus a parameter of bone quality, which is expected to be compromised in patients with a low-trauma fracture such as AFF. It has been shown that for typical osteoporotic fractures, TBS may improve fracture prediction on top of the lumbar spine BMD.[6-8] Low TBS is associated with both past and incident fracture in cross-sectional and prospective studies independently from BMD in population-based studies.[9] Therefore TBS can be especially useful in spe-

cific conditions in which the relationship between BMD and fracture risk is altered, such as in glucocorticoid-induced osteoporosis, diabetes mellitus type II and chronic kidney failure.[10] Abnormalities of the trabecular bone in AFF patients have been suggested by a small case series with iliac crest bone biopsies in four AFF cases that showed low trabecular bone volume.[11]

Yet, in this thesis no association was found between AFF and TBS. This is in line with other studies on trabecular microarchitecture in patients with AFF, including one study using high resolution pqCT-scanning[12] and two studies using TBS.[13,14] The outcome in **Chapter 2** and previous studies may be explained by the fact that bone quality in patients with AFF is only impaired at the fracture localization of AFF, i.e. the femoral shaft, while TBS is measured at the lumbar spine. Otherwise AFF may be related to decreased cortical bone quality rather than a condition that involves the trabecular bone.

A varus neck shaft angle in patients with AFF has been described in studies using conventional radiographs[15-17], but was not found in this study in **Chapter 2** nor in a Chinese case-control study using HSA in 31 AFF patients.[18] Possibly, any differences could not be detected because HSA is very sensitive to variation in femur rotation[19] and the low resolution of DXA scans may not allow exact measurements of the bone margins.[20]

In **Chapter 2**, a higher BMD and a more favorable hip geometry with regard to classical hip fracture risk were found in patients with AFF. These findings can be explained by the characteristics of the study population. First, a high proportion of glucocorticoid users (57% of AFF cases) suggests that these patients were treated for prevention instead of treatment of osteoporotic fractures. When adjusting for ethnicity, sex, age, height, weight, duration of bisphosphonate use and glucocorticoid use, the difference in BMD was no longer significant. Second, the lower buckling ratio (**Figure 1**) in AFF patients might be a result of the generalized increase in cortical thickness that has been described in patients with AFF, since this finding means that wall thickness of the cortex is large in comparison to the outer radius. However, it should be noted that higher cortical thickness in patients with AFF remains a controversial parameter, as discussed in the Introduction of this thesis. Lastly, the use of bisphosphonates for up to two years has been associated with improvement of HSA outcomes.[21,22] The longer treatment duration with bisphosphonates of the AFF cases (9.8 years) compared to controls (6.0 years) may have influenced the HSA parameters. On the other hand, it has been demonstrated that bisphosphonates do not increase BMD beyond three years of use, meaning that the bisphosphonate exposure is unlikely to be the main reason for difference in BMD between cases and controls.[23]

In summary, the findings in **Chapter 2** do not indicate that TBS and HSA could serve as useful screening parameters in the risk assessment for AFF.

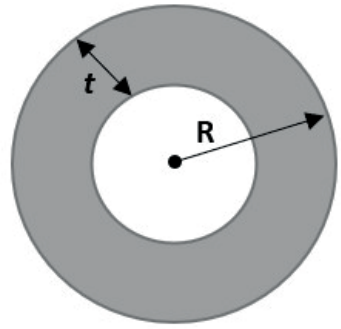

Figure 1. Buckling ratio is the ratio between the outer radius (R) and cortical thickness (*t*). Local buckling may occur with values of 10 or higher.

Chapter 3 entails a report on a 50-year-old female patient with fractures that fulfill the diagnostic criteria for AFF as formulated by the ASBMR Task Force, except for a medial localization instead of a lateral localization at the femoral cortex. She presented with prodromal pain and had subtrochanteric, non-traumatic bilateral localized periosteal reactions, confirmed on bone scintigraphy and MRI scanning. She was initially diagnosed with metastatic bone disease from primary breast cancer. She received intravenous bisphosphonate treatment and radiation therapy on one femur, after which a fracture line developed. At the Bone Centre from Erasmus University Medical Centre, she was subsequently diagnosed with 'atypical' AFFs. Based on this case study, medially located AFFs should be considered in a spectrum of bisphosphonate-related stress fractures.

A medially located AFF has been reported in another female patient after the use of denosumab.[24]

Stress or fatigue fractures are usually a result of excessive loading and repetitive stress in athletes and observed in weight-bearing bones such as the femur, tibia, metatarsals and calcaneus. AFFs resemble stress fractures, but are seen in patients without excessive physical activity. Bone remodeling is suggested to be low and healing is absent in a large proportion of AFF cases. Insufficiency fractures such as in patients with osteomalacia are therefore similar to AFF, since they are characterized by low bone quality and normal loading.

During walking under physiological conditions, the highest tensile strains occur at the anterolateral femur and the highest compressive forces along the medio-posterior femur.[25-29] Exercise-induced fractures usually have a medial localization at the femoral neck or femoral shaft as a result of weight-bearing and compression. Especially walking and stair descent have been found to increase lateral strains at the fracture localization of lateral AFFs (**Figure 2**).[30] It is thought that bowing of the femora further expands the lateral strain.[31,32]

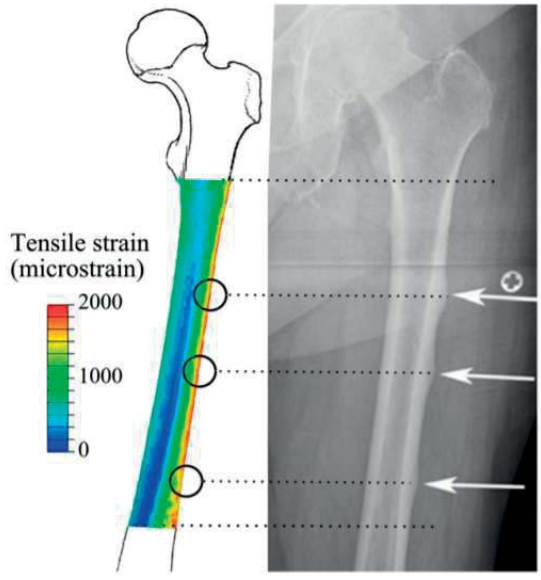

Figure 2. Tensile strain pattern during walking in comparison with three frequent AFF localizations along the femoral cortex indicated by the white arrows.[30]

This case study indicates that in (a subset of) patients, AFFs may occur at the medial cortex of the thigh bone due to yet unknown biomechanical factors. Speculatively, such unknown factors might involve differences in levels and type of physical activity and fitness, femoral bowing and alignment of the femoral axes, leg length or neck shaft angle.

It is important to recognize this specific entity within the spectrum of AFF, since misdiagnosis led to the wrongful conclusion of bone metastases. This resulted in unnecessary radiation therapy and potentially further deterioration of the fracture by administering bisphosphonate therapy in this patient, instead of cessation of bisphosphonates. In some instances, the clinical significance of focal irregularities of the medial cortex is unclear and might be a physiological phenomenon, as was found in three out of 282 patients with imaging of the upper legs (**Chapter 4**).

In addition to medial AFFs, bisphosphonate-induced fractures might arise at other skeletal sites such as the pelvis, ankle, metatarsals, humerus, fibula and tibia.[33-40] Another patient from the Bone Centre clinic has likewise presented with a nontraumatic tibia fracture just prior to the presentation with AFF, as described in **Chapter 6**. In a questionnaire amongst 81 patients with AFF, over a third of patients reported metatarsal fractures.[41] All these anecdotal reports of non-femoral fractures are suggestive of a relationship with bisphosphonates, but hard evidence is lacking.

Based on these findings in **Chapter 4** and **6** and the aforementioned reports in literature, we recommend to consider the possibility of medial AFF in patients on (long-term) bisphosphonate treatment with pain and cortical reactions at the medial femoral cortex.

In **Chapter 4** and **Chapter 5**, the potential of DXA scanning as a screening tool for incomplete AFFs is investigated. The length of a routine hip DXA is extended to depict the whole femur.

Chapter 4 entails a prospective study performed at the Bone Centre clinic, in which 10 incomplete AFFs were found in 282 patients in 2.3 years' time by screening all patients on bisphosphonate or denosumab regardless of duration of treatment. The diagnosis of AFF was confirmed by conventional radiographs of the femur. The early detection of AFF by DXA scanning had therapeutic consequences, such as the discontinuation of antiresorptive therapy, prescription of alternative osteoporosis medication or even prophylactic surgery in some cases.

Only two other prospective studies are available on screening for incomplete AFF using femur scans by DXA. These were both performed by McKenna *et al.* in Ireland and found a prevalence of 2.7% and 0%, respectively.[42,43] This is lower than the prevalence of 3.2% in our clinic, despite stricter inclusion criteria for screening in the Irish studies. Their study population was limited to patients above the age of 50 years who were taking bisphosphonates for at least five years. The difference in prevalence could be the result of a unique patient population with a higher disease burden in a Dutch tertiary care setting versus an Irish community based study. The 0% prevalence might even be an underestimation of the occurrence of incomplete AFF, because this is the second study that has been repeated within the same geographical region in which the existing AFF cases were already discovered by the previous screening.

Chapter 4 and the two studies by McKenna *et al.*, served as important hallmarks for the use of DXA scanning in the detection of incomplete AFF in clinical practice. A position statement on the use of DXA scans with regard to incomplete AFF is described in **Chapter**

5 and is the result of a working group from the International Society of Clinical Densitometry (ISCD). A systematic review of the literature and review by an external panel of experts, led to the following recommendations. Foremost, DXA scans of the femur should be inspected for abnormalities in the spectrum of AFF, including focal periosteal and endosteal thickening at the lateral cortex, with or without the presence of a fracture line. Preferably the whole femur is depicted by full-length femur imaging, since AFFs can also occur at the distal femoral shaft. The ISCD recommends the performance of full-length femur imaging in patients who undergo routine DXA scanning and who currently are or have been on antiresorptive therapy in the past year for a cumulative period of three or more years, especially in those with concomitant use of glucocorticoids.

There are several limitations and unknown aspects of densitometer-based screening for AFF.

The femur images are subject to individual interpretation, although interobserver agreement was excellent in an examination of 232 DXA images including 33 images with incomplete AFFs by three observers.[44] Recently, automated software to establish localized cortical thickening has become available to enable objective measurement, but a clinically relevant cut-off value has not been determined. Single energy scans may have better spatial resolution to depict fracture lines, but there are insufficient data to assess superiority of single energy over dual energy scans. When abnormalities are found on DXA images, there is uncertainty about the appropriate diagnostic modalities for assessment of the fracture, ranging from conventional radiographs, CT-scanning, MRI-scanning to bone scintigraphy. Ideally, screening would only be applied in a selected patient population with the biggest yield for AFF to ensure a favorable cost-benefit ratio. The AFF risk is notably increased after three years of oral bisphosphonate use[45], but scientific foundation of the advice for screening is lacking for users of denosumab or intravenous bisphosphonates. Moreover, this adverse event can also occur in patients with short exposure to antiresorptive therapy, thus screening in all patients on antiresorptive treatment of any duration can be justified. The presence of pain does not appear to be a reliable indicator of the presence of incomplete AFF, since pain in the hip, groin or upper legs are common in the elderly population. Moreover, in the study population of **Chapter 4** only two out of nine patients with incomplete AFF had spontaneously reported pain in the upper leg or thigh.

Even though AFFs mostly occur in female patients, which is most likely due to the large proportion of women amongst bisphosphonate users, AFFs also occur in men and the exclusion of men in screening by DXA is therefore not recommended. Specific diseases that could confer a significant risk of AFF are not apparent, although one study suggests

an increased risk in patients with autoimmune diseases taking long-term glucocorticoids .[46]

In conclusion, DXA scanning of the femur can serve as a clinically relevant screening tool for incomplete AFFs, although the selection of the screening population and the benefit cost ratio are factors that need further evaluation.

Part 2: Genetics in relation to atypical femur fractures

The pathophysiology of AFF remains unclear, but following lines of evidence from the literature and observations from clinical practice, we may presume that AFF patients have a genetic predisposition to develop AFF. Especially the clustering of AFF in families is suggestive that AFF is a genetic condition. We now know that many - if not all - complex common diseases such as osteoporosis and their risk factors such as BMD have genetic components. Genome Wide Association Studies (GWAS) have been very successful in identifying such genetic factors.[47] The yields of GWAS have now reached a level that so-called polygenic risk scores (PRS) are being constructed, which allow to stratify subjects into different risk categories based on the proportion of risk alleles they carry.[48] In addition, whole exome sequencing (WES) and whole genome sequencing (WGS) approaches have allowed to identify more rare variants involved in complex traits and diseases in the population[49] and in families.[50] All of these approaches allow for identification of genetic factors underlying AFF. A point of consideration is that osteoporosis has been shown to have a truly polygenic architecture with hundreds of genes involved determining risk of low BMD or fracture.[47] At the same time, Mendelian bone disease is caused by mutations in individual genes which sometimes overlap with those found in GWAS. Finally, given the suspected involvement of bisphosphonates, pharmacogenetic factors that determine the response to bisphosphonates could also be involved in the genetic architecture of AFF. Ergo, the genetic basis for AFF can be a mixture of such different genetic predispositions in individual AFF patients.

In **Chapter 6**, an 18-year-old man is reported who experienced an AFF after intravenous bisphosphonates from age nine until 16 for the treatment of what was at that time considered to be osteogenesis imperfecta, but later turned out to be X-linked osteoporosis due to a PLS3 mutation.[50] Even though the majority of AFF cases are seen in postmenopausal women, this case demonstrates that also adolescents and males can develop this adverse event. This risk should be taken into account for the decision-making on bisphosphonate treatment in children with a monogenetic bone disease.

The occurrence of AFF at such a young age and in a patient with a rare monogenetic bone disease, support the hypothesis that genetic factors play a role in the pathogenesis

of AFF, but naturally does not prove a causal relationship between the PLS3 mutation and AFF.

In search of more documentation with regard to AFF in relation to monogenetic bone disease, we performed a systematic review as reported in **Chapter 7**. We found that AFFs were documented in 23 patients representing seven different monogenetic bone diseases, including osteogenesis imperfecta, pycnodysostosis, hypophosphatasia, X-linked osteoporosis, osteopetrosis, X-linked hypophosphatemia and osteoporosis pseudoglioma syndrome. Of those 23 patients, 16 had never used any bisphosphonates and eight were diagnosed with the genetic bone disease only after the occurrence of AFF. These observational data are suggestive that monogenetic bone disease is a risk factor for AFF, although it is not established whether AFFs truly occur more frequently in patients carrying such mutations.

In the search of a genetic cause for AFF, either candidate sequencing, exon array analysis or exome sequencing can be performed in non-familial and familial cases. A whole-genome approach has not been documented in AFF patients.

Most targeted sequencing studies have focused on *ALPL*, a gene involved in hypophosphatasia. Hypophosphatasia is a heterogeneous disorder caused by a defect in enzyme function of alkaline phosphatase, leading to accumulation of substrates such as inorganic pyrophosphate. This inhibits bone mineralization and can result in skeletal and dental manifestations in a wide clinical spectrum, ranging from a lethal form in infants to a mild presentation in adulthood. This disease is of particular interest for the genetic study of AFF, since bisphosphonates are synthetic analogs of inorganic pyrophosphate and hypophosphatasia is associated with spontaneous pseudofractures of the thigh that may resemble AFF. Four small case series[51-54] with targeted sequencing of the *ALPL* (n=43) have been performed in mostly women (n=41, 95%) and none have confirmed the diagnosis of hypophosphatasia. Sum *et al.* did report one heterozygous carrier of a mutation in one AFF patient, which is associated with lethal hypophosphatasia when a second mutation on the other chromosome is present.[53] Peris *et al.* found a heterozygous variant of unknown significance in the *ALPL* gene in another AFF patient who had a normal total alkaline phosphatase value.[51]

One exon array analysis has been done as a pilot study that aimed to find risk variants for AFF in 13 women with AFF versus 268 controls that included 87 healthy women and 181 women with postmenopausal osteoporosis. This study lacked statistical power to find variants of interest.[55] One small GWAS has been performed on AFF cases compared to controls, conducted on 51 AFF patients compared to 324 controls matched on

bisphosphonate use, as well as to 4891 population-based controls.[56] No signals were genome-wide significant and, thus, much larger population is needed for GWAS. Therefore, no common genetic variants underlying AFF have been identified at this moment.

WES has been applied in a family-based setting in the search of a genetic cause for AFF.

Roca-Ayats *et al.* highlighted a variant in the *GGPS1* gene from a WES in a Spanish family with three siblings with AFFs, since this gene is involved in the mevalonate pathway that is targeted by bisphosphonates.[55,57] However, pathogenic variants in *GGPS1* have so far not been replicated in other AFF cases, such as in a cohort study of 17 unrelated women with AFF.[51] *GGPS1* mutations might only be contributing genetic factors in this one family from Spain. Moreover, it should also be noted that in this study 36 other exonic variants were found, of which the causal role cannot be excluded.

Chapter 8 describes the family-based approach with WES data applied to three patients from two Asian families with AFFs and found heterozygous variants of interest in *CYP1A1*, *PLOD2* and *TMEM25*. One family consisted of two sisters with AFFs. The other family was comprised of a woman with AFF of whom the mother had also sustained AFF, but was deceased and thus no DNA sample was available from the mother. Two *CYP1A1* variants were detected, one rare variant shared by two sisters, and one common variant by all three cases. *CYP1A1* is of interest because it has been reported in the Spanish WES study.[57] This gene is involved in metabolism of steroid hormones including 17β-estradiol and vitamin D, but has no known link to drug metabolism of bisphosphonates. A rare variant in *TMEM25* was shared by the sisters, a gene of which the potential link to bone physiology is unclear. Another variant in *TMEM25* was also identified in the Spanish family.[57] The *PLOD2* variant we observed in one of the Asian families was rare, and found only in the single patient of the mother-daughter pair. The clinical significance of a heterozygous mutation in this gene is unclear, but might predispose to AFF since *PLOD2* codes for the telopeptide lysyl hydroxylase, which is involved in crosslinking of bone collagen.[58] Homozygous mutations in *PLOD2* can lead to Brück syndrome, a rare form of osteogenesis imperfecta.[59] The significance of the findings in this WES study from one family and one individual with "familial AFF" is hard to assess. The study is limited by the large number of potential variants of interest given a big genotypic overlap in a small family, a lack of replication in other AFF families or unrelated AFF cases, and absence of functional studies.

In **Chapter 9** we present the clinical evaluation and preliminary WES results from a larger, Caucasian family. This family involves three siblings with bisphosphonate-associated AFFs and a fourth sibling with a femoral fracture after low trauma that might belong to

the spectrum of AFF. In total six siblings and a cousin had osteoporosis, but all family members had lifestyle factors, comorbidities or medication use that can contribute to low bone mass. The three siblings with AFF shared 78 rare exonic variants in 74 genes. One of these variants is located in a gene involved in bone biology, but this variant of interest for AFF did not segregate with osteoporosis in this family.

Based the work presented in **Chapter 7, 8** and **9**, it appears that AFF has a heterogeneous genetic background. Only some genetic factors have been identified, but these are limited to individual pedigrees and do not seem to be relevant for AFF in general.

The problem investigating the genetic background of AFF is that on the one hand, AFF and osteoporosis may have a different genetic predisposition, while on the other hand AFF could be the result of the same underlying genetic bone disease. The review of the literature in **Chapter 7** has already shown AFF is reported in patients with monogenetic bone diseases. Another possibility to explain the difficulty in finding monogenic causes for AFF, is that AFF may only develop on top of a genetic predisposition for osteoporosis or a Mendelian bone disease. In a family-based study, it should also be taken into consideration that family members without AFF cannot be used as reference (non-carriers) of a AFF risk variant, because they might have developed AFF when exposed to bisphosphonates. Although WES may identify many potentially disease-causing genetic variants within a family, prioritization of the variants should be based on segregation of a carefully selected phenotype, predicted pathogenicity, allele frequency, and function in bone biology. In addition, validation of conclusions on the involvement of a gene and variant thereof should always take place by replication of the findings in independent families or case series, and functional analysis to understand the underlying biological mechanism.

Part 3: medical treatment of atypical femur fractures

Patients with AFF may still be at high risk of typical fragility fractures caused by osteoporosis, even after long-term bisphosphonate use. Continuation of bisphosphonates in these patients is not an appealing option, since this may worsen the healing of AFF and increase risk of a second AFF of the contralateral leg. Given the availability of several osteoporosis drugs which intervene in different biological pathways, the influence of these drugs on AFF might be different and thereby provide options for therapy. In **Chapter 10**, the effects were asssessed of teriparatide, denosumab, raloxifene, romosozumab and abaloparatide on both the healing and occurrence of AFF using all documented cases, cohort studies and prospective studies in a systematic review. Based on a pooled analysis of these observational data, the majority of operated incomplete (n=9, 90%) and complete (n=44, 76%) AFFs healed within six months of teriparatide use, whilst non-

operated incomplete fractures with teriparatide (n=13, 43%) and complete AFFs without teriparatide (n=34, 51%) did not. New AFFs did occur during and after teriparatide treatment, either as a first event or a second AFF of the other femur, but always after bisphosphonate exposure. The numbers of patients with AFF associated with raloxifene (n=3), denosumab (n=22) and romosozumab (n=3) were very low, suggesting a low absolute risk although no epidemiological studies have been performed and these drugs are less commonly prescribed in comparison to bisphosphonates. There were no AFF cases linked with the use of abaloparatide, another anabolic drug which is currently not available in Europe.

An evidence-based recommendation is not possible in absence of randomized controlled trials, but based on the observational data and expert opinion of a group of European specialists in the field of bone health from the European Calcified Tissue Society (ECTS), a position statement was formulated for medical treatment after AFF. In AFF patients at high risk of fragility fractures, treatment with teriparatide, SERMs, romosozumab or abaloparatide should be considered. Alternatively, hormone replacement therapy or tibolone can be prescribed to high risk patients, but preferably in women under 65 years of age without an increased risk of venous thromboembolism, myocardial infarction, stroke or breast cancer. A last choice of treatment for patients at high risk of fracture, would be calcitonin. When AFF occurs during denosumab treatment, a short course of bisphosphonates or SERMS should be considered in patients with surgically treated AFF to prevent a rebound effect. Discontinuation without follow-up treatment can be considered after short-term denosumab use in absence of vertebral fractures. In case of bilateral, operated AFFs, bisphosphonates or denosumab may be continued since the worst adverse outcome has already taken place, although it should be kept in mind that there might be a small chance of bisphosphonate related stress fractures at other skeletal sites or below surgical material. For enhanced fracture healing of AFF itself, the observational data in our systematic review suggest, but do not prove, that teriparatide might accelerate the healing of surgically treated AFF, but nonunion after one year can still occur.

In conclusion we observed that indeed different osteoporosis drugs have different influences on healing and occurrence of AFF, but several drugs might provide good options for therapy where AFF is involved.

Future perspective

AFF is a potentially debilitating complication linked to bisphosphonate treatment for osteoporosis and deserves further research on the genetic and non-genetic predictors.

Based on **Chapter 2**, TBS of the lumbar spine and HSA parameters of the hip do not appear to be useful as predictors of AFF, but for more definite conclusions would ideally need evaluation in a larger population and in a prospective setting. To assess the trabecular microarchitecture at the femur, it may be possible in the future to investigate regions of interest at the femoral shaft instead of the lumbar vertebrae.[60]

The "atypical" AFF from **Chapter 3**, highlights the importance of careful recording of fracture history in patients on long-term bisphosphonate therapy may reveal a potential spectrum of bisphosphonate-associated stress fractures. Further studies on alignment of the femora in AFF patients by the use of standing long-leg radiographs could help in the identification of biomechanical risk factors for the development of AFF.

Chapter 4 and **5** shows that DXA scans can be used as a screening tool for incomplete AFFs, but future research is needed to evaluate how soon incomplete AFFs may develop over time to determine an optimal interval for follow-up DXA scanning. It also remains unclear how automated screening software performs in comparison to visual inspection and if single-energy DXA scans should be preferred over dual-energy femur scanning. A comprehensive analysis is needed to determine the advantages of early detection of AFF and reassurance in the patient management of long-term users of antiresorptive drugs. These benefits need to be weighed against the impact of additional imaging that requires time, radiation exposure, costs and education of the medical staff, plus the possibility of coincidental findings that may necessitate further examinations.

Part 2 of this thesis is dedicated to the role of genetics in the etiology of AFF and exposed several challenges in finding the genetic causal factors for AFF in **Chapter 8** and **9**. In spite of these difficulties, genetic studies are important because understanding of the genetic nature of AFFs could in the future serve as an important screening tool in clinical practice. When patients with a genetic risk of this severe adverse event can be identified before prescribing (long-term) bisphosphonate therapy, AFFs can be prevented. A genetic screening may also improve bisphosphonate compliance in low-risk patients and thus contribute to decreasing the treatment gap in osteoporosis.

The recommendations in **Chapter 10** on the medical management of patients who experienced AFF are based on expert opinion in absence of clinical trials. Future trials are needed to evaluate the use of teriparatide for the fracture healing of all forms of AFF, distinguishing incomplete forms, postoperative outcomes for complete AFF and electively operated incomplete AFF and non-healing AFF. Currently, one placebo-controlled trial is registered for the use of teriparatide in patients with conservatively managed incomplete AFF.[61]

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12

Summary / Samenvatting

SUMMARY

Introduction

Bisphosphonates are used by millions of patients worldwide for the treatment of metabolic bone disease such as osteoporosis. Bisphosphonates can effectively reduce the number of osteoporotic fractures, preventing morbidity and even mortality.

Since 2005, bisphosphonates have been associated with atypical femur fractures, which are very rare but severe adverse events. These fractures of the thigh bone can occur spontaneously in both upper legs, often followed by a delayed healing process or even absence of fracture healing. They are different from osteoporotic fractures because of the spontaneous character. They also have distinct radiological diagnostic criteria, such as the horizontal fracture line and a localization at the thigh bone below the hip where osteoporotic hip fractures normally occur.

A longer duration of bisphosphonate use corresponds with a higher risk of atypical femur fractures. The cause of atypical femur fractures is unknown, but part of the explanation might be that bisphosphonates lead to brittle bone due to suppression of the bone metabolism.

Fear of this severe adverse event, creates a treatment dilemma for the optimal (duration of) osteoporosis treatment with bisphosphonates. The negative publicity on atypical femur fractures has been linked to a decrease in bisphosphonate use in recent years. However, the benefits of bisphosphonates in persons with a high fracture risk clearly outweigh the risk of atypical femur fractures.

The aim of our research in this thesis is to improve the identification of patients with (a high risk of) atypical femur fractures by using diagnostic imaging and advanced genetic tests. In addition, we evaluate the best treatment options for those patients who already sustained an atypical femur fracture.

Part 1 Diagnostic imaging of atypical femur fractures

Patients with atypical femur fractures may have a different anatomy of the hip and lower bone quality than those without atypical femur fractures. A DXA scanner measures bone mineral density in patients to diagnose osteoporosis and evaluate the effect of treatment with bisphosphonates. In **Chapter 2**, the trabecular bone score is estimated from a DXA scan of the lumbar spine and used as an indirect measure of bone quality. Hip structural analysis is performed with a special software programme on DXA scans of the femur and can be used to analyze the geometry of the hip. When comparing the outcomes

of these two methods between a group of patients with atypical femur fractures and a group of control patients, none of the expected differences in bone quality of the spine and geometry of the hip were found. There is no evidence that trabecular bone score and hip structural analysis can distinguish patients with atypical femur fracture from those without, meaning that based on our findings these parameters will not serve as an adequate screening method.

Although atypical femur fractures are defined to occur at the outside of the femur (lateral cortex), the patient report in **Chapter 3** shows that these fractures in rare circumstances may also develop on the inner side of the femur (medial cortex). Therefore, the presented case could be considered an 'atypical' atypical femur fracture. It concerns a woman with a history of breast cancer who used a bisphosphonate. She was misdiagnosed with metastatic bone disease because of the femur fracture and consequently treated with a potent bisphosphonate infusion as well as radiation therapy on the femur. This worsened the atypical femur fracture. Her story shows the importance of knowledge on rare adverse events such as atypical femur fractures, preventing a misdiagnosis followed by an inappropriate treatment.

It is challenging to diagnose atypical femur fractures at an early stage before it becomes a complete fracture, not only because they are rare but also because patients or doctors do not always pay attention to potential symptoms preceding this type of fracture, such as pain in the groin and upper leg. In **Chapter 4**, it is shown that DXA scans, routinely performed in patients on bisphosphonate treatment, could serve as an adequate and relevant screening tool to identify incomplete atypical femur fractures. The length of the hip DXA, routinely performed at the hip, can be extended to the full length of the femur to assess local thickening of the bone as seen in incomplete atypical femur fractures. This screening method can lead to the prevention of a complete fracture by immediate cessation of bisphosphonates and sometimes preventive surgery. **Chapter 5** comprises a guideline for the use of DXA scanning for detection of atypical femur fractures, written in collaboration with the International Society of Clinical Densitometry.

Part 2 Genetics in relation to atypical femur fractures

The risk of atypical femur fractures may be influenced by genetic factors.

In **Chapter 6**, an adolescent boy is reported who had an atypical femur fracture after years of bisphosphonate infusions for the treatment of a genetic form of osteoporosis: X-linked osteoporosis based on a mutation in the *PLS3* gene. The question remains if the atypical femur fracture is a result of intensive bisphosphonate exposure or a manifestation of his genetic bone disease, or a combination of those two. Importantly, the risk

of atypical femur fractures should be considered in the duration of treatment of bone disease not only in adults, but also in children and teenagers.

An overview of atypical femur fractures in relation to genetics is given in **Chapter 7.** A systematic review of the literature shows that atypical femur fractures are linked with seven different genetic bone diseases and that not all patients have used bisphosphonates. This indicates that genetic bone disease could be an independent risk factor for atypical femur fractures.

Furthermore, the diagnosis of a genetic disease was sometimes made after the atypical femur fracture had occurred. This could mean that an unrecognized, mild form of genetic bone disease is present in other patients with atypical femur fractures.

Another argument for a genetic predisposition for atypical femur fractures, is the occurrence of atypical femur fractures in families. In **Chapter 8** two Asian families with atypical femur fractures are presented and analyzed for a genetic cause of the atypical femur fractures by using whole-exome sequencing. Because the families are small it was not possible to draw definite conclusions, but the results may in the future be used for replication in other families.

In **Chapter 9** a large Caucasian family is described with atypical femur fractures and osteoporosis. Many potential genetic risk variants are found with whole-exome sequencing, but further filtering of the results is needed to find the genetic cause of atypical femur fractures in this family. One variant was of particular interest, because it is located in a gene that is linked with bone biology. However, the difficulty lies in proving that this genetic variation is causally related to the fractures and osteoporosis in this family. Animal studies or laboratory experiments at the cellular level can give insight into the effect of a gene or variant. In addition, the presence of this genetic variant in other patients with atypical femur fractures may strengthen the evidence that it is a causal mutation.

Another point of interest in the further analysis of this family is if osteoporosis and atypical femur fractures have the same genetic cause. Both atypical femur fractures and osteoporosis are complex diseases, since the development is not just related to genetics but also medication use or other non-genetic factors. This makes the genetic analysis of this family particularly challenging.

Part 3 Medical treatment of atypical femur fractures

Patients who have had an atypical femur fracture, often after long-term bisphosphonate use, may still be at high risk of osteoporotic fractures. Bisphosphonates need to be

stopped because this confers a risk of worsening the atypical femur fracture or a second atypical femur fracture.

In **Chapter 10**, all available information in literature is systematically assessed on the safety of other osteoporosis drugs with regard to atypical femur fractures, including denosumab, teriparatide, raloxifene and romosozumab. Denosumab use is associated with a risk of atypical femur fractures as well, although no large cohort data are available to establish the exact risk. There is no evidence that teriparatide can cause nor prevent atypical femur fractures, although it appears that atypical femur fractures heal faster after surgery with the use of teriparatide. Unfortunately non-union of atypical femur fractures is also reported in some patients treated with teriparatide. Raloxifene and romosozumab have both sporadically been related to atypical femur fractures, but the risk seems to be very low. **Chapter 10** provides recommendations for medical treatment after a patient had had an atypical femur fracture, based on available data and expert opinion, supported by the European Calcified Tissue Society. It must be noted that no hard evidence is available for these recommendations, given the absence of randomized controlled trials.

Conclusion

Understanding the pathophysiology including clinical, genetic and radiological aspects of atypical femur fractures is relevant to prevent the occurrence of this rare but devastating fracture. When those at high risk of atypical femur fractures can be identified by genetic tests or imaging techniques, alternative osteoporosis medication can be prescribed whilst bisphosphonate treatment can be used in low risk groups. Several alternative osteoporosis drugs can be used in those patients with (a high risk of) atypical femur fractures. This approach of personalized medicine could play an essential role in closing the current treatment gap in osteoporosis.

Future research

Resulting from our own research in this thesis, we have several suggestions for future research with regard to atypical femur fractures. The trabecular bone score of the lumbar spine was perhaps not representative for the femur and therefore it would be interesting to study bone quality parameters at the fracture localization of atypical femur fractures. Biomechanical studies could help to understand why atypical femur fractures develop at the outside of the thigh bone and sporadically at the inside of the thigh bone. The screening with DXA scanning in patients using bisphosphonates can help to prevent atypical femur fractures, but the timing interval and selected population for screening can be further investigated keeping the cost-benefit ratio in mind. Genetic research requires large populations and more families with atypical femur fractures to establish

causal genetic factors. Lastly, there is a need of randomized controlled trials to evaluate the best medical treatment of patients with atypical femur fractures, especially to study the role of teriparatide and the treatment options in those patients with non-union fractures.

SAMENVATTING

Introductie

Bisfosfonaten worden door miljoenen mensen wereldwijd gebruikt voor de medicamenteuze behandeling van botaandoeningen zoals osteoporose (ook wel botontkalking genoemd). Bisfosfonaten zijn niet alleen effectief in het verminderen van osteoporotische botbreuken, maar ook de daarmee gepaard gaande ziektelast en sterfte. Sinds 2005 worden bisfosfonaten in verband gebracht met een atypische breuk van het bovenbeen, oftewel een atypische femurfractuur. Dit is een zeldzame maar ernstige complicatie van bisfosfonaatgebruik. Deze breuk van het dijbeen kan spontaan optreden in allebei de bovenbenen en geneest vaak langzaam of zelfs helemaal niet. De atypische femurfractuur onderscheidt zich van osteoporotische botbreuken door de spontane ontstaanswijze. Daarnaast zijn er specifieke radiologische kenmerken, waaronder een horizontale 'schone' breuklijn in de schacht van het dijbeen onder de heuphals, in tegenstelling tot de klassieke heupfractuur die juist ter plekke van de heuphals optreedt.

Hoe langer de behandelduur met bisfosfonaten, des te hoger is het risico op atypische femurfracturen. De oorzaak van atypische femurfracturen is niet bekend, maar mogelijk speelt onderdrukking van de botombouw door bisfosfonaten een rol wat zou kunnen leiden tot broosheid van het bot.

Angst voor deze ernstige bijwerking stelt de behandelend arts voor een dilemma wat betreft de optimale behandelduur van osteoporose met bisfosfonaten en leidt ook tot een afname in het gebruik van bisfosfonaten door patiënten, hoewel de voordelen voor patiënten met een hoog fractuurrisico ruimschoots opwegen tegen het kleine risico op een atypische femurfractuur.

Wij beogen met ons onderzoek om de identificatie van patiënten met een (hoog) risico op atypische femurfracturen te verbeteren door middel van diagnostische beeldvorming en geavanceerde genetische screening. Daarnaast is een deel van dit proefschrift gewijd aan de beste medicamenteuze behandeling voor die patiënten die al een atypische femurfractuur hebben doorgemaakt.

Deel 1: diagnostische beeldvorming van atypische femurfracturen

Patiënten met een atypische femurfractuur hebben mogelijk een andere geometrie (anatomie) van de heup en een lagere botkwaliteit vergeleken met patiënten zonder atypische femurfracturen. Een DEXA-scanner meet de botmineraaldichtheid in patiënten om osteoporose vast te stellen dan wel het effect te evalueren van behandeling met bisfosfonaten.

In **Hoofdstuk 2** wordt de *trabecular bone score* gebruikt als een indirecte maat van de botkwaliteit op DEXA-scans van de lendenwervels en *hip structural analysis* uitgevoerd met behulp van speciale software voor DEXA-scans van het femur om de heupgeometrie te bestuderen. Wanneer de uitkomsten van deze twee methodes worden vergeleken tussen een groep van patiënten met atypische femurfracturen en een groep van controle patiënten, wordt geen van de verwachte verschillen gevonden tussen beide groepen in heupgeometrie noch botkwaliteit van de lendenwervels. Er is geen bewijs dat de *trabecular bone score* en de *hip structural analysis* toegepast kunnen worden om patiënten met en zonder atypische femurfracturen te onderscheiden. Hiermee zijn deze parameters waarschijnlijk geen geschikte screeningsmethode om patiënten met een hoog risico op atypische femurfracturen te detecteren.

Hoewel atypische femurfracturen volgens de officiële definitie aan de buitenzijde van het femur ontstaan (laterale cortex), laat de casus in **Hoofdstuk 3** zien dat deze botbreuken in zeldzame gevallen ook aan de binnenzijde van het femur kunnen ontstaan (mediale cortex). Deze casus kan dus beschouwd worden als een “atypische” atypische femurfractuur. Het betreft een vrouw die een bisfosfonaat gebruikte en in het verleden behandeld was voor borstkanker. Vanwege de femurfractuur werd zij ten onrechte gediagnosticeerd met een uitzaaiing in het bot van de borstkanker en aansluitend behandeld met een krachtig bisfosfonaatinfuus en bestraling van het femur. Dit verergerde de atypische femurfractuur. Haar verhaalt toont ons het belang van kennis over een zeldzame bijwerking van bisfosfonaten zoals de atypische fractuur, wat een misdiagnose en een inadequate medische behandeling kan voorkomen.

Het is een uitdaging om atypische femurfracturen zo vroeg mogelijk te diagnosticeren voordat een complete botbreuk ontstaat, niet alleen vanwege de zeldzaamheid maar ook omdat artsen en patiënten niet altijd aandacht besteden aan mogelijke symptomen zoals pijn in de liezen en bovenbenen. In **Hoofdstuk 4**, wordt aangetoond dat DEXA-scans, die routinematig worden verricht bij patiënten die bisfosfonaten gebruiken, een adequate en relevante screeningsmethode kunnen opleveren om incomplete atypische femurfracturen te identificeren. De lengte van een standaard DEXA-scan van de heup kan verlengd worden om het gehele bovenbeen af te beelden en beoordeeld worden op een plaatselijke verdikking van het bot zoals gezien wordt bij een incomplete atypische femurfractuur. Deze screening kan leiden tot het voorkómen van een complete botbreuk door bisfosfonaten onmiddellijk te staken en soms ook door een operatie te verrichten

Hoofdstuk 5 is een richtlijn voor de toepassing van DEXA-scans in het detecteren van atypische femurfracturen, opgesteld in samenwerking met de *International Society of Clinical Densitometry.*

Deel 2: Genetica en atypische femurfracturen

Er zijn aanwijzingen dat het risico op een atypische femurfractuur mede wordt bepaald door genetische factoren.

Hoofdstuk 6 beschrijft een jongeman die een atypische femurfractuur doormaakt na een jarenlange behandeling met bisfosfonaatinfusen in zijn jeugd vanwege een erfelijke vorm van osteoporose: X-gebonden osteoporose op basis van een *PLS3* mutatie. Het blijft de vraag of de atypische femurfractuur het gevolg is van de intensieve bisfosfonaatbehandeling of een uiting van zijn erfelijke botziekte, dan wel een combinatie van beide. Deze casus demonstreert dat niet alleen rekening moet worden gehouden met het ontstaan van atypische femurfracturen tijdens een behandeling met bisfosfonaten bij volwassenen, maar ook bij kinderen en tieners.

Een overzicht van de erfelijke botziekten die ook in verband zijn gebracht met atypische femurfracturen wordt gegeven in **Hoofdstuk 7.** Een systematische beoordeling van de wetenschappelijke literatuur heeft opgeleverd dat in zeven verschillende genetische botaandoeningen ook atypische femurfracturen worden gerapporteerd, waarbij een aanzienlijk deel van deze patiënten nooit bisfosfonaten heeft gebruikt. Dit impliceert dat een genetische botziekte op zichzelf mogelijk een risicofactor is voor het ontwikkelen van een atypische femurfractuur. Bovendien werd de diagnose van de genetische botziekte soms pas gesteld nadat de patiënt al een atypische femurfractuur had doorgemaakt. Dit kan erop wijzen dat een milde, nog niet herkende vorm van een genetische botaandoening aanwezig is bij patiënten met atypische femurfracturen.

Een ander argument dat pleit voor een genetische aanleg voor atypische femurfracturen, is het bestaan van families met meerdere familieleden met een atypische femurfractuur. In **Hoofdstuk 8** worden twee Aziatische families gepresenteerd met atypische femurfracturen en zoeken wij naar een genetische oorzaak hiervan middels *whole-exome sequencing*. Helaas zijn de families te klein om definitieve conclusies te trekken, maar de resultaten kunnen mogelijk in de toekomst worden benut voor replicatie in andere families. In **Hoofdstuk 9** wordt een grote Europese familie beschreven waarbij meerdere familieleden een atypische femurfractuur hebben doorgemaakt en/of osteoporose hebben. Ook bij deze familie worden vele mogelijke genetische varianten aangetroffen door middel van *whole-exome sequencing*, maar is er verdere filtering van de resultaten nodig om de oorzakelijke verandering in het DNA te vinden. Een specifieke erfelijke variant die wij vonden was extra interessant vanwege de lokalisatie in een gen dat in verband staat met het botmetabolisme. De uitdaging is echter om aan te tonen dat deze genetische variant daadwerkelijk van invloed is op het skelet bij mensen. Dierproeven of laboratoriumstudies op cellulair niveau meer inzicht geven in het effect van

het desbetreffende gen. Ook zou de aanwezigheid van de gevonden genetische variant in andere patiënten met atypische femurfracturen een sterke aanwijzing zijn dat dit een causale mutatie is voor de atypische femurfractuur. Een ander punt van aandacht in de verdere analyse van deze familie is de vraag of de osteoporose en atypische femurfracturen dezelfde genetische oorzaak hebben. Beide zijn complexe aandoeningen, omdat de ontstaanswijze van zowel osteoporose als de atypische femurfractuur niet alleen berust op erfelijkheid maar ook op medicatiegebruik en andere niet-genetische factoren. Dit maakt de genetische analyse van deze familie complex.

Deel 3: Medicamenteuze behandeling na een atypische femurfractuur

Patiënten die een atypische femurfractuur gehad hebben, vaak na langdurig bisfosfonaatgebruik, kunnen nog steeds een verhoogd risico hebben op osteoporotische botbreuken. Bisfosfonaten moeten worden gestaakt vanwege een verhoogde kans op verergering van een incomplete atypische femurfractuur of op het onstaan va een tweede atypische femurfractuur.

In **Hoofdstuk 10** worden alle beschikbare wetenschappelijke bronnen geanalyseerd op de veiligheid van andere osteoporosemedicatie ten aanzien van atypische femurfracturen, waaronder denosumab, teriparatide, raloxifeen en romosozumab. Denosumab wordt ook in verband gebracht met een hoger risico op atypische femurfracturen, hoewel er geen getallen zijn van grote patiëntencohorten om dit statistisch te bevestigen. Er is geen bewijs dat teriparatide een atypische femurfractuur kan veroorzaken noch voorkómen, hoewel de genezing na een operatie van een atypische femurfractuur wel sneller lijkt te verlopen met teriparatide. Helaas wordt ook bij gebruik van teriparatide soms gerapporteerd dat een atypische femurfractuur helemaal niet geneest. Raloxifeen en romosozumab zijn allebei sporadisch in verband gebracht met het optreden van atypische femurfracturen, maar het risico lijkt erg laag te zijn. **Hoofdstuk 10** geeft aanbevelingen voor de medicamenteuze behandeling na een atypische femurfractuur gebaseerd op de observationele data en *expert opinion*, ondersteund door de *European Calcified Tissue Society.* Hierbij moet wel opgemerkt worden dat er geen harde bewijzen zijn voor deze adviezen in de afwezigheid van gerandomiseerde gecontroleerde trials.

Conclusie

Inzicht in de pathofysiologie van atypische femurfracturen waaronder de klinische, genetische en radiologische aspecten is relevant om deze zeldzame doch ernstige botbreuk te voorkomen.

Wanneer het mogelijk is om hoog-risico patiënten te identificeren door middel van beeldvormende technieken of genetische testen, kan alternatieve osteoporosemedicatie

worden voorgeschreven en kan bisfosfonaattherapie voorbehouden blijven aan de laag-risico patiënten. In patiënten met (een hoog risico op) atypische femurfracturen kunnen alternatieve medicijnen voor osteoporose voorgeschreven worden. Deze behandeling op maat kan een essentiële rol spelen in het bestrijden van de onderbehandeling van osteoporose en de daarmee gepaard gaande ziektelast en sterfte.

Toekomstig onderzoek

Voortvloeiend uit de artikelen in dit proefschrift, doen wij meerdere suggesties voor toekomstig onderzoek naar atypische femurfracturen. De *trabecular bone score* van de lendenwervels is wellicht niet representatief voor het bovenbeen en derhalve is het interessant om parameters van de botkwaliteit specifiek ter plekke van de fractuurlokalisatie van de atypische femurfractuur te bestuderen. Biomechanische studies zouden inzicht kunnen geven in de ontstaanswijze van de atypische femurfractuur aan respectievelijk de buitenzijde van het femur en in sporadische gevallen aan de binnenzijde van het femur. Screening door middel van DEXA-scans in patiënten die bisfosfonaten gebruiken kan weliswaar atypische femurfracturen voorkómen, maar het tijdsinterval en de beste selectiecriteria voor de screeningspopulatie kunnen nog verder onderzocht worden waarbij ook aandacht moet zijn voor de kosten-baten ratio. Genetisch onderzoek naar atypische femurfracturen vereist een grote studiepopulatie en ook meer families met atypische femurfracturen om oorzakelijke genetische factoren vast te stellen. Ten slotte is er behoefte aan gerandomiseerde gecontroleerde onderzoeken om de beste medicamenteuze behandeling voor patiënten die al een atypische femurfractuur hebben doorgemaakt te bepalen, in het bijzonder studies naar de rol van teriparatide evenals de opties voor de patiëntengroep met niet-genezende atypische femurfracturen.

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List of publications
PhD portfolio
About the author
Dankwoord

LIST OF PUBLICATIONS

1. Delli Paoli G*, van de Laarschot DM*, Friesema ECH et al. *Short-Term, Combined Fasting and Exercise Improves Body Composition in Healthy Males*. Int J Sport Nutr Exerc Metab. 2020 Sep 30;30(6):386-395. doi: 10.1123/ijsnem.2020-0058.
2. Van de Laarschot DM, McKenna MJ, Abrahamsen et al. *Medical Management of Patients After Atypical Femur Fractures: a Systematic Review and Recommendations From the European Calcified Tissue Society.* J Clin Endocrinol Metab. 2020 May 1;105(5):1682-1699. doi: 10.1210/clinem/dgz295.
3. Cheung AM, McKenna MJ, van de Laarschot DM et al. *The Official Positions of the International Society for Clinical Densitometry: Detection of Atypical Femur Fractures*. J Clin Densitom. Oct-Dec 2019;22(4):506-516. doi: 10.1016/j.jocd.2019.07.003.
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5. Nguyen HH*, van de Laarschot DM*, Verkerk JMH, Milat F, Zillikens MC, Ebeling PR. *Genetic risk factors for atypical femoral fractures (AFFs): a systematic review*. JBMR Plus. 2018 Jan 3;2(1):1-11. doi: 10.1002/jbm4.10024.
6. van de Laarschot DM, Smits AA, Buitendijk SK, Stegenga MT, Zillikens MC. *Screening for atypical femur fractures using extended femur scans by DXA*. J Bone Miner Res. 2017 Aug;32(8):1632-1639. doi: 10.1002/jbmr.3164.
7. van de Laarschot DM, Zillikens MC. *Atypical femur fracture in an adolescent boy treated with bisphosphonates for X-linked osteoporosis based on PLS3 mutation*. Bone. 2016 Oct;91:148–51. doi: 10.1016/j.bone.2016.07.022.
8. van de Laarschot DM, Somford MP, Jager A, Oei EH, Bos PK, Zillikens MC. *"Atypical" atypical femur fractures and use of bisphosphonates.* Clin. Cases Miner. Bone Metab. 2016 Sept-Dec. 13(3):204–8. doi: 10.11138/ccmbm/2016.13.3.204.

   * Shared first author

PHD PORTFOLIO

Name PhD student:	Denise M. van de Laarschot
Erasmus MC Department:	Internal Medicine
PhD period:	July 2016 – July 2021
Promotors:	prof. dr. M.C. Zillikens
	prof. dr. A.G. Uitterlinden

	General courses	**Workload (ECTS)**
2019	Scientific Integrity Course	0.3
2016	Biostatistical Methods I, Netherlands Institute for Health Sciences	5.7
	Specific courses	
2017	SNP course XIV, Molecular Medicine Research School	2.0
2017	NGS in DNA diagnostics, Molecular Medicine Research School	1.0
2017	Diagnostic data for dummies, Erasmus MC Summer School	0.7
2016	Basic Human Genetics course, Molecular Medicine Research School	0.5
	Seminars and workshops	
2019	Herbert Fleischer Workshop, Bruges, Belgium (International Federation of Musculoskeletal Research Societies)	0.7
2019	Bone Academy meeting, Amsterdam (AmGen, UCB Pharma)	0.4
2017	PhD day, Erasmus MC	0.3
	National conferences: oral presentations	
2019	Dutch Society of Rheumatology work group osteoporosis, Arnhem	0.2
2019	Dutch Endocrine Meeting, Noordwijkerhout	0.4
2018	Interdisciplinary Work Group Osteoporosis (IWO), Utrecht	0.2
2018	VF&O symposium, Antonius Hospital, Nieuwegein	0.2
2018	Dutch Society for Calcium and Bone Metabolism meeting, Zeist	0.6
2018	Dutch Endocrine Meeting, Noordwijkerhout	0.4
2017	Young Dutch Society of Endocrinology meeting, Leiden	0.3
2017	Dutch Society for Calcium and Bone Metabolism meeting, Zeist	0.6
2016	Dutch Society for Calcium and Bone Metabolism meeting, Zeist	0.6
	International conferences	
2019	European Calcified Tissue Society, Budapest, Hungary (oral poster)	1.3
2019	International Osteoporosis Foundation, Paris, France (oral poster)	0.9
2018	American Society for Bone and Mineral Research, Montréal, Canada (poster)	1.0
2017	American Society for Bone and Mineral Research, Denver, Colorado, USA (oral presentation and poster)	1.0
2017	European Calcified Tissue Society, Salzburg, Austria (oral presentation)	1.3

Teaching activities

Nov 2020	Bone Health TeleECHO program, University of New Mexico	0.2
April 2019	Lecture for Endocrinology fellows	0.2
Nov 2017 – Apr 2018	Supervision of master student (Dorith Vermeulen) Relation between TBS and BMD in liver disease	3.0
Dec 2017 – Jan 2018	Supervision of 2nd year medical students, systematic review (Kyra Compagne, Maud van Leeuwen)	0.4
2016-2017	Supervision of master student (Sanne Buitendijk)	3.0
2016	Lecture Erasmus University: College MA1.B15	0.1

Other

2017	Organizing Committee PhD-day, Erasmus MC	0.5
2018-2019	Member Task Force, International Society for Clinical Densitometry	1.2
2018-2019	Dutch Society of Endocrinology - Bone meetings	0.2
2018-2019	TrAFFic consortium, analyst Genetic working group	1.0
2017-2019	Academic Centre of Excellence - musculoskeletal diseases	0.8
2016-2019	Research meetings of Genetic Laboratory of Internal Medicine	4.2
2016-2019	Multidisciplinary meetings of the Bone Centre, Erasmus MC	2.5
2016-2019	National meetings of musculoskeletal experts (AmRoLei)	1.0
2016-2019	Reporting DXA scans of Bone Centre, Erasmus MC	29
2016-2019	Outpatient clinic Bone Centre, Erasmus MC	38

ECTS = European Credit Transfer and Accumulation System
1 ECTS represents 28 hours

ABOUT THE AUTHOR

Denise Milou van de Laarschot was born in Rotterdam on the 2nd of October 1991.

After graduating *cum laude* from Erasmiaans Gymnasium in Rotterdam in 2009, she studied Medicine at the Erasmus University of Rotterdam. She did a minor in Global Health and Tropical Medicine in Zambia. She participated in several committees of the Medical Students' Association. Between 2010 and 2016 she worked as a student assistant at the outpatient department of Internal Medicine of the Erasmus Medical Centre. As a Master student, she started research on atypical femur fractures (AFFs) under supervision of Prof. Carola Zillikens.

After obtaining her medical degree in 2016, she started working as a physician at the Bone Centre of Endocrinology at the Erasmus Medical Centre. She combined this with her PhD project on AFFs, guided by Prof. Carola Zillikens and Prof. André Uitterlinden.
She was part of a genetic working group for an AFF consortium (TrAFFic) in collaboration with Prof. Peter Ebeling from Australia, also including researchers from Singapore, the U.S.A. and the U.K.
She was member of a Task Force by the International Society for Clinical Densitometry for a position statement on detecting incomplete AFF with DXA scanning (Prof. Angela Cheung, Dr. Michael Lewiecki). She worked on recommendations for medical management of patients with AFF, enhanced by the European Calcified Tissue Society (Prof. Malachi McKenna, Prof. Bo Abrahamsen, Prof. Bente Langdahl, Prof. Martine Cohen-Solal, Prof. Núria Guañabens, Prof. Richard Eastell, Prof. Stuart Ralston).
In 2019, she started working as a physician at the inpatient clinic of Internal Medicine in Reinier de Graaf Gasthuis, Delft. Since 2020, she has worked at the Maasstad Hospital in Rotterdam for her traineeship in Internal Medicine.

DANKWOORD

Toen ik een jaarcontract aangeboden kreeg als basisarts bij het Botcentrum in 2016, was het allesbehalve zeker dat ik de kans zou krijgen om een volledig promotietraject te doorlopen.

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