



Accumulation of microdamage at complete and incomplete fracture sites in a patient with bilateral atypical femoral fractures on glucocorticoid and bisphosphonate therapy

Ken Iwata¹ · Tasuku Mashiba¹ · Satoshi Mori² · Tetsuji Yamamoto¹

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Introduction

Atypical femoral fractures are defined in the second report by the Task Force of the American Society for Bone and Mineral Research in 2014 [1] as stress and fragility fractures caused by deterioration of bone quality resulting from suppressed bone turnover associated with exposure to bisphosphonates and/or glucocorticoids, abnormal alignment of the lower extremities because of femoral lateral bowing or genu varum, and other skeletal diseases such as hypophosphatasia, pycnodysostosis, and osteopetrosis. Odvina et al. reported that marked suppression of bone turnover by long-term bisphosphonate therapy increased the risk of atypical femoral fracture [2]. Recently, we reported accumulation of microdamage in the lateral cortical bone adjacent to an atypical femoral fracture site and marked suppression of cancellous bone turnover on an iliac bone biopsy at this site in a patient who had received long-term bisphosphonate therapy [3]. Our report indicated that the stress fracture occurred because accumulated microdamage was not repaired because of severely suppressed bone turnover. In the present study, we performed biopsy of the cortical bone around bilateral atypical femoral fractures (complete fracture of one leg and incomplete fracture of the other) in a patient with rheumatoid arthritis and neurosarcoidosis on glucocorticoid and bisphosphonate therapy for 10 years and 20 months, respectively, and herein report our pathological findings. The patient and

her family provided written informed consent for data from her case to be submitted for publication.

Case report

A 68-year-old woman was transported to our hospital because she was unable to stand after a fall while walking. She had underlying rheumatoid arthritis treated with glucocorticoids for 10 years since diagnosis and with etanercept (50 mg/week) for approximately 5 years. She also had underlying osteoporosis that was treated with alendronate (35 mg/week) for 20 months since diagnosis and neurosarcoidosis that was treated with pulse glucocorticoid therapy 9 months before presentation and prednisone (7.5 mg/day). She was diagnosed with an atypical right femoral fracture. Plain X-ray imaging showed a transverse subtrochanteric fracture of the right femur and lateral cortical thickening (Fig. 1). Intramedullary nailing was performed the following day, with biopsies of the lateral thickened cortical bone adjacent to the fracture site and cancellous bone at the nail insertion site in the greater trochanter (Fig. 2a, d). Blood tests performed 4 days after surgery showed a tartrate resistant acid phosphatase 5b level of 89 mU/dL (normal range 120–420 mU/dL), which was abnormally low compared with the level seen immediately after a fracture [4]. Bone mineral density (BMD) was evaluated by dual-X-ray absorptiometry using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA). BMD at the lumbar spine and left femoral neck was 0.818 g/cm² (*T* score –1.8) and 0.493 g/cm² (*T* score –2.7), respectively. 5 months after surgery, the inserted nail was found to have broken due to incomplete bone union; this was replaced with a long intramedullary nail and an allogeneic bone graft was performed. Finally, complete bone union was achieved 1 year after revision surgery (Fig. 2e). During the period of revision, an atypical incomplete fracture of the left femur, without prodromal

✉ Ken Iwata
kiwata@med.kagawa-u.ac.jp

¹ Department of Orthopedic Surgery, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-Gun, Kagawa 761-0793, Japan

² Department of Orthopedic Surgery, Seirei Hamamatsu Hospital, 2-12-12 Sumiyoshi, Nakaku, Hamamatsu City, Shizuoka 430-0906, Japan



Fig. 1 Plain radiograph of the right femur taken immediately after the fracture showing a transverse subtrochanteric fracture and lateral cortical thickening (white arrow)

pain, was detected on plain X-ray and was judged to be at high risk of complete fracture, so prophylactic intramedullary nailing was performed and a biopsy was taken (Fig. 3a,

d). All bone biopsy specimens were stained in bulk and the extent of microdamage was assessed using bone morphology analysis software (System Supply, Nagano, Japan); the area was measured at least 1.5 mm from the edge of the collected bone to avoid the biopsy needle infringing on the resection margin, as described in our previous study [3]. A small number of microcracks were observed at the site of nail insertion (the cancellous bone of the greater trochanter, Fig. 2b) and many were found in the cortical bone adjacent to the femoral fracture (Fig. 2c). In the area corresponding to the incomplete fracture of the left femur, a fracture line was confirmed running across the entire area of the cortical bone specimen (Fig. 3b), with many microcracks (Fig. 3b, c). The method reported by Burr et al. for evaluation of microdamage [5] showed that the cortical bone adjacent to the right femoral fracture had an estimated microcrack density of $1.9/\text{mm}^2$, microcrack length of $109\ \mu\text{m}$, and microcrack surface density of $210\ \mu\text{m}/\text{mm}^2$, while the cortical bone adjacent to the left femoral incomplete fracture had a microcrack density of $6.8/\text{mm}^2$, microcrack length of $168.6\ \mu\text{m}$, and microcrack surface density of $1149.8\ \mu\text{m}/\text{mm}^2$ (Table 1). Mean estimated crack length at the complete femoral fracture site on the right was comparable, but the mean microcrack density was considerably larger in our patient than in a report by

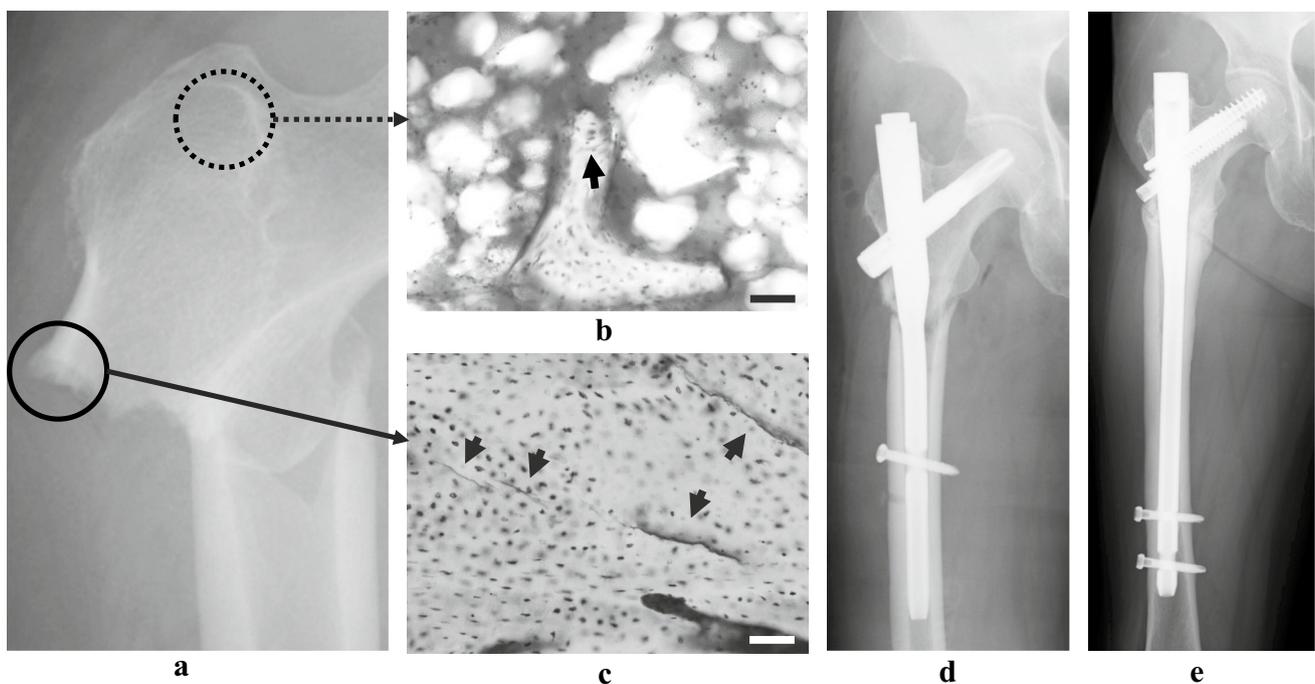


Fig. 2 **a** A cortical bone specimen was taken from the area of thickened lateral cortical bone adjacent to the fracture site (solid line circle). A cancellous bone specimen was taken from the greater trochanter, which had been hollowed to allow insertion of an intramedullary nail (dotted line circle). **b** A few microcracks can be observed in the cancellous bone of the greater trochanter on light micros-

copy (black arrow). Scale bar $100\ \mu\text{m}$. **c** Many microcracks can be observed at the fracture site in the right femur (black arrows). Scale bar $100\ \mu\text{m}$. **d** Postoperative X-ray film shows fixation of the fracture by a short femoral nail. **e** 1 year after revision surgery. X-ray film shows complete bone union by means of a long femoral nail

Fig. 3 **a** A cortical bone specimen taken from the thickened lateral cortical bone showing the incomplete fracture site (solid line circle). **b** A large fracture line crossing the cortical bone completely (white arrows) and many microcracks are observed around it (black arrows). Scale bar 500 μm . **c** Numerous and extremely long microcracks are observed at the site of the incomplete fracture of the right femur (black arrows). Scale bar 100 μm . **d** Postoperative X-ray film shows that the fracture was fixed using a long femoral nail

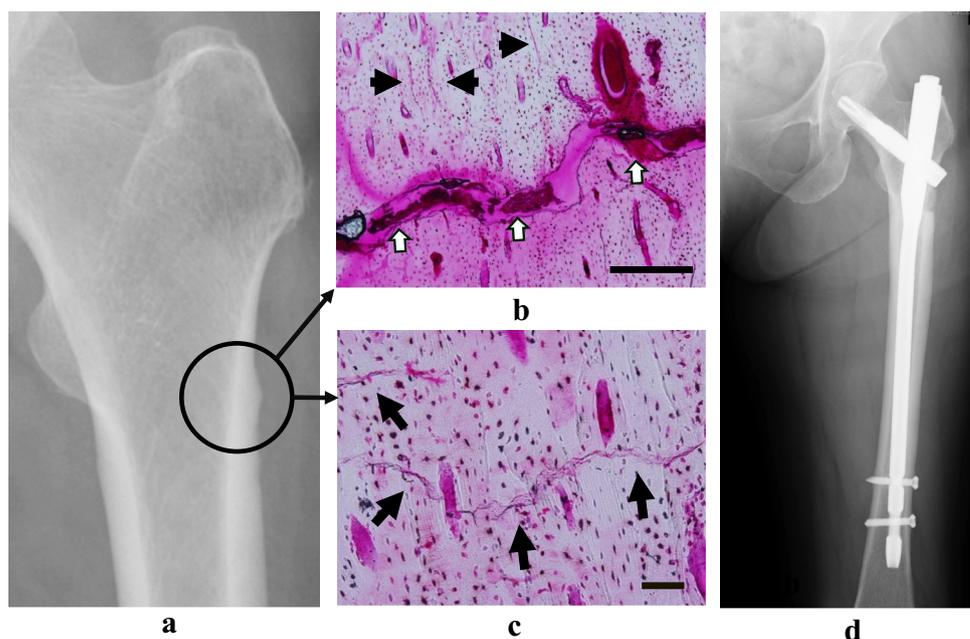


Table 1 Assessment of microdamage in cortical bone

Microdamage parameter	Present case		Norman and Wang
	Right femur	Left femur	
Crack density (mm^2)	1.9	6.8	0.21
Crack length (mm)	109	168.6	92.9
Crack surface density (mm/mm^2)	210	1149.8	19.5

More severe microdamage accumulation was observed in the present case than in the 27 cadavers assessed by Norman and Wang [6]

Norman and Wang [6], who determined the degree of microdamage in cortical bone adjacent to a right femoral fracture site in autopsy specimens (Table 1). However, the mean microcrack length and microcrack density in the cortical bone were both larger at the site of the incomplete left femoral fracture in our patient than in the report by Norman and Wang [6]. Moreover, frequency histograms of the number of microcracks in the cortical bone at each femoral fracture site according to microcrack length showed a similar pattern (Fig. 4a, b). In addition, many microcracks measuring over 200 μm were observed at the incomplete fracture site in the left femur. Microcracks at this site were significantly longer than those at the complete fracture site in the right femur ($p < 0.0005$, unpaired *t* test). Furthermore, a microcrack density of $0.9/\text{mm}^2$, a microcrack length of 28.8 μm , and a microcrack surface density of $25 \mu\text{m}/\text{mm}^2$ in the cancellous bone of the greater trochanter were confirmed in the present study. These findings are comparable with those obtained by Fazzalari et al. [7] in their study of cancellous bone microdamage at the proximal femur in autopsy specimens (Table 2).

Discussion

Use of bisphosphonates is a well-known risk factor for atypical femoral fracture [8–13]. We have previously reported that the pathophysiology of atypical femoral fractures occurring in patients on long-term bisphosphonate therapy involves stress fractures caused by accumulation of microdamage that is not repaired by bone remodeling because of severe suppression of bone turnover [3]. Previously, we showed that exposure to high doses of bisphosphonates for 1–3 years suppressed remodeling in vertebral trabecular bone and rib cortical bone, thereby inhibiting microdamage repair, resulting in increased microdamage accumulation in normal beagle dogs [14–18]. We recovered the lumbar spines from beagle dogs for in vitro loading test and showed that the incidence of microdamage was significantly higher in those that received alendronate than in the control group [19]. Furthermore, comparing ovariectomized cynomolgus monkeys exposed to alendronate with those exposed to minodronate, the degree of microdamage accumulation in the lumbar spines was higher, despite milder suppression of remodeling, underpinning the notion that the degree of repair suppression is not the sole determinant of microdamage accumulation, rather agents administered also influence the level of damage accumulation [20].

Several studies have demonstrated an association between atypical femoral fractures and use of glucocorticoids. For example, in studies by Thompson et al. [11] and Dell et al. [13], atypical femoral fractures occurred, respectively, in 8 out of 27 (27%) patients and 17 out of 142 (12%) patients on glucocorticoid therapy. It is not clear whether microdamage accumulation from long-term glucocorticoid therapy, based

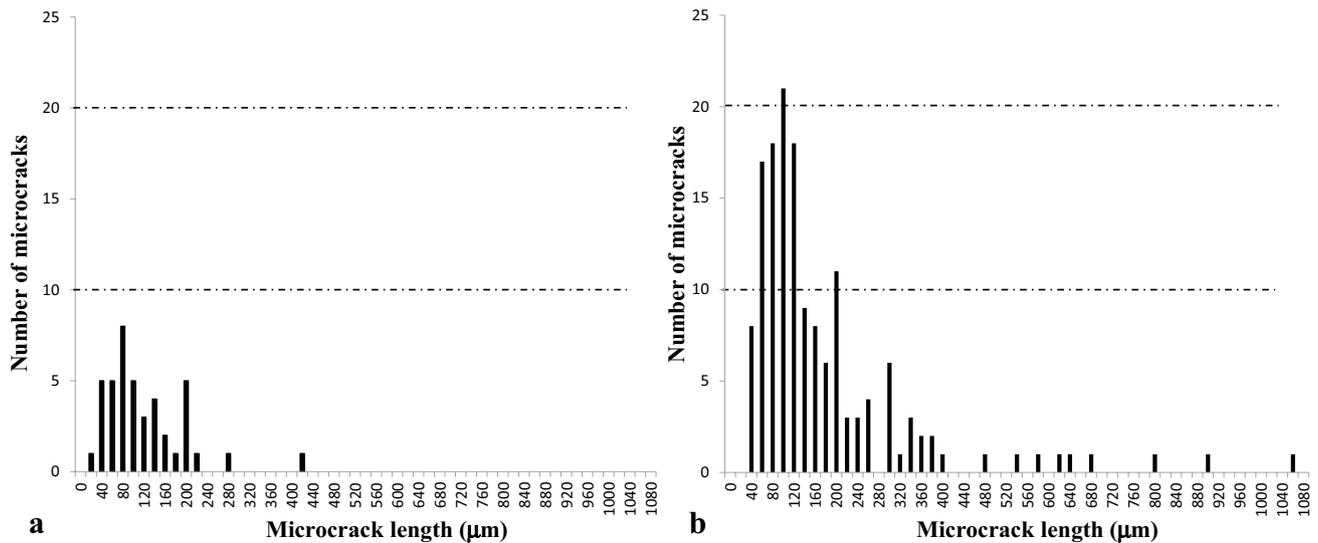


Fig. 4 Frequency histogram of the number of microcracks in each cortical bone in the site of the fracture in the femur. **a** Histogram of the right femur shows the peak frequency is 80–100 μm . **b** Histogram of the left femur shows the peak frequency is 100–120 μm . Both histograms are similar in shape, but the peak of the histogram appears

at a longer microcrack length for the left femur compared with the right femur. In addition, many long microcracks over 200 μm were observed in the left femur. Microcracks in the left femur at the incomplete fracture site are significantly longer than those in the right femur at the complete fracture site ($p < 0.0005$, unpaired t test)

Table 2 Assessment of microdamage in cancellous bone

Microdamage parameter	Present case	Fazzalari et al.
Crack density (mm^2)	0.9	0.51
Crack length (mm)	28.8	63
Crack surface density (mm/mm^2)	25	32.9

Accumulation of microdamage in the present case was comparable with that in the 12 cadavers assessed by Fazzalari et al. [7]

on glucocorticoid-induced osteocyte apoptosis, decreases osteocyte and the lacunar-canalicular network sensing system of microdamage to repair [21, 22], but long-term glucocorticoid and bisphosphonate therapy is thought to be a risk factor for atypical femoral fracture [23]. In addition, Girgis et al. reported that rheumatoid arthritis and use of glucocorticoids were risk factors for atypical femoral fractures [8]. In our patient, there were multiple risk factors for atypical femoral fracture, including rheumatoid arthritis, long-term use of glucocorticoids, and use of bisphosphonates. We have previously reported finding extensive accumulation of microdamage in the cortical bone adjacent to a femoral fracture site. In our present patient, we found similar accumulation of microdamage in the femoral cortical bone adjacent to the fracture site at a level considerably higher than that in the autopsy specimens reported by Norman and Wang [6]. However, in the present patient, the level of accumulation at the nail insertion site in the cancellous bone of the greater trochanter was comparable with that in the autopsy specimens described by Fazzalari et al. [7]. These

findings suggest that such microdamage accumulates at the site where stress is concentrated during load bearing rather than throughout the entire femur. Hagen et al. examined the neck-shaft angles in patients with atypical femoral fractures, and reported that these angles were smaller in the group with fractures than in the group without, suggesting a higher risk of fracture in individuals with genu varum [24]. Sasaki et al. reported that the degree of femoral curvature was significantly higher in patients with an atypical femoral fracture than in those without [25]. In addition, Saita et al. showed that the location of the fracture correlated with the standing femorotibial angle and that the location of the fracture was roughly the same on both sides in patients with bilateral atypical femoral fractures [26]. Because the mechanical axis of the lower limb runs through the medial side of the proximal part of the femur [27, 28], tensile force is applied on the lateral side of the cortical bone of the femur. In individuals with femoral lateral bowing, coxa vara, or genu varum, this tilting tendency would be more severe, suggesting increased levels of tensile force on the lateral cortex. Burr et al. performed ex vivo four-point bending tests in canine femurs and demonstrated that cortices under tension were more prone to generating microcracks than cortices under compression, consistent with the notion that atypical femoral fracture is initiated from the lateral cortical side [29]. The patient described in the present study did not develop lateral bowing, but had atypical femoral fractures at roughly the same location on both sides, which is in good agreement with Saita et al. [26].

This is the first report to evaluate accumulation of microdamage at the site of an incomplete atypical femoral fracture, and demonstrates greater accumulation of microdamage around the fracture line in an incomplete fracture than in a complete fracture (Table 1). A possible explanation for this difference may lie in the different sites evaluated. That is, the bone sample on the side with the complete fracture was from the area adjacent to the fracture line whereas that on the side of the incomplete fracture included the fracture line. In a fatigue loading study using a compact tension specimen made from human and bovine cortical bone, Vashishth et al. identified a relationship between formation of microcracks and propagation of a fracture line [30, 31]. In their study, propagation of the main crack that created the fracture line was accompanied by microcracks adjacent to the main crack. Therefore, the parameters indicating microdamage on the side with the complete femoral fracture in our present patient may be underestimated because the area of bone evaluated on the side with the complete fracture was apart from the fracture line, and therefore, did not include many associated microcracks.

In this study, we found marked accumulation of microdamage in the cortical bone adjacent to the fracture site, but not in the cancellous bone of the greater trochanter. This suggests that accumulation of damage does not occur systemically even if use of steroids and/or bisphosphonates causes low bone turnover. The balance between generation and repair of the microdamage determines the degree of accumulation of damage, such that stress fractures occur without suppression of repair when underlying bone fragility is present, and conversely, these fractures can occur even with adequate bone strength in response to excessive stress concentration and severe suppression of bone remodeling. Although deterioration in bone quality was not found in the case, the pathology of her atypical femoral fractures could be explained by accumulation of insufficiently repaired microdamage that developed initially because of stress concentration due to bone geometry and weight-bearing.

This study had some limitations. We did not perform iliac bone biopsy nor did we investigate other bone turnover markers like parathyroid hormone, bone-specific alkaline phosphatase, total amino-terminal propeptide of type I collagen, and N-terminal telopeptide of collagen type I. Thus, we cannot state whether severely suppressed bone turnover occurred. Moreover, assessment of osteocytes was also not done, and so no inference can be made regarding the effect of glucocorticoids on osteocytes.

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Compliance with ethical standards

Ethical approval The study was approved by the local ethics commission. Written informed consent was obtained from the patient and her family.

Conflict of interest None of the authors has a conflict of interest associated with this study.

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