Osteoporos Int (2011) 22:2951–2961
DOI 10.1007/s00198-011-1804-x

REVIEW

# Pathophysiology of atypical femoral fractures and osteonecrosis of the jaw

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Received: 7 September 2011 / Accepted: 23 September 2011 / Published online: 14 October 2011


**Abstract** In recent years, atypical femoral fractures and osteonecrosis of the jaw have emerged as potential complications of long-term bisphosphonate therapy; osteonecrosis of the jaw has also been reported in patients receiving high doses of denosumab. The pathophysiology of both conditions is poorly defined, and the underlying mechanisms are likely to differ. The initiation of atypical fractures in the lateral femoral shaft suggests that reduced tensile strength, possibly secondary to alterations in the material properties of bone resulting from low bone turnover, may be an important pathogenetic factor. Osteonecrosis of the jaw is characterised by infection, inflammation, bone resorption and bone necrosis, but the sequence in which these occur has not been established. However, the observation that bone resorption occurs in close proximity to microbial structures suggests that infection may be the most important trigger, often as a result of dental disease. Other possible pathogenetic factors include suppression of bone turnover, altered immune status and adverse effects of bisphosphonates on the oral mucosa.

**Keywords** Atypical femoral fractures · Bisphosphonates · Denosumab · Microdamage · Osteonecrosis of the jaw · Suppression of bone turnover

In the last decade, atypical femoral fractures (AFFs) and osteonecrosis of the jaw (ONJ) have emerged as potential complications of long-term bisphosphonate therapy. These conditions are rare, but are associated with significant morbidity. Both affect specific skeletal sites, may occur in bisphosphonate-naive patients, and in patients exposed to bisphosphonates, appear to be related to the duration of therapy. However, whilst ONJ has also been reported in patients taking denosumab, AFFs have so far only been associated with bisphosphonate therapy, and whereas the incidence of ONJ is higher in patients receiving larger doses of bisphosphonates or denosumab for oncological indications than in those receiving smaller doses for osteoporosis, this dose response is not apparent for bisphosphonates and AFFs. These latter differences indicate distinct pathophysiological mechanisms, and in this review, the two conditions will be considered separately.

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## Atypical femoral fractures

### Clinical and radiological features

AFFs have strikingly characteristic clinical and radiological features that may provide some clues as to their pathogenesis. They occur in the subtrochanteric or diaphyseal region of the femur and have a simple transverse or oblique pattern, without comminution. They occur after minimal trauma, are often preceded by prodromal pain, which may be of several months' duration, and are bilateral in approaching 50% of cases. Cortical thickening and medial beaking are commonly seen, and healing is often delayed [1]. Atypical fractures appear to originate in the lateral cortex, where they may show features characteristic of a stress fracture with a periosteal reaction (Fig. 1). Other characteristics shared with stress fractures include prodromal pain, bilaterality and the transverse configuration of the fracture. In contrast, the prodromal pain, bilaterality, cortical thickening and delayed healing associated with atypical fractures are not recognised characteristics of fragility fractures in patients with osteoporosis and argue for a different pathogenesis.

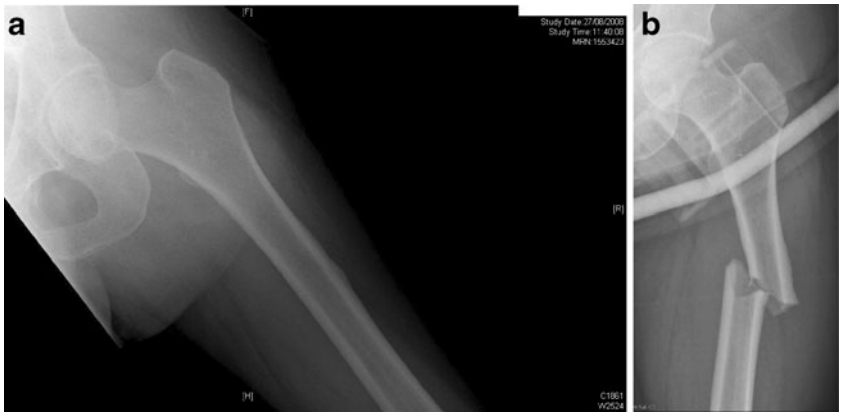


**Fig. 1** **a** Periosteal reaction in the lateral cortex of a 58-year-old woman who had been on bisphosphonate therapy for 5 years. **b** Two months later, she presented with a complete fracture with characteristics of an atypical fracture. Note the diffuse cortical thickening and medial beaking

## Association with bisphosphonate therapy

Atypical fractures were first described in a small group of patients treated with alendronate, sometimes in combination with other anti-resorptive agents; in all cases, iliac crest bone biopsies showed evidence of marked suppression of bone turnover [2]. This study and other case reports fostered the perception that bisphosphonate-induced suppression of bone turnover was responsible for the development of atypical fractures. However, a direct causal role for bisphosphonates has yet to be established.

Epidemiological data indicate that subtrochanteric and femoral shaft fractures account for approximately 10% of all hip and femoral fractures in the elderly [3]. Of these, around 15–30% are atypical, and AFFs thus probably account for only about 1% of all hip and femoral fractures [4]. In most studies that have utilised databases to establish the frequency of subtrochanteric and femoral shaft fractures and their association with bisphosphonate therapy, viewing of X-rays has not been possible, and thus, atypical fractures have not been specifically identified. In addition, the number of individuals receiving long-term bisphosphonate therapy has often been relatively small; the lack of a demonstrated association between bisphosphonate therapy and atypical femoral fractures in most of these studies may reflect these limitations [5, 6]. Similarly, in a secondary analysis of randomised controlled trials of bisphosphonates in postmenopausal women with osteoporosis, no excess of subtrochanteric and femoral shaft fractures was reported in treated versus control patients, but X-rays were unavailable, and the duration of treatment was relatively short [7]. However, results from a recent population-based case control study, again without adjudication of X-rays, showed a significant increase in risk of subtrochanteric and shaft fractures in women with long-term bisphosphonate use (≥5 years) compared to those with shorter-term use (odds ratio, 2.74; 95% CI 1.25, 6.02) [8].

Another large study from the USA demonstrated different trends in the age-related incidence of hip fractures and subtrochanteric fractures, the former decreasing and the latter increasing over recent years; data from a separate database indicated that the rise in subtrochanteric fractures was more or less paralleled by an increase in bisphosphonate prescribing [9]. Femoral shaft X-rays were not included in this analysis, and subtrochanteric fractures were not adjudicated; nevertheless, this study provides some indirect evidence for an association between bisphosphonate therapy and AFFs.

Studies in which features of AFFs have been identified from X-rays have mostly been relatively small, but have sometimes demonstrated an association between duration of bisphosphonate use and occurrence of atypical fractures [10–12]. These studies are also important in demonstrating that atypical fractures, with all the characteristic features noted above, may occur in individuals who have never been treated with a bisphosphonate. Schilcher et al. reviewed X-rays of 1,234 women with a subtrochanteric or shaft fracture sustained in 2008 and identified 59 women with atypical fractures, 78% of whom had received treatment with a bisphosphonate. In the cohort analysis, the age-adjusted relative risk of atypical fracture was 47.3 (95% CI 25.6, 87.3); however, it should be noted that data on drug use were not available before 2005 and that substantial but unmeasured bisphosphonate exposure prior to this time may have resulted in overestimation of risk. Case control analysis demonstrated increasing risk with increasing duration of bisphosphonate therapy and a 70% reduction in risk/year after stopping bisphosphonate therapy [13].

Bisphosphonate-associated atypical fractures have most commonly been described in patients taking alendronate, most likely reflecting its predominance in the osteoporosis market. However, cases have also been described with other bisphosphonates including risedronate, etidronate, ibandronate, pamidronate, and zoledronic acid. At the present time, no cases have been reported in patients taking other anti-resorptive agents. The mean duration of bisphosphonate use varies between 3 months and 16 years [4]. A small number of so-called atypical fractures have also been reported at other sites including the metatarsals, ankle, tibia, fibula, pelvis, sacrum and pedicles of L5 [2, 14]. The vast majority of case reports of bisphosphonate-associated

atypical fractures have been in patients treated for osteoporosis; although there have been sporadic case reports in patients receiving higher doses of bisphosphonates for skeletal malignancy, there does not appear to be the clear dose response seen for osteonecrosis of the jaw [15, 16].

## Pathophysiology

### *Suppression of bone turnover*

The explanation most commonly advanced for the development of AFFs is that bone turnover is suppressed, resulting in adverse effects on bone material properties and strength. Before considering the potentially harmful effects of reduced bone turnover, however, it is important to recognise that suppression of bone turnover in the postmenopausal skeleton has well-documented beneficial effects on bone strength and fracture risk and provides the rationale for most of the pharmacological interventions currently used in the management of osteoporosis. Thus, reduction in fracture risk, accompanied by increased bone mineral density and preservation of bone microarchitecture, has been demonstrated for all approved anti-resorptive drugs during a minimum of 3 years of treatment. Furthermore, AFFs have not so far been reported in patients receiving non-bisphosphonate anti-resorptive agents, indicating that suppression of bone turnover per se may not be the sole pathogenetic mechanism. In addition, if AFFs are a consequence of suppressed bone turnover, they might be expected to occur in other conditions in which low bone turnover is a feature. The pseudofractures that occur in the femoral shaft in hypophosphatasia and in X-linked hypophosphataemia bear some resemblance to atypical femoral fractures although, in the latter condition, a medial location is more common [17]. In osteosclerotic bone diseases due to decreased bone resorption, however, AFFs have not been reported, nor have they been described in other conditions associated with low bone turnover such as hypothyroidism or hyperparathyroidism.

Nevertheless, there are a number of potential mechanisms by which suppression of bone turnover may reduce bone strength. These include increased mineralisation of bone and reduced heterogeneity of mineralisation, changes in collagen composition and accumulation of microdamage.

### *Mineral density and distribution*

Increases in the density and homogeneity of mineralisation have been reported in postmenopausal women treated with bisphosphonates for up to 3 years, although there is some evidence that both the density and distribution of mineral return to normal premenopausal status with longer-term therapy [18–21]. The increase in mineralisation observed in the first years of treatment contributes to the increase in BMD and is associated with increased strength and stiffness of bone; however, greater homogeneity of mineralisation may allow microcracks to propagate more rapidly. Most studies have been conducted in iliac crest trabecular bone, but recent data reported in women treated with long-term bisphosphonates indicate that the changes in mineralisation density and distribution in cortical bone of the proximal femoral may be more pronounced [22]. Conversely, Somford et al. reported that mineralisation was not increased in bone obtained from the subtrochanteric region of the femur close to the site of an atypical fracture [23].

### *Collagen maturity and cross-linking*

Bisphosphonates have been shown to increase collagen maturity and cross-linking. An increase in the ratio of PyrD/DPD following bisphosphonate therapy has been reported in dogs, an effect associated with increased stiffness and strength of bone [24–27]. However, suppression of bone turnover was also associated with increased production of advanced glycation end products, with a reduction in the toughness of bone [24]. Data in humans are sparse, and it remains uncertain whether changes in collagen due either to suppression of bone turnover or, more specifically, to bisphosphonates contribute to treatment-induced changes in bone strength.

### *Microdamage*

In both humans and preclinical models, age-related increases in microdamage accumulation have been reported [28]; in dogs, it has also been shown that suppression of bone turnover with bisphosphonates increases microdamage and reduces bone toughness [29–31]. In an ovine model, long-term administration of high doses of zoledronic acid resulted in more microcracks in metacarpal bone than in ovariectomised or control animals, although these cracks were shorter [32]. Two studies in iliac crest bone from women treated with bisphosphonates have produced conflicting results, one suggesting increased microdamage in iliac crest bone obtained from postmenopausal women treated with alendronate whilst another showed no evidence for such an effect [33, 34]. In the former group of patients, a subsequent study demonstrated that treatment with teriparatide for 2 years was associated with significantly decreased crack density, surface density and length in previously alendronate-treated patients whereas only crack length was significantly reduced in those who were previously treatment naive. No significant correlation was found between biochemical or histomorphometric indices of bone turnover and microdamage in teriparatide-treated patients; however, increased bone turnover in response to teriparatide is maximal in the first year to 18 months of treatment, whereas microdamage was only assessed after 2 years of treatment [35]. Accurate demon-

stration of microcracks in bone sections is challenging; in addition, there is evidence that marked variations in microdamage occur between skeletal sites. The reduction in toughness demonstrated in bisphosphonate-treated dogs is not closely related to microdamage accumulation, suggesting that effects of bisphosphonates and/or suppression of bone turnover are also mediated through other mechanisms.

Accumulation of bisphosphonates at sites of microdamage could inhibit repair of microcracks, resulting in their propagation and the development of a stress fracture. Unlike conventional fractures, stress fractures heal by bone remodelling, and bisphosphonate therapy might therefore delay their healing and allow progression to more complete fractures. Data on the effects of bisphosphonate therapy in humans with stress fracture are sparse; in a study of military recruits, risedronate did not reduce the development of stress fractures, although its effects on healing of established fractures were not assessed [36]. Two recent studies in animals support the view that bisphosphonates adversely affect the healing of stress fractures. In rats treated with high doses of risedronate, there was a reduction in the volume of bone formed and resorbed during healing of large stress fractures, resulting in a significantly lower length of repair along the fracture crack [37]. In another study in rats, relatively small doses of alendronate were also shown to delay stress fracture repair, whereas parathyroid hormone expedited repair [38]. In both these studies, stress fractures were induced by mechanical loading in the ulna in adult rats; whether the results can be extrapolated to humans requires further study.

*Biopsy studies*

Suppression of bone turnover is an expected finding in patients treated with bisphosphonates, and its presence in those with AFFs therefore does not necessarily implicate it as a pathogenetic factor. Biochemical markers of bone turnover may be reduced, normal or elevated in affected patients [2, 39–41]; this variability is likely to reflect, at least in part, the presence of fracture. Histomorphometric data are relatively sparse and mainly limited to the iliac crest; however, double tetracycline labelling has been reported in approximately 30% of the biopsies studied, indicating that bone turnover is not severely suppressed in all patients [4]. Moreover, bone resorption, when assessed, has been reported to be normal or increased in the majority of cases, and in one report in which a biopsy was obtained from the fracture site soon after fracture, the presence of osteoclasts and resorption was noted [4]. There is therefore no evidence from these studies that bone turnover is more suppressed in bisphosphonate-treated patients with AFFs than in those without these fractures, although no direct comparisons have been made.

*Angiogenesis*

Anti-angiogenic actions of bisphosphonates have been demonstrated in preclinical studies [42], and in patients with cancer, zoledronate and pamidronate have been reported to reduce serum levels of vascular endothelial growth factor (VEGF); a transient reduction in serum platelet derived growth factor was also observed in one study of patients treated with zoledronic acid [43, 44]. If present, inhibition of angiogenesis could impair the repair of stress fractures by preventing the increase in periosteal vascularity required for their healing. In addition, since capillaries formed at sites of bone remodelling are a source of osteoclast and possibly also osteoblast precursors, inhibition of angiogenesis may further suppress bone remodelling [45]. However, whether the doses of bisphosphonates used for the treatment of osteoporosis have significant anti-angiogenic effects in humans remains to be established.

## Risk factors

Other than long-term bisphosphonate therapy, no risk factors for the development of AFFs have been convincingly demonstrated. A high prevalence of co-morbidities has been reported in women with AFFs, particularly chronic pulmonary disease, rheumatoid arthritis and diabetes, and concomitant use of anti-resorptive drugs, glucocorticoids or proton pump inhibitors has also been reported in many cases [4]. However, in the case control analysis conducted by Schilcher et al., no excess of any of these proposed risk factors was seen in cases compared with a control population with ordinary subtrochanteric or shaft fractures; in addition, the risk of atypical fractures was independent of age [13]. Vitamin D deficiency has also been reported to increase the risk of subtrochanteric fractures [46]. Finally, whilst it might be expected that low bone turnover prior to bisphosphonate therapy increases the risk of developing AFFs, there is currently no evidence to support this proposition.

## Location of AFFs and possible biomechanical implications

The origin in the lateral femoral cortex of AFFs contrasts with the medial location of proximal femur stress fractures seen in athletes, at sites of high compressive strains. Koh et al. reviewed X-rays of 48 patients with AFF, in all of which periosteal and cortical stress lesions occurred in the lateral cortex and were clustered in the region of maximal tensile loading [41]. There may therefore be specific changes in bone material properties in patients with AFFs that predispose to tensile failure. In addition, conformational abnormalities of the lower limbs due to osteoarthritis or other conditions might increase the risk of AFFs by increasing tensional strains in the lateral cortex of the femur.

### Cortical thickening

Generalised cortical thickening of the femoral shaft, distinct from the localised periosteal reaction, is a characteristic feature of AFFs. The available evidence does not support a role for bisphosphonate therapy in cortical thickening, since it is observed in bisphosphonate-naive patients with AFFs [47]. In addition, a study in which cortical thickness in the subtrochanteric region of the femur was measured by DXA at baseline and after a mean follow-up period of 7.3 years showed no significant difference between patients treated with long-term alendronate and untreated controls [48]. These observations are consistent with the known effects on bone remodelling of bisphosphonates, which inhibit bone resorption but do not have the anabolic effects necessary to stimulate new bone formation on periosteal or endosteal surfaces. It therefore appears most likely that cortical thickening precedes the development of atypical fracture but for how long is unknown. The underlying mechanisms of cortical thickening and its significance as a risk factor for the development of AFFs remain to be established.

### Conclusions

Recent studies indicating a relationship between duration of bisphosphonate therapy and risk of AFFs, together with epidemiological data demonstrating divergent trends in hip versus subtrochanteric fracture incidence, support the contention that there is an association between bisphosphonate therapy and AFFs. However, although there are biologically plausible mechanisms by which bisphosphonates may impair bone strength, a direct causal association has yet to be established. The vast majority of patients treated with bisphosphonates do not develop AFFs despite suppressed bone turnover, and, moreover, there is no evidence that suppression is greater in those with than without atypical fractures. Other data that challenge a causal association with bisphosphonates include the well-documented occurrence of AFFs in bisphosphonate-naive patients and the relative rarity of these fractures in patients treated with higher doses for skeletal malignancy as opposed to osteoporosis. In addition, if suppression of bone turnover is proposed as the main mechanism by which bisphosphonates are implicated, the reason for the apparent absence of AFFs in patients treated with other anti-resorptive drugs is unclear.

Although it has been suggested that AFFs represent one end of the spectrum of osteoporotic fractures, characteristics such as prodromal pain, bilaterality and cortical thickening clearly distinguish these fractures from other fragility fractures associated with osteoporosis and indicate different underlying pathophysiological mechanisms. The diffuse cortical thickening of the femoral shaft observed in patients with AFFs is particularly intriguing, since it appears to be unrelated to bisphosphonate therapy and predates the development of fractures. The location of AFFs at sites of maximum tensile stress may also provide valuable clues. Current evidence suggests that bisphosphonates, whilst not a prerequisite for the development of AFFs, may contribute to their development in some cases. Identification of the factors that predispose individuals to develop these rare fractures may hold the key to uncovering their pathophysiology.

## Osteonecrosis of the jaw

### Definition and incidence

Unlike atypical femoral fractures, the clinical manifestations of ONJ are rather non-specific, and the definitions that have been proposed relate to relatively advanced disease. The most widely accepted definition requires the presence of exposed bone in the maxillofacial region for at least 8 weeks in the absence of previous radiation to the jaw [49]. ONJ has been reported in patients treated with both nitrogen-containing and non-nitrogen-containing bisphosphonates including zoledronic acid, pamidronate, alendronate, ibandronate, risedronate and clodronate. It has also more recently been described in patients treated with denosumab. Like AFFs, ONJ can occur in individuals never exposed to these drugs.

There is evidence for a dose response, the incidence in patients treated with the higher doses of bisphosphonates used for oncological indications being reported at between 1% and 10%, at 3 years, whereas in patients receiving lower doses of bisphosphonates for osteoporosis, the incidence is estimated at between 1 in 10,000 and <1 in 100,000 patient treatment years and is positively related to duration of treatment [49–52]. The incidence of ONJ in patients treated for skeletal malignancy with denosumab appears to be similar to that observed with bisphosphonates. In a large prospective study of nearly 4,000 patients with metastatic bone disease, ONJ occurred in 2% of denosumab-treated patients and 1.4% of those treated with zoledronic acid [53]. Similar rates of 2% and 1%, respectively, were reported in a trial of denosumab versus zoledronic acid in men with castration-resistant prostate cancer and bone metastases [54]. ONJ appears to be rare in patients receiving denosumab therapy for osteoporosis; in the FREEDOM study of 7,868 postmenopausal women treated for 3 years, ONJ was not seen [55] although two cases have subsequently been reported in the extension study, both in women who were switched from placebo to denosumab.

### Local and systemic risk factors

The mandible is affected by ONJ approximately two times more frequently than the maxilla, although both may be

affected in some cases. Bone in the mandible and maxilla is subjected to high levels of mechanical stress, has a rich blood supply and, particularly in the intracortical compartment, exhibits higher bone turnover than other skeletal sites. Bone in the jaw also differs in other respects from much of the skeleton, being formed by intramembranous rather than endochondral ossification; furthermore, there is evidence for site-specific regulation of osteoblastic and osteoclastic formation and activity [56]. Finally, the mouth is heavily colonised by a variety of pathogenic microorganisms which can come into contact with bone if the mucosal barrier is breached.

A number of dental and systemic risk factors for ONJ have been described. Dental risk factors are seen in the majority of cases and include periodontal disease, tooth extraction, current or past oral infection, trauma caused by dentures, implant procedures and failed root canal treatment with retained periapical infection. Reported systemic risk factors include malignant disease, chemotherapy, glucocorticoid treatment, diabetes and older age. Breast cancer is the most common malignancy associated with ONJ, followed by multiple myeloma and prostate cancer. Tobacco use and alcohol abuse have been reported in some, but not all studies [51, 57]. The risk of developing ONJ is strongly related to the dose and duration of bisphosphonate therapy and appears to be greatest in patients treated with more potent bisphosphonates. Thus, in patients with myeloma, the risk of developing ONJ was 9.5-fold greater in patients treated with zoledronic acid than in those treated with pamidronate [57].

## Pathophysiology

A number of potential mechanisms have been implicated in the development of ONJ, including suppression of bone turnover, infection and inflammation, inhibition of angiogenesis and immunomodulatory effects. However, the relative contributions of these factors and the sequence in which they operate remain poorly defined. In particular, it is uncertain whether bone necrosis precedes or is a consequence of infection. Many recent reviews have focused on mechanisms by which bisphosphonates could contribute to the development of ONJ; however, the recently reported association between denosumab and ONJ requires that proposed mechanisms are consistent not only with known effects of bisphosphonates but also those of denosumab.

### Suppression of bone turnover

Although the association between ONJ and potent anti-resorptive drugs might indicate a major pathogenetic role for suppression of bone turnover, current evidence is conflicting. ONJ appears to be most common after treatment with potent anti-resorptive drugs, and its incidence increases with higher doses; in addition, case reports suggest that teriparatide may hasten healing of ONJ [58]. However, histological studies of tissue removed from patients with ONJ have demonstrated the presence both of osteoclasts and bone resorption. Lesclous et al. [59] reported the presence of TRAP-positive mononuclear and multinucleate cells in perinecrotic tissue, and in other studies, the presence of increased numbers of osteoclasts and active bone resorption have been reported. Furthermore, isotope studies using technetium-labelled diphosphonate or F-18 FDG PET have shown increased uptake in the regions surrounding the necrotic jaw [60–63]. Finally, ONJ does not occur in other conditions associated with low bone turnover; in autosomal dominant osteopetrosis (ADO), osteomyelitis of the jaw has been reported in approximately 13% of affected individuals [64], but distinguishing osteomyelitis and ONJ can be difficult, and it is uncertain whether the condition described in patients with ADO conforms fully to the clinical definition of ONJ currently in use.

Results obtained using an animal model provide some support for the hypothesis that suppression of bone turnover plays a role in the pathogenesis of ONJ [65, 66]. A proportion (up to one third) of beagles treated with high doses of bisphosphonates develop areas of necrotic bone in the mandible, although the exposed bone characteristic of ONJ in humans is not seen. The absence of basic fuchsin staining in the osteocytic lacunae within necrotic bone indicates that the lacunar spaces have become filled with mineral and that the osteocytes are non-viable. The authors hypothesise that bisphosphonates are taken up in high concentrations in mandibular bone and directly affect osteocytes, increasing their apoptosis [67]. Alternatively, accumulation of non-viable osteocytes could be secondary to suppression of bone turnover. The same authors have also demonstrated that following tooth extraction in beagles, cortical bone remodelling at the extraction site is much higher than at other sites in the mandible, favouring preferential bisphosphonate uptake [68].

Since ONJ only affects the jaw, a primary role for suppression of bone turnover can be advanced for bisphosphonate-treated patients on the assumption that bisphosphonate uptake may be greater in the jaw, particularly at sites of dental extraction, than elsewhere in the skeleton. However, the recent evidence that, at least in patients treated for skeletal malignancy, ONJ occurs with similar frequency in denosumab-treated patients [53, 54] makes this explanation less plausible because of the different pharmacokinetics of denosumab and lack of evidence that it promotes osteocyte apoptosis.

### Angiogenesis

As discussed earlier, bisphosphonates have been shown to have anti-angiogenic effects [36–38]. However, histological

studies of affected tissue from patients with ONJ have generally shown the presence of normal vasculature [59, 69], and furthermore, there is no evidence that denosumab has effects on angiogenesis. ONJ has been described in a few patients treated for cancer with bevacizumab, an anti-angiogenic agent that targets VEGF and is used in the treatment of advanced metastatic disease. Guarneri et al. [70] have recently analysed data from three large prospective trials that included 3,560 patients receiving bevacizumab-containing therapy for breast cancer, some of whom were also receiving bisphosphonate therapy. The overall incidence of ONJ of 0.3–0.4% was not significantly greater than the incidence observed in placebo-treated patients; there was a non-significant trend for a higher incidence in patients also receiving bisphosphonates (0.9% versus 0.2%). Current evidence therefore does not support an increased risk of ONJ with bevacizumab, although further studies are needed. Worsening of ONJ has been reported in a patient treated with another anti-angiogenic agent, sunitinib, but in this case, ONJ was already present before initiation of sunitinib therapy [71].

### Infection/inflammation

The colonisation of the mouth by a variety of microflora, the presence of these in ONJ tissue and the strong association between dental disease and ONJ point strongly to a pathogenetic role of infection. Bacterial aggregates, mainly actinomyces, and polymorphonuclear leucocytes with marrow fibrosis are commonly seen in ONJ tissue. However, whether infection is a primary event or secondary to others has not been established. Lesclous et al. [59] postulated that inflammation occurs first, weakening the mucosal barrier and hence predisposing to infection and necrosis. Alternatively, it is possible that necrosis is the primary event and is followed by infection, or that infection results in inflammation and necrosis. Histological studies are confined to advanced disease and cannot establish the preceding sequence of events; bacterial aggregates, mainly actinomyces, and polymorphonuclear leucocytes with marrow fibrosis are commonly seen in ONJ tissue. Bisphosphonates may be implicated in the inflammatory response, since nitrogen-containing bisphosphonates activate γδ T cells and stimulate the release of pro-inflammatory cytokines [72–74].

The ability of bacterial products to stimulate osteoclastic bone resorption has been known for many years [75, 76], and osteoclasts and active bone resorption in resected ONJ tissue have been described in several studies, sometimes in close proximity to necrotic bone. Sedghizadeh et al. [77] described the presence of microbial biofilms in bone resected from patients with ONJ, consisting of microbial organisms embedded in an extracellular polymeric substrate. Active bone resorption was seen on the surface of bone, with large numbers of bacteria in the resorption cavities. Resorption occurred in close proximity to the biofilms, supporting a role for the microorganisms in bone necrosis. Multiple bacterial types were seen including actinomyces and streptococci, and fungal organisms similar in appearance to *Candida albicans* were also present.

In addition to damage to the mucosal barrier of the mouth by trauma or dental disease, there is evidence that bisphosphonates have adverse effects on proliferation, viability, migration and survival of oral keratinocytes [78–83]. In an in vitro model of wound healing, pamidronate significantly delayed wound closure, and studies in rodents indicate that bisphosphonates impair early healing after dental extraction, although these adverse effects are not seen at later time points [78]. In beagles, although the amount of bone formed within extraction sockets was similar in control and zoledronate-treated animals, the formation of a thick cortical shell in the apical cortex in controls was not seen in the treated dogs [68]. Conversely, in an in vivo study in rats, zoledronate did not affect wound healing in the oral cavity [82]. Whether and how oral epithelial cells become exposed to bisphosphonates are uncertain; it is possible that bisphosphonate in bone could be released by surgical dental procedures, as a result of periodontal disease or as a consequence of active bone resorption associated with bacterial infection. Effects of denosumab on oral epithelial cells have not been reported.

### Other possible mechanisms

#### *Altered immune status*

The increased risk of ONJ in patients receiving glucocorticoid therapy and/or chemotherapy suggests that immunosuppression may be implicated in its pathogenesis, possibly by impairing the response to infection and delaying wound healing.

It has also been proposed that the adverse effects of nitrogen-containing bisphosphonates on macrophage viability may impair the immune response, acting on the mevalonate pathway to induce apoptosis [84, 85]. Vitamin D deficiency is associated with reduced macrophage differentiation and decreased production of the antibacterial protein, cathelicidin, and may further suppress the response to infection [86]. Although RANKL and OPG play important roles in the development of the immune system in rodents, clinical studies in human adults have not indicated significant effects of denosumab on the immune system [87, 88].

#### *Genetic predisposition*

Several genetic polymorphisms have been reported to be associated with an increased risk of developing ONJ,

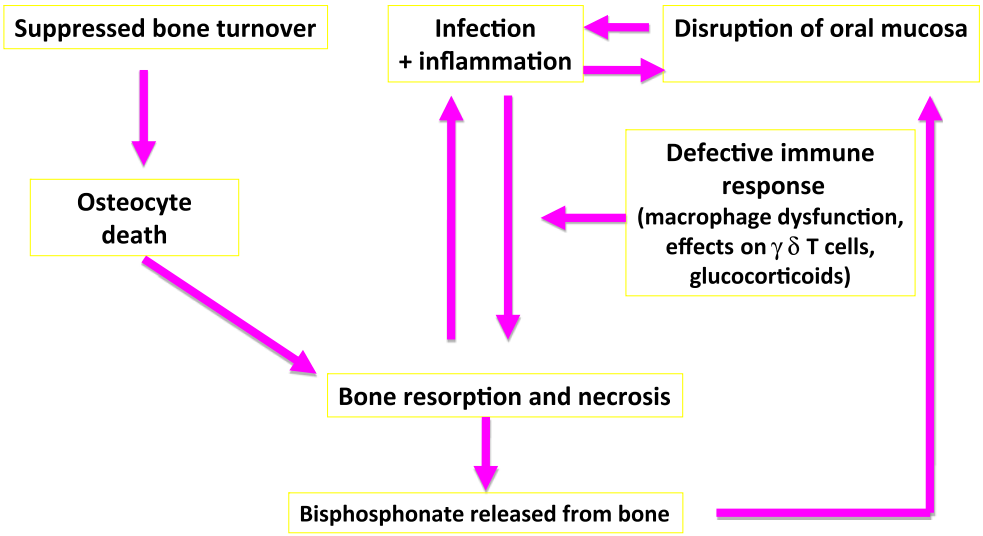


**Fig. 2** Possible events underlying the development of ONJ. The primary event is unknown but unlikely to be either infection or bone resorption and necrosis

although the number of cases in these studies has been small. Polymorphisms of the cytochrome P450 CYP2C8 gene were significantly associated with ONJ risk in bisphosphonate-treated patients with multiple myeloma, but not in bisphosphonate-treated men with prostate cancer [89, 90]. It has also been suggested that a polymorphism of the farnesyl pyrophosphate synthase gene may increase the risk of developing ONJ in bisphosphonate-treated patients with cancer. In a study of 68 patients treated with high doses of zoledronic acid over 18–24 months for myeloma, breast cancer or prostate cancer, the AA genotype carrier status was significantly associated with the occurrence of ONJ [91].

## Conclusions

Despite a number of potential mechanisms, the pathophysiology of ONJ is poorly defined. Until recently, studies focused mainly on the role of bisphosphonates, but the recent finding that denosumab therapy is also a risk factor for ONJ indicates the need to search for mechanisms that are common to both. An obvious candidate is suppression of bone turnover, since denosumab and those bisphosphonates most commonly associated with ONJ are potent inhibitors of resorption, and the incidence of ONJ increases with higher doses of these drugs.

A major challenge in understanding the mechanisms underlying the development of ONJ is unravelling the sequence of events leading up to the clinical syndrome of exposed bone. Thus, it is unknown whether bone necrosis is the initial event, followed by infection and inflammation, or if infection and/or inflammation produce the primary insult, to be followed by necrosis of underlying bone. If the former is the case, suppression of bone turnover would be the most likely mechanism although its focal nature is hard to explain, particularly in denosumab-treated patients. Conversely, stimulation of bone resorption by microorganisms embedded in biofilms in areas of infection, often secondary to dental trauma or disease, provides a more plausible explanation for localised necrosis. However, if necrosis is caused by increased bone resorption, it is difficult to invoke suppression of bone turnover as a pathophysiological mechanism (Fig. 2).

Apart from their anti-resorptive effects, it is possible that bisphosphonates and denosumab could contribute to the development of ONJ in different ways (Table 1). The inhibitory effects of bisphosphonates on oral epithelial cell proliferation and viability may contribute, enhanced by the

**Table 1** Possible pathogenetic factors in ONJ and potential contributions of bisphosphonates and denosumab

| Factor | Effect of bisphosphonates | Effect of denosumab |
|---|---|---|
| Suppression of bone turnover | Yes | Yes |
| Decreased osteocyte viability | Yes | Not demonstrated |
| Infection | Possibly via effects on oral mucosa and effects on macrophage function | Possibly via altered immune status |
| Inflammation | Activation of gamma delta T cells | Not demonstrated |
| Adverse effects on oral mucosa | In vitro evidence, conflicting evidence in vivo | Not demonstrated |
| Anti-angiogenic effect | Yes, but normal vasculature reported in ONJ lesions in many studies | Not demonstrated |
| Altered immune status | Possible effects on macrophage function | Possible but not demonstrated |

release of bisphosphonates from bone undergoing necrosis. Anti-angiogenic effects of bisphosphonates might also be involved, although most studies indicate that this is not an important pathogenetic factor. Bisphosphonates may increase the inflammatory response, and both denosumab and bisphosphonates may reduce immune defence mechanisms. Finally, the greater incidence of ONJ in patients treated with higher doses of these drugs may not simply reflect a dose response, but might rather reflect increased predisposition to the development of ONJ by factors associated with malignancy per se. It is thus likely that multiple factors are involved in the pathogenesis of ONJ, but their relative contribution remains to be established.

**Conflicts of interest** Prof. Compston has served on advisory boards for MSD, Novartis, Sanofi Aventis, GlaxoSmithKline and Amgen and has received speaking fees from MSD, Amgen and Warner Chilcott.

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