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ORIGINAL ARTICLE

Importance of microcracks in etiology of bisphosphonate-related osteonecrosis of the jaw: a possible pathogenetic model of symptomatic and non-symptomatic osteonecrosis of the jaw based on scanning electron microscopy findings

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Abstract The aim of this study was to evaluate a possible role of microcracks in the pathogenesis of bisphosphonate-related osteonecrosis of the jaw (ONJ) and to discuss an etiological model. Bone samples from 35 patients with ONJ were analyzed. Control samples were taken from five patients with osteomyelitis (OM), ten patients with osteoradionecrosis, seven patients with osteoporosis and bisphosphonate medication without signs of ONJ, and six osteoporotic elderly patients. Samples were examined using scanning electron microscopy. In 54% of the bone samples of patients with ONJ, microcracks were seen. Inflammatory and connective tissue reactions within the microcracks were evident in 82% of the cases, indicating that these cracks were not artificial. In contrast, only 29% of samples from patients with oral bisphosphonate medication without ONJ, no sample from patients with OM, none of the osteoradionecrosis group, and only 17% from patients with osteoporosis showed microcracks. Statistically significant differences could be found between the ONJ group and the group after irradiation and the group with OM, respectively. The evidence of microcracks could be a first step in the pathogenesis of bisphosphonate-related ONJ. The accumulation of these microcracks leads to a situation that could be named "non-symptomatic ONJ". Disruptions of the mucosal integrity may then allow bacterial invasion, leading to jawbone infection with exposed bone, fistulas, and pain. This state could be called "symptomatic ONJ". Furthermore, an assumed local immunosuppression as indicated by various studies could explain the severe courses of therapy-resistant ONJ as regularly observed.

Keywords Osteonecrosis of the jaw · Jawbone · Bisphosphonates · Microcracks · Scanning electron microscopy

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Introduction

In 2003, Marx described 36 cases of a new phenomenon of osteonecrosis of the jaw (ONJ) secondary to bisphosphonate (BP) therapy [36]. Since then, the number of cases is still rising [1, 22, 36, 39, 50]. In 2007, the risk of osteonecrosis in patients with cancer treated with high doses of intravenously applied BPs was in the range of one to ten per 100 patients [31] in contrast to 0.05 to one case each 100,000 persons-years of exposure for oral BPs [31, 49].

ONJ lesions are persistent and do not respond well to conventional treatment modalities such as debridement, antibiotic therapy, or hyperbaric oxygen therapy [40]. Despite the growing awareness of ONJ, there is still a lack of knowledge of pathogenesis, prevention, and treatment.

The histological examination of bone samples of ONJ patients showed inflammation with osteomyelitis (OM), areas with empty osteocyte lacunae, necrotic areas, and bacteria colonies, mainly actinomyces [1, 4, 20, 22].

In contrast, osteoradionecrosis (ORN) of the jaw is a well-described phenomenon after irradiation (of the jawbone). This lesion is particularly found in the mandible. A devitalization of bone and a lack of blood supply are typical [7, 19, 21, 30]. Similar to ONJ, the infection of the affected bone is a serious complication [7, 19, 21]. The incidence of ORN varies from 0.4% to 56% in literature [30].

Long-term administration of BP should lead to microcrack accumulation in bone. Allen and Burr, Day et al., and Li et al. have described this phenomenon of microcracks in bone mostly in animal models. The accumulation of microcracks was related to reduced bone remodeling caused by BP-suppressed osteoclast function [2, 13, 35]. Earlier histological examinations of ONJ bone samples showed a major number of microcracks sometimes with bacteria and leukocytes visible within. Therefore, the increase in the number of microcracks was suspected to allow microorganisms to penetrate deeply into the bone after they entered the bone, e.g., through an extraction socket [26].

The aim of this study was to analyze microcracks in ONJ-affected bone in contrast to samples of patients under BP treatment without signs of ONJ, patients with irradiation or ORN, and to patients with osteoporosis or OM not linked to BP, irradiation, or immunodeficiency. The examination with scanning electron microscopy was used to exclude artificial cracks from in vivo cracks. The existence of cells in the fractures was regarded as a sign of preexistence before operation or preparation of the bone samples. A model of "non-symptomatic" and "symptomatic ONJ" based on scanning electron microscopy results is presented, and a model of an insufficient local immune reaction as a promoter of ONJ disease is discussed with regards to the literature.

Materials and methods

Bone samples of the jaw from 63 patients from our clinic were analyzed between the years 2004 to 2007. These patients were divided in five different groups (Table 1): The first group included patients with ONJ and a history of BP treatment for more than 1 year with oral findings like minimal fistula, multiple fistulas, or areas with exposed necrotic bone lasting for more than 3 months (*ONJ group*, Fig. 1; Tables 2 and 3); 30 of these patients had BP therapy because of malignant diseases (*ONJ neoplasm group*, Table 2), 23 (77%) of these patients were female, and seven (23%) were male; five patients in this group received BP for osteoporosis treatment (*ONJ osteoporosis group*, Table 3), four of these patients (80%) were female, and one patient was male. Both the ONJ osteoporosis and the ONJ neoplasm patients had a median age of 69 years. BP medication lasted from 1 year up to 6 years. Thirteen patients received zoledronate; two, pamidronate; one, alendronate; one, clodronate; and two patients, ibandronate. Eleven patients were treated with different BPs at different times including seven patients with pamidronate/zoledronate and one patient with ibandronate/zoledronate, alendronate/zoledronate, and clodronate/zoledronate each. One patient received a triple combination of pamidronate, zoledronate, and ibandronate. The ONJ osteoporosis group had two patients with alendronate and one patient with risedronate therapy. Two patients received a combination of both BPs. The collective of patients with malignant tumors consisted of

Table 1 Classification of patient groups

Group	Description
Neoplasm osteonecrosis of the jaw group (ONJ neoplasm)	Osteonecrosis of the jaw
	Bisphosphonate treatment for more than 1 year
	Oral clinical findings: exposed necrotic bone; minimal fistula, multiple fistulas
	Malignant disease
Osteoporosis osteonecrosis of the jaw group (ONJ osteoporosis)	Osteonecrosis of the jaw
	Bisphosphonate treatment for more than 1 year
	Oral clinical findings: exposed necrotic bone; minimal fistula, multiple fistulas
	Osteoporosis
	No malignant disease
Osteomyelitis group (OM)	Osteomyelitis of the jaw
	Exposed bone as oral clinical finding
	No irradiation in medical history
	No bisphosphonate medication in medical history
	No immunodeficiency
	No immunosuppressive medication in medical history
Radiation group (RA)	Clinical signs of osteoradionecrosis or
	No signs of osteoradionecrosis but
	Irradiation history of more than 50 Gy
Bisphosphonate group (BP)	Bisphosphonate treatment for more than 1 year
	No clinical findings like oral pain, fistula, or exposed bone
	No immunosuppressive medication in medical history
Osteoporosis group (OP)	General osteoporosis
	No bisphosphonate medication in medical history
	No immunosuppressive medication in medical history

Description of inclusion criteria and the characteristics of the different groups

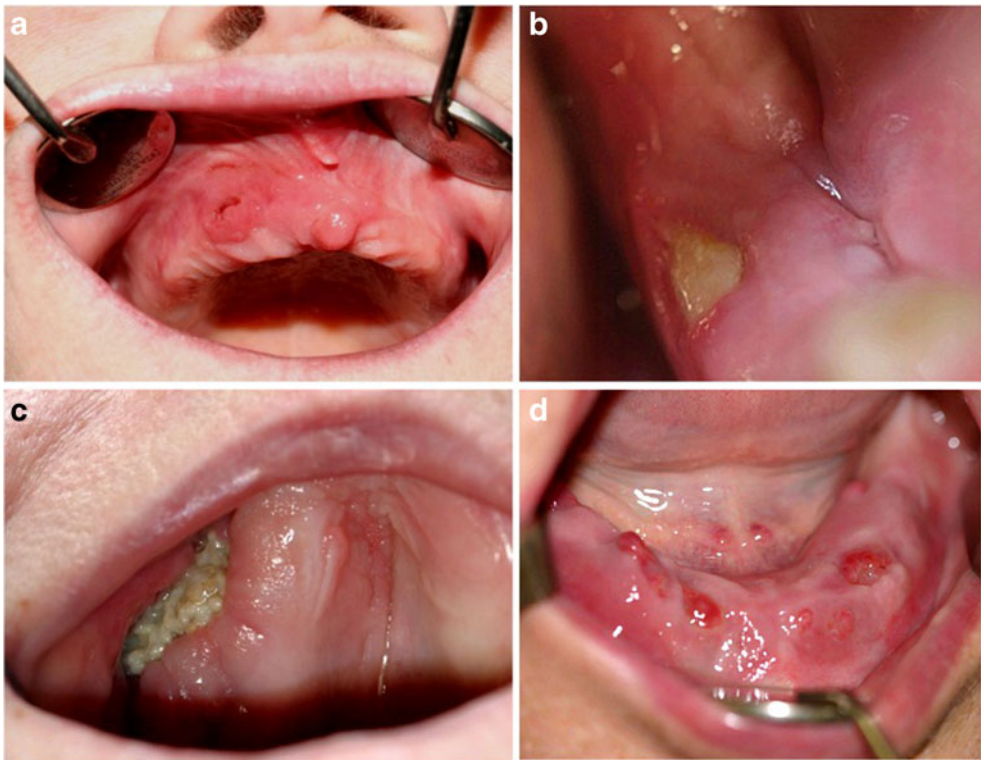

Fig. 1 Clinical oral findings of active osteonecrosis of the jaw. **a** A 61-year-old patient with multiple myeloma and a history of zoledronate medication: She developed a fistula after insertion of new dentures. **b** Exposed bone at the lingual alveolar ridge in a 60-year-old patient with multiple myeloma and ibandronate medication: She showed a non-healing bone area after extraction of a lower molar 2 months prior to the investigation. **c** Almost entirely exposed bone of the right upper alveolar ridge in a 51-year-old patient with zoledronate and ibandronate medication: The teeth were removed 15 months prior to the investigation, causing non-healing sockets with fistulas that led to extensive exposure of necrotic bone. **d** A 64-year-old patient receiving pamidronate and zoledronate medication for metastatic breast cancer: Multiple fistulas showed purulent discharge. There was no history of recent tooth extractions

seven patients having multiple myeloma, 17 having breast cancer, four having lung cancer, two having prostate cancer, one having Hodgkin's disease, one having intestine cancer, and one having uterine cancer (Table 2). None of the patients of the ONJ osteoporosis group had malignant diseases (Table 3).

Bone samples of ten patients who underwent dental extractions after irradiation of the jaws were also included (*RA group*; Table 4). Seven patients had ORN. Two were female, eight were male, and the median age was 69 years. All these patients had received radiation doses between 50 and 110 Gy after the diagnosis of oral cancer.

Another group included five patients with OM of the jaw. One patient was female, four were male, and the median age was 51 years. These patients had localized OM of the mandible with exposed bone with no history of BP medication, irradiation, or immunosuppression (*OM group*; Table 5).

All patients underwent standardized surgical therapy. Oral antibiotics were given before surgery. Bone samples were taken during surgery with marginal bone resection, decortication, removal of sequesters, bone modeling, and extraction of teeth with caries or parodontitis. Intravenous antibiotic medication followed for 1 week after surgery, and prolonged oral medications were given in cases where the jawbone was infected with actinomyces.

Further samples of seven patients, all female, who had received oral BP medication, were also analyzed. The median age was 72 years. Six patients had alendronate, and one patient had risedronate medication. Individual BP therapy lasted up to 9 years. In all patients, osteoporosis had been diagnosed. These patients had no signs of ONJ, particularly, no fistulas, exposed bone, or pain (*BP group*; Table 6).

Another six patients with osteoporosis, four female and two male with a median age of 83 years, were also included. These patients had no medical history of BP medication (*OP group*; Table 7).

The patients in the BP and OP groups required oral surgery. Patients in the BP group received a smoothing of the alveolar bone surface and tight soft tissue coverage with short intravenous or oral antibiotic treatments. Patients in the OP group required smoothing of the bone surface. Samples from these patients included only the small amount of alveolar bone that was smoothed down.

None of the patients underwent additional bone harvesting to gain samples for this study. All samples were taken from bone segments that would have been removed anyway,

Table 2 Patients' characteristics of the osteonecrosis of the jaw (ONJ neoplasm) group with intravenous bisphosphonate therapy and malignoma

Patient	Diagnosis	Bisphosphonate	Duration of treatment (years)	Additional chemotherapy	Microcracks	Oral findings
59 years; f	Multiple myeloma	Pamidronate	2	Yes	Yes	Exposed bone mandible
69 years; f	Breast cancer	Pamidronate Zoledronate	6 2	Yes	Yes	Exposed bone mandible
71 years; m	Lung cancer	Pamidronate	2	Yes	Yes	Exposed bone mandible
76 years; f	Breast cancer	Alendronate	4	Yes	No	Exposed bone mandible
73 years; f	Breast cancer	Pamidronate Zoledronate	2 2	Yes	No	Exposed bone mandible
78 years; f	Breast cancer	Ibandronate Zoledronate	1 3	Yes	Yes	Exposed bone mandible
80 years; f	Breast cancer	Pamidronate Zoledronate	3 2	Yes	Yes	Exposed bone mandible
70 years; f	Breast cancer	Clodronate Zoledronate	2 1	Yes	Yes	Fistula mandible
69 years: f	Multiple myeloma	Pamidronate Zoledronate	3 5	Yes	Yes	Exposed bone mandible
63 years; f	Multiple myeloma	Ibandronate	7	No	No	Exposed bone mandible
46 years; f	Breast cancer	Pamidronate Zoledronate	3 3	Yes	No	Exposed bone mandible
67 years; m	Hodgkin's disease, lung cancer	Zoledronate	2	Yes	Yes	Multiple fistulae mandible
84 years; f	Multiple myeloma	Pamidronate Ibandronate Zoledronate	2 1 3	Yes	Yes	Exposed bone mandible
66 years; f	Breast cancer	Zoledronate	3	Yes	No	Exposed bone mandible
77 years; f	Breast cancer	Zoledronate	2	Yes	Yes	Exposed bone mandible
47 years; f	Breast cancer	Zoledronate	4	Yes	No	Exposed bone mandible
74 years; f	Breast cancer	Pamidronate Zoledronate	Once 5	Yes	Yes	Exposed bone mandible
79 years; m	Multiple myeloma	Pamidronate Zoledronate	1 3	Yes	Yes	Exposed bone mandible
59 years: f	Breast cancer	Zoledronate	3	Yes	Yes	Exposed bone mandible
66 years; m	Lung cancer	Zoledronate	1	Yes	No	Exposed bone mandible and maxilla
55 years; f	Breast cancer	Zoledronate	2	Yes	No	Exposed bone maxilla
63 years; f	Breast cancer	Zoledronate	4	Yes	Yes	Exposed bone mandible
79 years; m	Lung cancer, intestine cancer	Zoledronate	2	Yes	No	Exposed bone mandible
34 years; f	Breast cancer	Zoledronate	2	Yes	No	Exposed bone mandible
63 years; f	Breast cancer	Alendronate Zoledronate	6 2	Yes	No	Fistula mandible
87 years; f	Multiple myeloma	Ibandronate	6	Yes	No	Exposed bone mandible
74 years; m	Prostate cancer	Zoledronate	1	Yes	No	Exposed bone mandible and maxilla
81 years; f	Breast cancer	Zoledronate	5	Yes	Yes	Exposed bone mandible
66 years; m	Prostate cancer	Zoledronate	1	Yes	No	Exposed bone mandible
84 years; f	Multiple myeloma, uterine cancer	Clodronate	3	–	Yes	Exposed bone mandible

Descriptions of age, sex, diagnosis, bisphosphonate medication, duration of bisphosphonate treatment, chemotherapy in patients history, microcracks in scanning electron microscopy, and clinical appearance of the osteonecrosis of the jaw (oral findings)

f female, *m* male

Table 3 Patients' characteristics of the osteonecrosis of the jaw (ONJ osteoporosis) group with an oral bisphosphonate medication and osteoporosis (OP)

Patient	Diagnosis	Bisphosphonate	Duration of treatment (years)	Microcracks	Oral findings
53 years; f	OP	Risedronate	2	No	Exposed bone mandible
76 years; f	OP	Risedronate Alendronate	4 1	Yes	Exposed bone maxilla
62 years; f	OP, chronic obstructive pulmonary disease, celiac disease	Risedronate Alendronate	3 2	Yes	Exposed socket mandible
69 years; m	OP, chronic obstructive pulmonary disease, cardiac arrhythmia	Alendronate	4	Yes	Exposed bone mandible
80 years; f	OP, anticoagulation therapy	Alendronate	3	No	Exposed bone mandible

Descriptions of age, sex, diagnosis, bisphosphonate medication, duration of bisphosphonate treatment, microcracks in scanning electron microscopy, and clinical appearance of the osteonecrosis of the jaw (oral findings)

f female, *m* male

due to clinical reasons. All patients agreed to a possible use of their bone samples for scientific research. All samples were taken by two surgeons using the same surgical technique, giving all samples similar mechanical stress.

Samples taken during surgery were immediately rinsed in 0.9% sodium chloride infusion solution (Baxter, Unterschleissheim, Germany) to remove blood components (5–10 min) and fixed with 4% phosphate-buffered saline formalin (SAV Liquid Production, Flintsbach a. Inn, Germany) until further preparations.

Scanning electron microscopic investigation samples were rinsed in phosphate buffer pH 7.2–7.4 (3×15 min), dehydrated in a series ethanol solutions of increasing concentrations (30% to 100%), and then critical point dried (CPD 030, Baltec, Liechtenstein). Dried samples were fixed onto Thermanox slices (Plano, Wetzlar, Germany) with Leit C Tabs (Plano) and sputtered with gold (40 nm, Sputter Coater S150B, Edwards, North Walsham, UK). Scanning electron microscopic examinations were done with the DSM 982 Gemini (Zeiss, Oberkochem, Germany). The complete surface of the samples was analyzed and measured (Fig. 2a). Microcracks were counted at ×100 to ×200 magnification (Fig. 5a, c). Microcracks were not counted when seen at the periphery of specimens (Fig. 2b). Additionally, the lengths of microcracks were measured in the ONJ group. Magnifications of 200 up to 2,000 times were used for the detection of cellular ingrowth into the fractures (Fig. 3a–b, 5c-e). Statistical analysis was performed by JUMP 5.0.1 (SAS Institute, Cary, NC, USA). The Fisher's exact test was used.

Results

The median size of all bone samples ranged from 29.3 to 61.3 mm^2 (Table 8). Bone surfaces did not show any significant differences in all groups.

Table 4 Characteristics of patients with a history of irradiation (RA) with or without osteoradionecrosis (ORN): a dose of 50 up to 110 Gy irradiation was applied in their medical history

Patient	Diagnosis	Dose (Gy)	Reason for operation	Microcracks	Oral findings
67 years; m	ORN, oral cancer	70.0	ORN	No	Exposed bone mandible
59 years; m	ORN, oral cancer	70.0	ORN	No	Exposed bone mandible
52 years; m	ORN, oral cancer	70.0	ORN	No	Exposed bone mandible
54 years; m	Oral cancer	60.0	Extractions	No	Closed mucosal surface
76 years; m	Oral cancer	50.0	Extractions	No	Closed mucosal surface
81 years; m	Oral cancer	50.4	Extractions	No	Closed mucosal surface
85 years; f	ORN, oral cancer	110	ORN	No	Exposed bone mandible
90 years; m	ORN, oral cancer	60.0	ORN	No	Exposed bone mandible
70 years; m	ORN, oral cancer	110	ORN, fracture of titanium plate	No	Exposed bone mandible
58 years; f	ORN, oral cancer	50.4	ORN	No	Exposed bone mandible

Descriptions of age, sex, diagnosis, irradiation dose, reason for operation, microcracks in scanning electron microscopy, and oral findings

f female, *m* male

Table 5 Patients' characteristics of the osteomyelitis (OM) group

Patient	Diagnosis	Etiology of osteomyelitis	Microcracks	Oral findings
84 years; m	OM	Unknown	No	Exposed bone mandible
50 years; m	OM (actinomyces)	Unknown	No	Exposed bone mandible
47 years; m	OM	Unknown	No	Exposed bone mandible
30 years; f	OM	Unknown	No	Exposed bone mandible
52 years; m	OM (actinomyces)	Unknown	No	Exposed bone mandible

These patients had no history of irradiation, bisphosphonate medication, or immunosuppression. Descriptions of age, sex, diagnosis, etiology of OM, microcracks in scanning electron microscopy, and oral findings

f female, *m* male

Microcracks were found in 19 (54%) specimens of the ONJ patients (Figs. 4, 5, and 7; Table 9). The ONJ neoplasm group showed microcracks in 16 (53%) cases, and the ONJ osteoporosis group showed microcracks in three (60%) cases. An ingrowth of cells into these fractures could be seen in 82% of samples. Seventy-four percent of these fractures had a length of less than 500 µm, 5% between 500 µm and 1 mm, and 21% were greater than 1 mm (Figs. 3a, b and 8). Clinical findings of the ONJ patients included minimal solitary fistula, multiple fistulas, or larger areas with exposed bone (Fig. 1). In 19 (54%) patients, ONJ followed extraction of teeth, three (9%) patients had ONJ caused by denture-related pressure-ulcers, and three (9%) patients developed ONJ after treatment of dental infections. Ten (28%) patients had ONJ without any etiological history.

Two samples from the five patients in the OM group were infected with actinomyces. No microcracks were found in these samples (Figs. 5 and 7; Tables 5 and 9). None of the 10 patients of the RA group showed microcracks in any of the samples (Fig. 7; Tables 4 and 9). The seven patients of the BP group showed microcracks in two (29%) of the samples (Figs. 4 and 7; Tables 6 and 9). None of these patients developed ONJ by a 1-year follow-up examination. The six patients of the OP group showed microcracks in only one (17%) sample (Fig. 7; Tables 7 and 9).

Statistical analysis showed significant differences between the ONJ neoplasm group and the OM and RA groups, as well as between the ONJ osteoporosis group and the RA group. The p value between the ONJ osteoporosis group and the OM group was less than 0.2. The p value between both ONJ groups and the OP group was less than 0.2.

Discussion

ONJ has been defined as "exposed bone in the mandible, maxilla, or both that persists for at least 8 weeks, in the absence of previous radiation and of metastases in the jaws" recently [31, 49]. The patients in our study groups showed these symptoms for at least 12 weeks.

Li et al. defined microcracks as microscopic cracks with lengths of 30–80 µm [35]. Most of our samples showed crack lengths below 500 µm. Therefore, most of our cracks could be considered to be microcracks or could have been microcracks in the beginning. Principally, microcracks have

Table 6 Patients' characteristics of the bisphosphonate (BP) group without osteonecrosis of the jaw

Patient	Diagnosis	Bisphosphonate	Duration of treatment (years)	Microcracks	Oral findings
72 years; f	Osteoporosis	Alendronate	>1	No	Closed mucosal surface
85 years; f	Osteoporosis	Alendronate	2	No	Closed mucosal surface
69 years; f	Osteoporosis	Alendronate	4	No	Closed mucosal surface
55 years; f	Osteoporosis, renal insufficiency, hyperparathyroidism	Alendronate	1	No	Closed mucosal surface
74 years; f	Osteoporosis	Risedronate	9	Yes	Closed mucosal surface
64 years; f	Osteoporosis	Alendronate	10	No	Closed mucosal surface
84 years; f	Osteoporosis, anticoagulation therapy	Alendronate	2	Yes	Closed mucosal surface

These patients had bisphosphonate medication and no signs of fistulae, exposed bone, or pain. Description of age, sex, diagnosis, bisphosphonate, duration of medication, microcracks in scanning electron microscopy, and oral appearance

f female, *m* male

Table 7 Patients' characteristics of the osteoporosis (OP) group: These patients had an osteoporosis and no oral bisphosphonate medication in their history for osteoporosis treatment

Patient	Diagnosis	Bisphosphonate	Reason for operation	Microcracks	Oral findings
75 years; f	Osteoporosis, anticoagulation therapy	None	Extractions	No	Closed mucosal surface
88 years; f	Osteoporosis	None	Extractions	Yes	Closed mucosal surface
77 years; m	Osteoporosis, anticoagulation therapy	None	Extractions	No	Closed mucosal surface
86 years; m	Osteoporosis	None	Extractions	No	Closed mucosal surface
79 years; f	Osteoporosis	None	Extractions	Yes	Closed mucosal surface
87 years; f	Osteoporosis anticoagulation therapy	None	Extractions	No	Closed mucosal surface

Description of age, sex, diagnosis, reason for operation, microcracks in scanning electron microscopy, and oral appearance
f female, *m* male

sharp edges and are larger than canaliculi but smaller than vascular canals [10]. A variety of methods for detecting microcracks in bone has been described in literature [53]. These include light microscopy, fluorochromes, laser scanning confocal microscopy, micro-CT, and en bloc staining with basic fuchsin, which has become the method of choice to demonstrate bone microdamage produced in vivo [2, 9, 10, 18, 28, 33]. Even the basic fuchsin staining method had limitations and did not stain all cracks [2, 9]. Scanning electron microscopy was used to verify the different histological staining methods in some of the experiments conducted by different authors [11, 33]. Schaffler et al. described in their back-scattered electron microscopy technique a significantly reduced number of microcracks in contrast to basic fuchsin [53]. An estimated crack numerical density and crack surface density may depend on the microscopy technique even when similar staining methods are used. In addition, staining of artificial damage or cracks in bone, possibly caused by processing, is still a problem. To minimize artificial damage, Burr and Hooser suggested a block staining method [9]. Even this method, like principally all methods, has a risk of detecting artificial damage caused by sample processing [33]. Therefore, we consider tissue cells in the gaps of the cracks to be a sign of existence in vivo and could be used to exclude artificial cracks. These cells could be bacteria with inflammatory tissue or inflammatory cells. In our opinion, scanning electron microscopy seems to be a good method for microcrack detection and that detection of tissue cells in fractures is a safe way to exclude artificial damage after harvesting or by processing of bone samples.

The skeleton is known to have the ability to renew itself throughout adult life. Old bone is continuously removed and replaced by new bone, a process known as bone remodeling. It involves osteoclasts that resorb bone and osteoblasts that form new bone. Bone remodeling is regulated by numerous systemic and local factors [44, 45]. Osteoclasts release cytokines, bone morphogenetic proteins, insulin-like growth factors (ILGFs), metallo-matrix proteins (MMP-2, MMP-9), and others like ILGF-1, ILGF-2, IL-6, CRP, and TNF-alpha [25, 36, 37, 50]. The jawbone is a frequently "used" bone with a high degree of mechanical stress by masticatory forces, especially with deformation in the mandible [6, 14, 40, 42]. This mechanical stress continuously leads to cracks [35, 37, 44] and even local necrotic regions [2]. A sufficient volume of necrotic bone may result from the accumulation of microcracks. In a normal bone, these microcracks are continuously repaired [2, 52]. Osteocytes, which are differentiated osteoblasts that are regularly spaced throughout the bone, are believed to detect bone microdamage and to transmit signals leading to its repair [11, 42, 44, 47]. The dying or apoptotic osteocyte is responsible for sending signals to initiate bone resorption and is associated with osteoclastic resorption [42]. Some

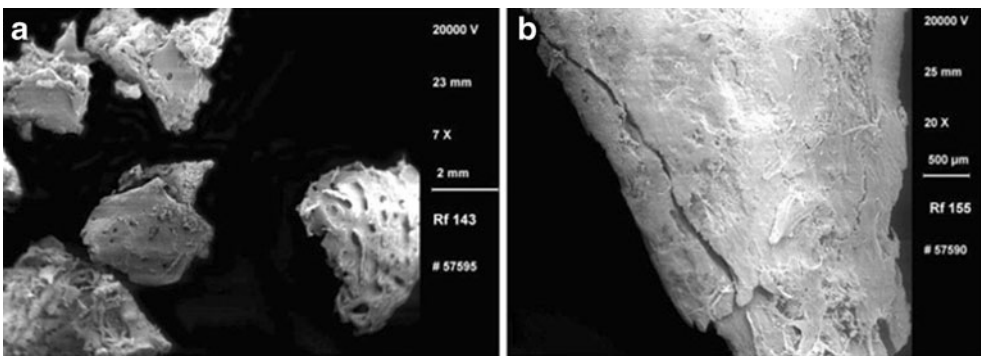

Fig. 2 **a** Scanning electron microscopy overview (×7) of bone samples. An overview was used to calculate the surface area analyzed. **b** A bone sample with a crack located at the border. Cracks like these were excluded

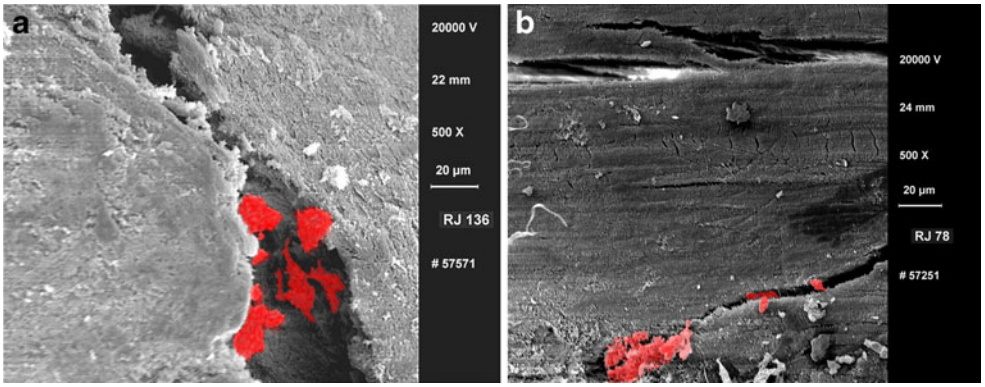

Fig. 3 Examples of cellular ingrowth in fracture gaps. **a** Tissue cells are visible (*colored*) along the edge and within the gap. **b** On top, a typical appearance of a microcrack without cells. At the bottom, a demonstration of a microcrack at the early stages of cellular ingrowth (magnification (×500) of Fig. 5a). Tissue cells in fracture gaps are signs of preexistence before harvesting of bone samples

authors [4, 52] described an increased number of empty osteocytic lacunae in ONJ bone. Therefore, the disruption of the osteocytic network could compromise bone remodeling and lead to microdamage accumulation and increased bone fragility [42, 47, 52] and weakness [44]. This may lead to local destruction of bony, vascular, and connective tissue necessary for self-repair within the remodeling process [14, 37, 40, 42, 52].

BP bound to mineral bone sites is subsequently liberated during resorption and uptake by osteoclasts [1, 45]. After taking alendronate for 5 years, the bone resorption and formation markers are described to remain suppressed for at least 5 years after discontinuation [44]. Most reported cases of ONJ of the jaw involve intravenous BP such as pamidronate and zoledronate, but there also seems to be a risk of ONJ under oral BP medication [1, 25]. About 170 cases of alendronate-induced ONJ have been reported worldwide in 2008 [52]. A total number between 443 and 600 cases of BP-induced ONJ were found in literature by other authors [49, 51].

Day et al. showed that the administration of risedronate and alendronate in high doses for 1 year leads to an accumulation of microcracks and microdamage of the first lumbar vertebra in beagle dogs [13]. Allen and Burr treated beagle dogs daily for 3 years with alendronate and described a reduced bone turnover and an increased incidence of matrix necrosis within the mandible. The majority of the necrotic regions were localized to the alveolar bone of the mandible mostly, a location with a turnover that is six times higher than that of the remainder of the mandible [2]. In earlier animal studies, Li et al. treated beagle dogs with risedronate and alendronate for 1 year and found increased microcrack accumulation and reduced remodeling [35]. Odvina et al. analyzed 15 patients who sustained non-spinal fractures while on alendronate therapy. Histomorphometric analysis of the cancellous bone showed markedly suppressed bone formation with reduced or absent osteoblastic surface. The number of osteoclasts was found to be low or low to normal, and the matrix synthesis was markedly diminished. They concluded that a severe suppression of bone turnover occurs during long-term alendronate therapy, resulting in increased susceptibility to and delayed fracture healing [43]. Earlier examination of 20 bone samples of ONJ patients by Hoefert et al. showed a large number of microcracks, sometimes with bacteria and leukocytes visible within [26].

Table 8 Distribution of the size of the samples of the different groups in scanning electron microscopy

Group	Median of size (mm^2)	Range of size (mm^2)
ONJ	34.5	6.6–360.0
ONJ cancer	29.3	9.0–360.0
ONJ osteoporosis	48.0	6.6–120.0
RA	49.6	7.0–106.6
OM	61.3	16.8–164.8
BP	29.6	14.3–69.2
OP	41.0	31.2–105.0

Description of median of size and range

In this study, we could see a significantly higher number of microcracks between all samples from the ONJ patients in contrast to samples from the RA and OM groups. No microcracks were detectable in either the RA or the OM group. In general, ORN is linked to devitalization of bone and disruption of vascular supply by irradiation [19, 21, 46]. The most likely affected cells are the vascular endothelium, fibroblasts, stromal, and parenchymal cells [46]. Consequently, the bone virtually becomes a non-vital tissue [19] having established the nature of hypocellularity, hypovascularity and, consequently, hypoxia of irradiated tissues [46]. After infection, the presence of actinomyces should promote the persistence of chronic non-healing processes [19, 21, 30]. Similar to ONJ, infection is a serious complication [25, 44]. ORN patients with jaw infections are described to have pain and show fistula, exposed bone, and signs of inflammation of the surrounding mucosa or skin [19, 46] similar to ONJ patients. In contrast to ONJ, mostly the mandible is involved because the blood supply may be less abundant than in the maxilla [38, 46]. Wannfors and

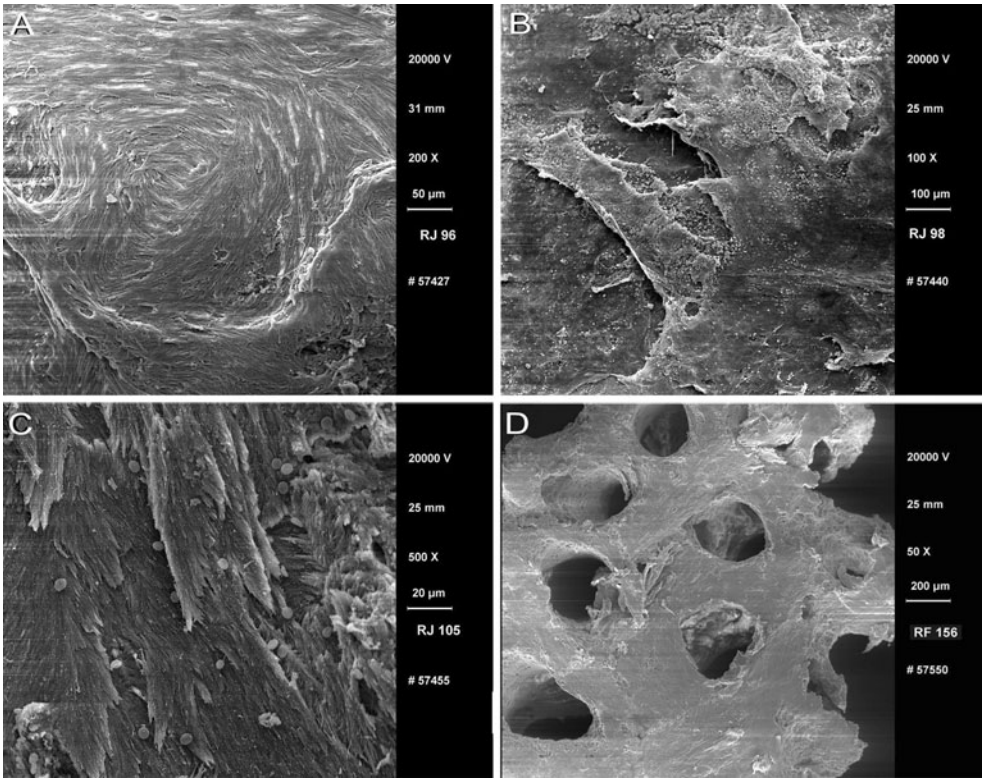

Fig. 4 Scanning electron microscopy of bone samples. **a** A 68-year-old female patient with a 4-year alendronate medication and osteoporosis without osteonecrosis of the jaw: circular arrangement of cellular bone without signs of microcracks (×200). **b** A 54-year-old female patient with osteoporosis, hyperparathyroidism, dialysis, and an alendronate medication for more than 1 year without osteonecrosis of the jaw: regular bone surface without microcracks (×100). **c** A 46-year-old male patient with osteomyelitis of the mandible of unknown etiology: regular bone surface with erythrocytes (×500). **d** A 65-year-old male patient with osteonecrosis of the jaw, metastatic prostate cancer, and no cracks in this overview (×50)

Hammarström mention that important factors contributing to OM without radiation include the virulence of microorganisms and anatomic possibilities for the infection to spread in spite of the immunological response. Deficiencies in vascular or immunological systems as predisposing factors were also assumed [57].

"Non-symptomatic osteonecrosis of the jaw", a new definition

In conclusion, our ONJ samples showed more microcracks than samples from the BP, ORN, and OP groups. The existing microcracks in OP patients could be explained by their high median age or a higher vulnerability of the bone due to osteoporotic disease [13].

Because of all these considerations and findings, microcracks can be considered as an "important first step" in the pathogenesis of ONJ. The accumulation of microcracks without any symptoms of ONJ could be named "non-symptomatic ONJ". It is possible that the lack of clinical symptoms is linked to a stable state in bone physiology within microcracks or simply to the absence of infection or any serious inflammation (Fig. 6).

"Symptomatic osteonecrosis of the jaw", a new definition

Extraction of teeth or other oral surgical procedures, pressure of dentures, local dental infection, other trauma, or a spontaneous breakdown (as a result) of the overlying mucosa will always lead to the invasion of bacteria into the bone from the oral cavity [26, 32, 49]. Additionally, Reid et al. and Rizzoli et al. suggested that BP could themselves be toxic to oral epithelium at pharmacologic concentrations [48] or that the oral epithelium could be compromised by immunosuppression, cytostatic agents, or steroids leading to a primary or continued bone exposure [49]. Landesberg et al. showed that BP pretreatment of oral mucosal cells inhibits proliferation and wound healing at clinically relevant doses [32]. When bacteria reach the bone, the cavitations caused by microcracks could give way for microbiological spreading. Empty osteocyte lacunae could also support the spreading as mentioned above [26]. Sedghizadeh et al. found the presence of bacteria along the inner surface of the bone, indicating the presence of microbial biofilms in the deeper cavities of the bone and not just on the surface exposed to the contaminated oral cavity [54]. Consequently, the blood supply may be obstructed by infection leading to the formation of ischemic bone sequesters [26]. This infected bone shows clinical findings like fistulas, more or less associated with necrotic bone areas, purulent discharge, or pain—nearly always without evidence of tumor cells [20, 37, 56]. However, pain is to be considered a sign of bacterial infection [37, 40].

Badros et al. described in their histological findings inflammation consistent with OM and areas of acellular necrotic bone [4]. Ruggiero et al. described necrotic bone

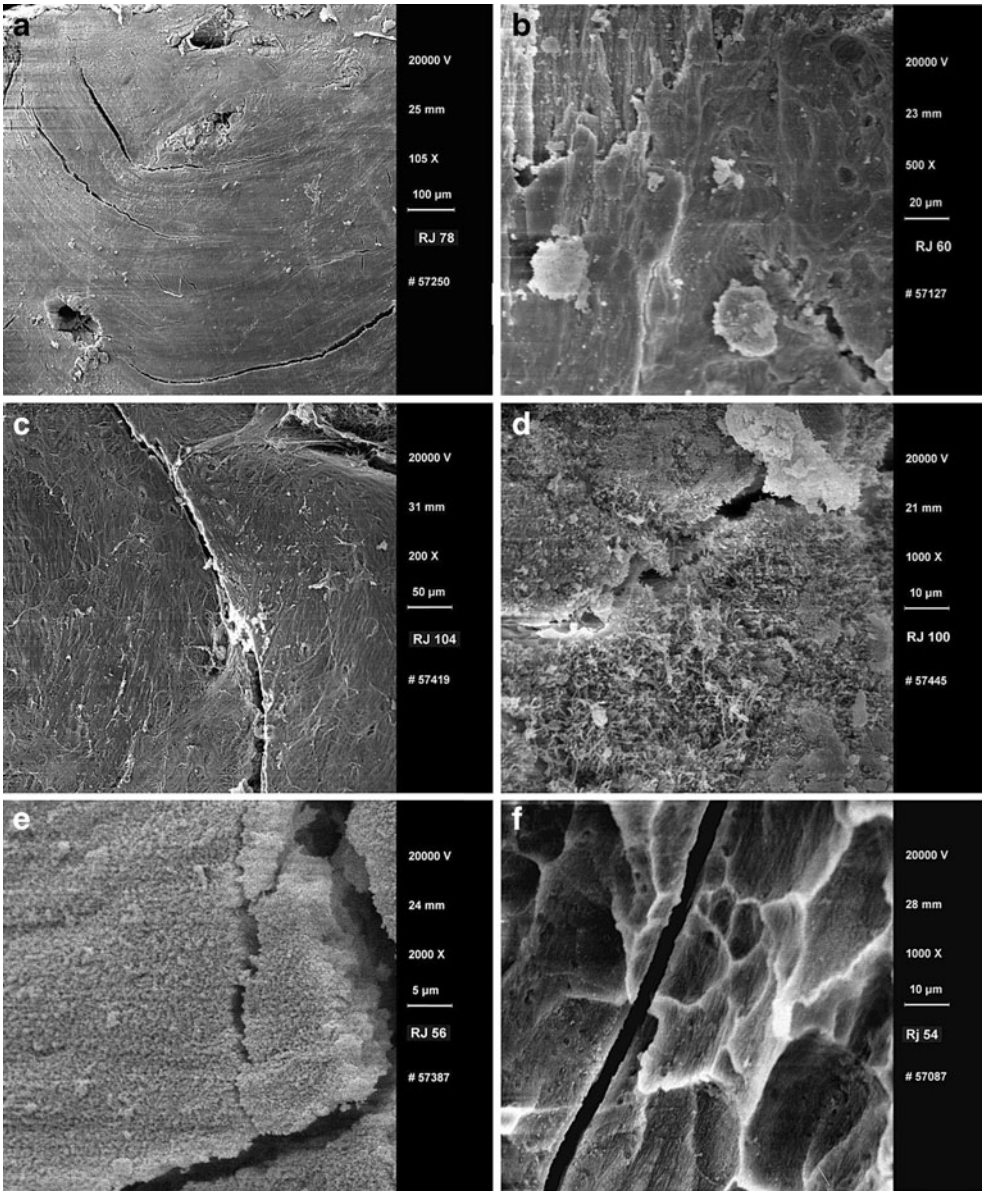

Fig. 5 Scanning electron microscopy of bone samples. **a** A 61-year-old patient with osteoporosis, 4-year risedronate, and 2-year alendronate medication and osteonecrosis of the jaw: multiple visible microcracks (×105; see also Fig. 3b). **b** A 70-year-old patient with metastatic lung cancer and with 4-year pamidronate medication. Beginning microcrack in the lower right (×500). **c** A 87-year-old patient with osteoporosis: visible long fracture line with cellular invasion (×200). **d** A 83-year-old patient with osteonecrosis of the jaw, multiple myeloma, and uterine cancer: bacterial and cellular overgrowth of a microcrack (×1000). **e** Example of cellular and bacterial overgrowth and microcracks with cellular ingrowth. Sample of a patient with oral cancer, prostate cancer, oral irradiation, and bisphosphonate medication (×2000; data excluded). **f** Sharp fracture line with no signs of cellular ingrowth (×1000), probably representing a preparation artifact (crack excluded) from a 58-year-old female patient with multiple myeloma and pamidronate medication

with associated bacterial debris and granulation tissue [51]. Abu-Id et al. and Hellstein and Marek described in their histopathological examination subepithelial bacterial colonies and small non-vital bone fragments. Inflammatory cells with bacterial colonies were dominant in acutely affected areas. In areas with vital bone, the vascularity of the connective tissue appeared to be intact [1, 22]. Hansen et al. described non-vital bone tissue, in contrast to ORN in multiple, partially confluent areas honeycombed with residual nests of vital bone. Only a few osteoclasts were detected in the majority of cases [20].

ONJ was also referred to originally as aseptic necrosis, ischemic necrosis, or avascular necrosis [22, 56]. These descriptions are based on an ischemic etiology of necrosis, as most authors concluded [5, 16, 20, 37, 39, 40, 50, 56]. Ruggiero et al. mentioned antiangiogenic properties by a diminished circulating level of vascular endothelial growth factor [51]. Dieli et al. mentioned that some BP has potent antiangiogenic activity that may contribute to anti-bone-resorptive effects as well as to the anti-tumor effect [16]. Other authors like Allen and Burr (in animal models), Hansen et al., and Hellstein and Marek could not see a lack of vessels [2, 20, 22].

In ORN, Hellstein and Marek described inflammation and bacteria around sequesters and on superficially exposed bone surfaces, but not in deeper portions of trabecular bone, where bacteria and signs of inflammation were not commonly seen [22]. Hansen at al. mentioned that actinomyces was almost exclusively found in the necrotic bone [20]. Bras et al. concluded that, for ORN, the hypoxic, hypovascular, and hypocellular tissue with tissue breakdown was most important. In their view, the microorgan-

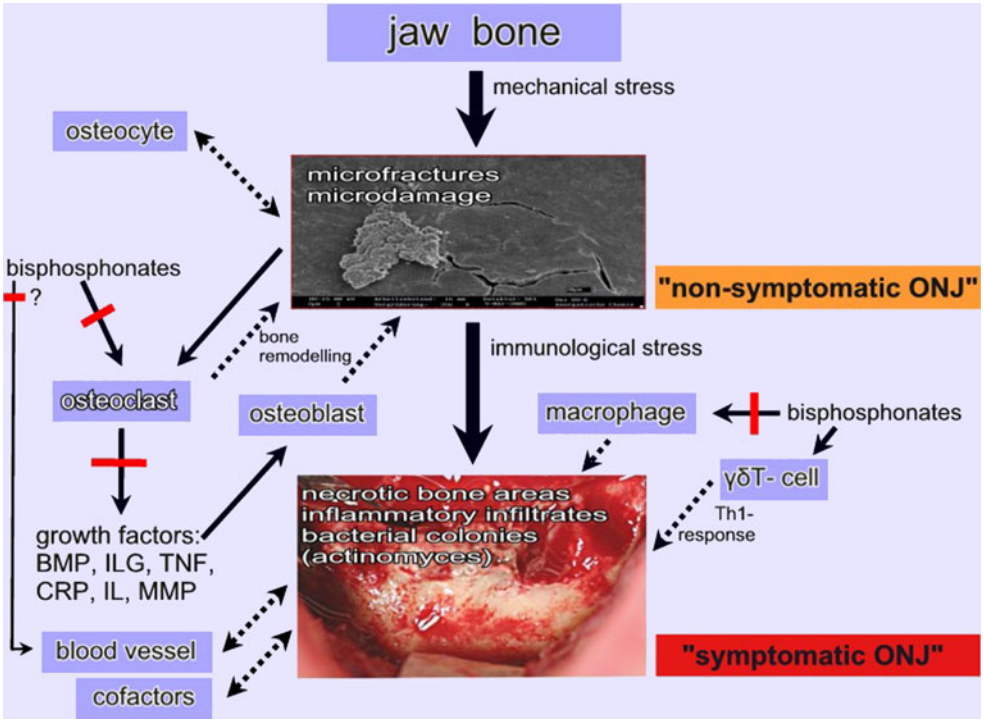

Fig. 6 A possible model of osteonecrosis of the jaw (ONJ) secondary to bisphosphonates: Mechanical stress (by masticatory forces) generally leads to microcracks of bone. Suppression of adequate repair action of osteoclasts, osteocytes, and osteoblasts under bisphosphonate medication could lead to an accumulation of microcracks and microdamage. This situation could be named "non-symptomatic ONJ". A bacterial invasion (e.g., after extractions) causes immunological stress by deep bacterial invasion, which is possibly increased by immunosuppression, and leads to bone necrosis and inflammatory infiltrates with the clinical appearance of ONJ. This situation could be named "symptomatic ONJ". Local depression of macrophage function and a possible overweight of a Th1 response could lead to local immunosuppression in addition to further negative systemic effects of other cofactors. There is also a possibility of an antiangiogenic activity of some bisphosphonates (e.g., zoledronate) compromising the bone by reduced blood supply. Additionally, a wide variety of cofactors may influence ONJ development or progression

isms play only a concomitant role. Histologically, they described necrosis of bone with or without sequestration characterized by the loss of osteocytes and by large areas of resorption filled with debris and granulocytes. In contrast to ONJ, they described the formation of new bone in the subperiosteal level and replacement of bone marrow with dense fibrous, less-vascularized tissue [7].

This stands in contrast to the distinct signs of an OM in ONJ after an exposition to bacteria has taken place. As a second step in ONJ etiology, the infected ONJ could be named "symptomatic ONJ" (Fig. 6).

Considering microcracks as an important first step in ONJ, there is still a lack of explanation why 46% of our ONJ samples show no cracks. This could be related to different sensitivity to cracks in different areas of the jawbone as mentioned by Allen and Burr [2] so that there could be a dependence of the area in the jaw where the sample was harvested. One the other hand, the number of cracks "needed" for ONJ could be influenced by cofactors like diabetes, steroid, and cytostatic agents or even by advanced age, arthritis, chronic inactivity, estrogen, female sex, hemodialysis, thrombophilic disorders, hyperlipidemia, hypertension, and hypercholesterolemia as named by Hess et al. and Sarin et al. These cofactors are believed to raise the risk of ONJ principally [23, 52]. Additionally, a lack of sufficient immune response, as discussed below, could explain a different "sensitivity" for the symptomatic ONJ. Besides this, microcracks were not obvious in the samples of OM and ORN in contrast to the ONJ samples.

A lack of sufficient immune response, a BP medication phenomenon?

There is no explanation as to why this microdamaged bone is highly sensitive to bacterial contamination and shows inflammation in a severity similar to OM in the pre-antibiotic era [26]. Hellstein and Marek even estimated that "bis-phossy jaw" might have more of a bacterial cofactor risk than ORN as mentioned above [22].

Furthermore, it is still unclear how much BP themselves interfere with the immune system, especially in tumor patients with widespread disease or additional chemotherapy [26].

A common adverse effect typically associated with BP therapy is transient fever in 34% to 38% of patients [15, 24,

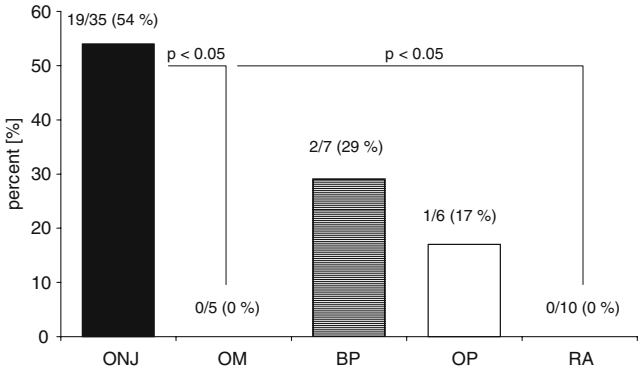

Fig. 7 Diagram of bone samples showing microcracks (percentage values given). *ONJ*: 35 patients with osteonecrosis of the jaw (ONJ neoplasm+ONJ osteoporosis); *OM*: five patients with osteomyelitis not linked to bisphosphonates or irradiation; *BP*: seven patients with bisphosphonate medication without clinical signs of ONJ; *OP*: six patients with osteoporosis without bisphosphonate medication; *RA*: ten patients with osteoradionecrosis or patients with irradiation of the jaws with more than 50 Gy. The ONJ group shows statistically significant differences to the OM and the RA group

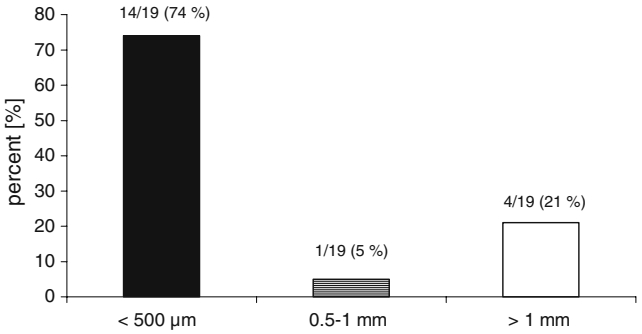

Fig. 8 Distribution of microcracks according to their length (percent) of 19 samples from the complete osteonecrosis of the jaw (ONJ) group

55]. The mechanism underlying this reaction is not fully understood, but increased production of proinflammatory cytokines like IL-1, IL-6, IFN-gamma, and TNF-alpha are thought to play a role [24, 55].

Mammalian CD3-lymphocytes can be separated into two lymphocyte subsets bearing T-cell receptors composed of either αβ or γδ heterodimers. Human γδT-cells represent a unique lymphocyte population with an unusual tissue distribution to lymphoid tissues including skin and intestine-associated lymphoid organs. In particular, the Vγ9Vδ2 subset represents the majority of peripheral blood γδT-cells and is involved in the immune response against intracellular pathogens and

Table 9 Absolute number and percentage of samples with microcracks of the osteonecrosis of the jaw group with neoplasm (ONJ neoplasm) and osteoporosis (ONJ osteoporosis), osteomyelitis (OM), irradiation (RA), bisphosphonate medication without ONJ signs (BP), and osteoporosis without bisphosphonate medication

Group	Total number of samples	Number of samples with microcracks	Percentage of samples with microcracks
ONJ	35	19	54
ONJ neoplasm	30	16	53
ONJ osteoporosis	5	3	60
OM	5	0[a]	0
RA	10	0[a,b]	0
BP	7	2	29
OP	6	1	17

[a] Significantly different from the ONJ neoplasm and both ONJ groups ($p<0.05$)

[b] Significantly different from the ONJ osteoporosis group ($p<0.05$)

hematological malignancies, therefore, heading to a Th1 immune response [39].

Studies have clearly demonstrated that some BPs activate γδT-cells [39, 40]. They promote proliferation of themselves and Th1 cytokine production as well as the stimulation of cytotoxic activity against selected tumor cells [39]. Conti et al. also mentioned a Th1 immune response triggered by some BPs [12]. In contrast, a Th2 immune response is needed for an antibody-mediated immunity. For symptomatic ONJ, a domination of a Th1 immune response may be important.

Furthermore, osteoblasts originate from mesenchymal stem cells; whereas, osteoclasts originate from haematopoietic precursors of the monocyte/macrophage line [45]. Dicuonzo et al. described by in vitro models that BP may depress the accessory function of monocytes in lymphocyte proliferation, inhibit mouse monocyte/macrophage growth from bone marrow precursors, and inhibit cytotoxic and migration activities of mouse peritoneal macrophages [15]. Huk et al. described an induction of macrophage apoptosis by pamidronate [29]. Moreau et al. demonstrated in vitro inhibition of proliferation and cell death. Alendronate and pamidronate caused macrophage apoptosis at very high concentrations. In contrast, apoptosis was evident with risedronate and zoledronate at lower concentrations. Furthermore, the authors estimated similar therapeutic concentrations could be reached in vivo as by their experiments in vitro [41]. Other authors used a clodronate-liposome model to eliminate macrophages experimentally [17, 34].

The question still remains as to why the immune response should only be compromised at the jawbones. Treatment of osteoclasts, monocytes, and tumor cells with BP leads to cell death, indicating that resorption of bone is not necessarily required for cellular BP ingestion. A locally high BP concentration at the bone site due to accumulation of (osteoclast) debris could be a possible explanation. Osteoclast precursors, osteoblasts, osteocytes, and bone lining cells would be exposed to similar BP concentrations as osteoclasts, so that the mechanism of osteoclast selectivity remains to be elucidated [8]. In addition, there

seems to be a higher BP concentration at the bone site [2, 8, 15, 16, 51, 52, 55]. There are observations that the proportion of BP absorbed by the skeleton may vary according to the bone turnover, so that the highest absorption should take place at the sites of active bone remodeling [3, 27, 37, 44, 52]. As described above, the jaws, especially the mandible, are bones with a high rate of remodeling [37]. It is possible that the local concentration of BP reach very high levels that may have cytotoxic effects on osteocytes [2]. Another explanation could be that the immune response is only locally modulated, as explained above.

All these results suggest that all BP may interact with the (local) immune system and influence the circulating levels of local cytokines [15]; in addition, a limitation of the local immune defense within the jaw should be taken into consideration.

Conclusions

Our findings show a high number of microcracks in the bone samples with ONJ. This stands in contrast to samples of RA and OM patients with no fractures and fewer fractures in bone samples of patients under BP medication and of those with OP. The state of accumulated microcracks without any clinical sign of ONJ could be named "non-symptomatic ONJ". A clinical obvious ONJ with fistulas, pain, or exposed bone could be named as "symptomatic ONJ". A compromised local immune system of the jaw could theoretically be postulated and may explain the severe inflammation seen in cases of active ONJ.

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Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Abu-Id MH, Acil Y, Gottschalk J, Kreusch T (2006) Bisphosphonatassoziierte Osteonekrose des Kiefers. Mund Kiefer Gesichtschir 10:73–81
2. Allen MR, Burr DB (2008) Mandible matrix necrosis in beagle dogs after 3 years of daily oral bisphosphonate treatment. J Oral Maxillofac Surg 66:987–994
3. Altundal H, Guevener OE (2004) The effect of alendronate on resorption of the alveolar bone following tooth extraction. Int J Oral Maxillofac Surg 33:286–293
4. Badros A, Weikel D, Salama A, Goloubeva O, Schneider A, Rapoport A, Fenton R, Gahres N, Sausville E, Ord R, Meiller T (2006) Osteonecrosis of the jaw in multiple myeloma patients: clinical features and risk factors. J Clin Oncol 24:945–952
5. Bamias A, Kastritis E, Bamia C, Moulopoulos LA, Melakopoulos I, Papadimitriou C, Terpos E, Dimopoulos A (2005) Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. J Clin Oncol 23:8580–8587
6. Bouvier M, Hylander WL (1981) Effect of bone strain on cortical bone structure in macaques (*Macaca mulatta*). J Morph 167:1–12
7. Bras J, de Jonge HKT, van Merkensteyn JPR (1990) Osteoradionecrosis of the mandible: pathogenesis. Am J Otolaryngol 11:244–250
8. Bukowski JF, Dascher CC, Das H (2005) Alternative bisphosphonate targets and mechanisms of action. Biochem Biophys Res Commun 328:746–750
9. Burr DB, Hooser M (1995) Alterations to the en bloc basic fuchsin staining protocol for the demonstration of microdamage produced in vivo. Bone 17:431–433
10. Burr DB, Stafford T (1990) Validity of the bulk staining technique to separate artificial from in vivo bone microdamage. Clin Orthop Relat Res 260:305–308
11. Burr DB, Martin RB, Schaffler MB, Radin EL (1985) Bone remodeling in response to in vivo fatigue microdamage. J Biomech 18:189–200
12. Conti L, Casetti R, Cardone M, Varano B, Martino A, Belardelli F, Poccia F, Gessani S (2005) Reciprocal activating interaction between dendritic cells and pamidronate-stimulated gammadelta T cells: role of CD86 and inflammatory cytokines. J Immunol 174:252–260
13. Day JS, Ding M, Bednarz P, van der Linden JC, Mashiba T, Hirano T, Johnston CC, Burr DB, Hvid I, Sumner DR, Weinans H (2004) Bisphosphonate treatment affects trabecular bone apparent modulus through micro-architecture rather than matrix properties. J Orthop Res 22:465–471
14. Dechow PC, Hylander WL (2000) Elastic properties and masticatory bone stress in the macaque mandible. Am J Phys Anthropol 112:553–574
15. Dicuonzo G, Vincenzi B, Santini D, Avvisati L, Rocci L, Battistoni F, Gavasci M, Borzomati D, Coppola R, Tonini G (2003) Fever after zoledronic acid administration is due to increase in TNF-alpha and IL-6. J Interferon Cytokine Res 23:649–654
16. Dieli F, Gebbia N, Poccia F, Gaccamo N, Montesano C, Fulfaro F, Arcara C, Valerio MR, Meraviglia S, Di Sano C, Sireci G, Salerno A (2003) Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. Blood 102:2310–2311
17. Enzendam J, Kosterman K, Spijkerboer H, Bleumink R, Hassing I, van Rooijen N, Vos JG, Pieters R (2005) Macrophages are involved in hexachlorobenzene-induced adverse effects. Toxicol Appl Pharmacol 209:19–27
18. Frost HM (1960) Presence of microscopic cracks in vivo in bone. Henry Ford Hosp Med Bull 8:25–35
19. Hansen T, Kunkel M, Kirkpatrick CJ, Weber A (2006) Actimomyces in infected osteoradionecrosis—underestimated? Human Pathol 37:61–67
20. Hansen T, Kunkel M, Weber A, Kirkpatrick CJ (2006) Osteonecrosis of the jaws in patients treated with bisphosphonates—histomorphologic analysis in comparison with infected osteoradionecrosis. J Oral Pathol Med 35:155–160
21. Hansen T, Wagner W, Kirkpatrick CJ, Kunkel M (2006) Infected osteoradionecrosis of the mandible: follow-up study suggests deterioration in outcome for patients with actinomyces-positive bone biopsies. Int J Oral Maxillofac Surg 35:1001–1004
22. Hellstein JW, Marek CL (2005) Bisphosphonate osteochemonecrosis (bis-phossy jaw): is this phossy jaw of the 21st century? J Oral Maxillofac Surg 63:682–689

23. Hess LM, Jeter NJ, Benham-Hutchins M, Alberts DS (2008) Factors associated with osteonecrosis of the jaw among bisphosphonate users. Am J Med 121:475–483
24. Hewitt RE, Lissina A, Green AE, Slay ES, Price DA, Sewell AK (2005) The bisphosphonate acute phase response: rapid and copious production of proinflammatory cytokines by peripheral blood gd T cells in response to aminobishosphonates is inhibited by statins. Clin Exp Immunol 139:101–111
25. Hoefert S, Eufinger H (2006) Nekrosen der Kieferknochen unter Bisphosphonattherapie. Orthopäde 35:204–209
26. Hoefert S, Wierich W, Eufinger H, Krempien B (2006) BP-associated avascular necrosis (AN) of the jaws: histological findings. Bone 38(Suppl):76
27. Hu JH, Ding M, Soballe K, Bechtold JE, Danielsen CC, Days JS, Hvid I (2002) Effects of short-term alendronate treatment on the three-dimensional microstructural, physical, and mechanical properties of dog trabecular bone. Bone 31:591–597
28. Huja SS, Hasan MS, Pidaparti R, Turner CH, Garetto LP, Burr DB (1999) Development of a fluorescent light technique for evaluating microdamage in bone subjected to fatigue loading. J Biomech 32:1243–1249
29. Huk OL, Zukor DJ, Antoniou J, Petit A (2003) Effect of pamidronate on the stimulation of macrophage TNF-alpha release by ultra-high-molecular-weight polyethylene particles: a role for apoptosis. J Orthop Res 21:81–87
30. Jereczek-Fossa BA, Orechia R (2002) Radiotherapy-induced mandibular bone complications. Cancer Treat Rev 28:65–74
31. Khosla S, Burr D, Cauley J, Dempster DW, Ebeling PR, Felsenberg D, Gagel RF, Gilsanz V, Guise T, Koka S, McCauley LK, McGowan J, McKee MD, Mohla S, Pendrys DG, Raisz LG, Ruggiero SL, Shafer DM, Lillian Shum L, Silverman SL, Van Poznak CH, Watts N, Woo SB, Shane E (2007) Bisphosphonate-associated osteonecrosis of the jaw: report of the task force of the American Society for Bone and Mineral Research. J Bone Miner Res 22:1479–1491
32. Landesberg R, Cozin M, Cremers S, Woo V, Kousteni S, Sinha S, Garrett-Sinha LA, Raghavan S (2008) Inhibition of oral mucosal cell wound healing by bisphosphonates. J Oral Maxillofac Surg 66:839–847
33. Lee TC, Mohsin S, Taylor D, Parkesh R, Gunnlaugsson T, O'Brien FJ, Giehl M, Gowin W (2003) Detecting microdamage in bone. J Anat 203:161–172
34. Leemans JC, Juffermans NP, Florquin S, van Rooijen N, Vervoordeldank MJ, Verbon A, Deventer SJH, van der Poll T (2001) Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. J Immunol 166:4604–4611
35. Li J, Mashiba T, Burr DB (2001) Bisphosphonate treatment suppresses not only stochastic remodeling but also the targeted repair of microdamage. Cacif Tissue Int 69:281–286
36. Marx RE (2003) Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg 61:1115–1118
37. Marx RE, Sawatari Y, Fortin M, Broumand V (2005) Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: risk factors, recognition, prevention, and treatment. J Oral Maxillofac Surg 63:1567–1575
38. Mendenhall WM (2004) Mandibular osteoradionecrosis. J Clin Oncol 22:4867–4868
39. Migliorati CA (2003) Bisphosphonates and oral cavity avascular bone necrosis. J Clin Oncol 21:253–254
40. Migliorati CA, Casiglia J, Epstein J, Jacobsen PL, Siegel MA, Sook-Bin MS (2005) Managing the care of patients with bisphosphonate-associated osteonecrosis. An American academy of oral medicine position paper. JADA 136:1658–1668
41. Moreau MF, Guillet C, Massin P, Chavalier S, Gascan H, Basle MF, Chapparad D (2007) Comparative effects of five bisphosphonates on apoptosis of macrophage cells in vitro. Biochem Pharmacol 73:718–723
42. O'Brien FJ, Brennan O, Kennedy OD, Lee C (2005) Microcracks in cortical bone: how do they affect bone biology? Curr Osteoporos Rep 3:39–45
43. Odvina CV, Zerwkh JE, Rao DS, Maalouf N, Gottschalk FA, Pak CY (2005) Severely suppressed bone turnover: a potential complication of alendronate therapy. J Clin Endocrinol Metab 90:1294–1301
44. Ott SM (2005) Editoral: long-term safety of bisphosphonates. J Clin Endocrinol Metab 90:1897–1899
45. Papapoulos SE (2001) Catabolic and anabolic signals in bone: therapeutic implications. Curr Opin Clin Nutr Metab Care 4:191–196
46. Peleg M, Lopez EAL (2006) The treatment of osteoradionecrosis of the mandible: the case for hyperbaric oxygen and bone graft reconstruction. J Oral Maxillofac Surg 64:956–960
47. Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T (1999) Prevention of osteocyte and osteoblast apoptosis by bisphophonates and calcitonin. J Clin Invest 104:1363–1374
48. Reid IR, Bolland MJ, Grey AB (2007) Is bisphosphonate-associated osteonecrosis of the jaw caused by soft tissue toxicity? Bone 47:318–320
49. Rizzoli R, Burlet N, Cahall D, Delmas PD, Eriksen EF, Felsenberg D, Grbic J, Jontell M, Landesberg R, Laslop A, Wollenhaupt M, Papapoulos S, Sezer O, Sprafka M, Reginster JY (2008) Osteonecrosis of the jaw and bisphosphonate treatment for osteoporosis. Bone 42:841–847
50. Ruggiero SL, Mehrotra B, Rosenberg T, Engroff SL (2004) Osteonecrosis of the jaws associated with the use of bisphosphonates: a review of 63 cases. J Oral Maxillofac Surg 62:527–534
51. Ruggiero SL, Fantasia J, Carlson E (2006) Bisphosphonate-related osteonecrosis of the jaw: background and guidelines for diagnosis, staging and management. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 102:433–441
52. Sarin J, DeRossi SS, Akintoye SO (2008) Updates on bisphosphonates and potential pathobiology of bisphosphonate-induced jaw osteonecrosis. Oral Dis 14:277–285
53. Schaffler MB, Pitchford K, Choi K, Riddle M (1994) Examination of compact bone microdamage using back-scattered electron microscopy. Bone 15:483–488
54. Sedghizadeh PP, Kumar SKS, Gorur A, Schaudinn C, Shuler CF, Costerton JW (2008) Identification of microbiofilms in osteonecrosis of the jaws secondary to bisphosphonte therapy. J Oral Maxillofac Surg 66:767–775
55. Takagi K, Takagi M, Kanangat S, Warrington KJ, Shigemitsu H, Postlethwaite AE (2005) Modulation of TNF-alpha gene expression by IFN-gamma and pamidronate in murine macrophages: regulation by STAT1-dependent pathways. J Immunol 174:1801–1810
56. van Poznak C (2006) The phenomenon of osteonecrosis of the jaw in patients with metastatic breast cancer. Cancer Invest 24:110–112
57. Wannfors K, Hammarström L (1985) Infectious foci in chronic osteomyelitis of the jaws. Int J Oral Surg 14:493–503