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# Osteonecrosis of the jaws in patients treated with bisphosphonates – histomorphologic analysis in comparison with infected osteoradionecrosis

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**BACKGROUND: Patients treated with bisphosphonates because of bone metastases have been shown to develop osteonecrosis of the jaws. In the present study, we examined the histologic findings of these cases. As similarities between this disorder and infected osteoradionecrosis (IORN) are described, both lesions were compared.**
**METHODS: We investigated eight patients with bisphosphonate treatment and osteonecrosis (four female, four male; median age: 65.6 years; cancer: multiple myeloma in five patients, breast cancer in three patients; mandibular involvement in five patients, maxillar involvement in three cases), and 10 patients suffering from IORN (all male; median age: 61.3 years; cancer: squamous cell carcinoma in nine patients, adenoid cystic carcinoma in one patient; mandibular involvement in all cases). Multicentric and bilateral involvement was common in the bisphosphonate group. Histologically, the bone revealed diffuse and patchy areas of necrosis in the bisphosphonate group, while in IORN osteonecrosis was larger and not diffusely distributed.**
**RESULTS: In all cases, we found *Actinomyces* attached to the necrotic bone tissue. In five of eight bisphosphonates cases, and in six of 10 IORN cases, numerous osteoclasts could be detected close to vital bone exhibiting signs of bone resorption. Pseudoepitheliomatous hyperplasia (PH) was revealed in five of eight bisphosphonate patients, and in seven of 10 IORN patients.**
**CONCLUSION: We conclude that *Actinomyces* is involved in the chronic, non-healing inflammatory processes as a characteristic feature of both diseases. Together with the associated presence of increased osteoclast numbers, we suggest that both factors may be involved in osteolytic mechanisms.**
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## Introduction

Patients sustaining osseous metastatic spread not only suffer substantial pain but also may develop pathologic fractures with devastating consequences. As it is known that the mechanisms of osteolysis associated with metastatic destruction of bone are essentially mediated by osteoclasts, inhibition of osteoclastic activity by biphosphonates has become a major target in the drug treatment of these patients. Bisphosphonates bind avidly to the mineralized bone tissue at the sites of osteoclast lacunae, and are then internalized by the osteoclasts. They inhibit both osteoclastic activity and osteoclast recruitment and, moreover, diminish the lifespan of these cells. The efficacy of bisphosphonates has been established in numerous studies. Based upon the guidelines by the American Society of Clinical Oncology, bisphosphonates are standard treatment in severe hypercalcemia associated with malignancy and bone metastases, mainly in patients suffering from multiple myeloma and breast cancer (1–4).

Recently, a conspicuous number of jaw osteonecrosis was reported for patients undergoing bisphosphonate treatment, especially with the nitrogen containing bisphosphonates pamidronate and zoledronate (4–8). Most authors favor the view that bisphosphonates induce obliteration of the regional blood vessels and thus lead to avascular bone necrosis (4–6) but there is also evidence of an inhibitory effect of bisphosphonates on the keratinocyte cell cycle, which might promote a mucosal breakdown as a second line of pathogenesis (9). The clinical symptoms and lesions are rather similar to the lesions seen in patients with osteoradionecrosis. Necrotic bone is exposed to the oral cavity. The lesions are often painless; however, the patients may suffer from pain because of surrounding inflammatory soft tissue

reactions and show symptoms and radiological signs of bone sequestration and/or osteomyelitis (4–6). Tooth extraction is very common in the history of these patients. Interestingly, this disorder seems to be less prominently localized in the mandible in comparison with osteoradionecrosis (4).

Concerning the histopathology of this disorder, Ruggiero et al. (4) mentioned that the microscopical examination of their study population revealed necrotic bone with bacterial debris and granulation tissue. Recently, it has been found that *Actinomyces* is detectable in a high percentage of patients suffering from infected osteoradionecrosis (IORN), which is one of the most severe complications of radiotherapy in the head and neck region (10, 11). Interestingly, Lugassy et al. (8) describe two patients with severe osteomyelitis and presence of *Actinomyces* colonies after bisphosphonate therapy. Recently, Melo and Obeid (12) reported on a similar case with *Actinomyces* osteomyelitis in a patient treated with zoledronate because of metastatic breast cancer.

In the present study, we investigated the light microscopical findings of tissue specimens of bisphosphonate-treated patients with osteonecrosis of the jaws and compared them with tissues from patients suffering from IORN.

## Materials and methods

*Patient data from the bisphosphonate group*

The characteristics of the patients are summarized in Table 1. This study population included eight patients (four female, four male; mean age: 65.6 years), who were treated because of bone metastases with bisphosphonates. Multiple myeloma was the underlying disease in five patients, while three patients suffered from breast cancer. All patients except for patient No. 1 received both pamidronate (Aredia; Novartis, East Hannover, NJ, USA) and zoledronate (Zometa; Novartis). Most commonly, therapy was started by pamidronate and followed by zoledronate. The mean duration time of therapy was 14 months in the case of pamidronate, and 19.6 months in the case of zoledronate. Patient No. 7 received ibandronate (Bondronat; Novartis). Patients mainly presented with necrotic bone exposed to the oral cavity and inflammatory reactions of the surrounding soft tissues. Three patients (37.5%) presented with mandibular bone involvement, three patients (37.5%) had maxillary involvement, and two patients (25%) presented with the disorder both in maxilla and mandible. In three patients, lesions occurred bilaterally. Most interestingly, six of eight patients (75%) had a tooth extraction in their history. Panoramic X-rays showed regions of osteolysis, sometimes with sequester formation.

*Patient data from the IORN group*

The characteristics of the patients are summarized in Table 2. This study population included 10 patients (all male; mean age: 61.3 years). Patients were treated by radiotherapy due to cancer of the head and neck region. Underlying malignancy was squamous cell carcinoma except for one case with an adenoid cystic carcinoma (patient No. 15). For diagnosis, IORN was defined according to previous studies as bone necrosis following irradiation of osseous tissue (i.e. the jaws) with signs of infection or sequester formation (13, 14). The patients presented with local abscess formation, chronic fistula, and necrotic bone exposed to the oral cavity. In all cases, bone necrosis was confirmed by radiography. In this group, only four patients had tooth extraction prior to radiotherapy.

*Tissue preparation and microscopy*

Biopsies were taken both from the bone tissue and the skin-exhibiting fistula. Tissue specimens were fixed in 4% phosphate-buffered saline (PBS)-formalin and

**Table 2** Data of the patients with IORN

| *Patient number* | *Age* | *Sex* | *Diagnosis* | *Radiation dose (Gy)* | *Tooth extraction* | *Site of necrosis* |
|---|---|---|---|---|---|---|
| 9 | 77 | M | SCC | 60 | Yes | Mandible |
| 10 | 59 | M | SCC | 60 | Yes | Mandible |
| 11 | 55 | M | SCC | 70 | No | Mandible |
| 12 | 49 | M | SCC | 60 | No | Mandible |
| 13 | 63 | M | SCC | 60 | No | Mandible |
| 14 | 62 | M | SCC | 60 | Yes | Mandible |
| 15 | 57 | M | ACC | 80 | Yes | Mandible |
| 16 | 47 | M | SCC | 60 | No | Mandible |
| 17 | 74 | M | SCC | 60 | No | Mandible |
| 18 | 70 | M | SCC | 70 | No | Mandible and maxilla |

ACC, adenoid cystic carcinoma; SCC, squamous cell carcinoma; IORN, infected osteoradionecrosis; M, male.

**Table 1** Data of the patients treated with bisphosphonates

| *Patient number* | *Sex* | *Age* | *Diagnosis* | *Bisphosphonate (treatment duration in months)* | *Tooth extraction* | *Site of necrosis* |
|---|---|---|---|---|---|---|
| 1 | F | 84 | MM | Zometa (18) | No | Mandible |
| 2 | M | 44 | MM | Aredia (14) and Zometa (12) | Yes | Mandible |
| 3 | M | 58 | MM | Aredia (9) and Zometa (48) | No | Mandible |
| 4 | F | 70 | MM | Aredia (9) and Zometa (12) | Yes | Maxilla |
| 5 | F | 56 | BC | Aredia (18) and Zometa (13) | Yes | Maxilla |
| 6 | M | 65 | MM | Aredia (13) and Zometa (25) | Yes | Mandible and maxilla |
| 7 | F | 81 | BC | Zometa (22) and Bondronat (7) | Yes | Mandible |
| 8 | F | 68 | BC | Aredia (21) and Zometa (7) | Yes | Maxilla |

BC, breast cancer; MM, multiple myeloma; F, female; M, male.

processed according to standard protocols. Decalcification was performed either in trichloric acid (for 24–72 h) or in ethylenediaminetetraacetic acid (EDTA; for 3–8 days) *ad libitum* (it should be mentioned that no differences could be observed between these two procedures, especially concerning the histomorphology of the bone). The specimens were embedded in paraffin, and 4 μm thick slides were cut. Slides were stained by hematoxylin and eosin as well as special histochemical techniques to detect *Actinomyces*, such as periodic-acid Schiff (PAS), gram, and Grocott reaction. These stains were applied according to standard protocols (15).

Slides were examined and photographed with a Zeiss microscope (type Axiophot Oberkochen, Germany; Olympus camera Camedia, Hamburg, Germany).

## Results

The results of the histologic examination are summarized in Table 3. Histologic examination revealed non-vital bone tissue in all cases. However, the gross pattern of necrosis differed between IORN and bisphosphonate-associated lesions. The typical IORN lesions showed extended homogenous regions of complete bone necrosis whereas the bisphosphonate lesions consisted of multiple, partially confluent areas of necrotic bone honeycombed with residual nests of vital bone (Fig. 1a,b).

Inflammatory infiltrates were found in all cases. These consist of neutrophilic granulocytes and often lymphocytes and plasma cells. Besides inflammatory infiltrates, fibrosis of the medullary spaces occurred in nearly all cases.

Obliteration of blood vessels was detected only in a few specimens (one in the bisphosphonate group and three patients in the IORN group). Mainly, these obliterations occurred in segmental arteries. In the bisphosphonate group, an increased cellularity was observed both in the intima and media of the artery (Fig. 2a), while in IORN, intima and media were hyalinized and exhibited less number of cells (Fig. 2b).

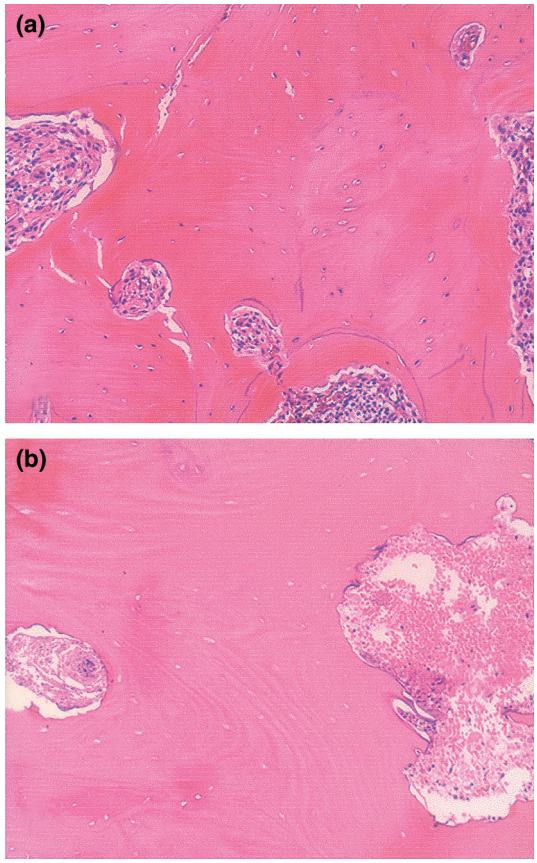


**Figure 1** (a) Tissue specimen from a patient treated with bisphosphonates shows osteonecrosis with a diffuse pattern: besides empty osteocytic lacunae viable osteocytes are seen (hematoxylin and eosin, original magnification ×100). (b) Tissue specimen from a patient with infected osteoradionecrosis (IORN) demonstrates an extended complete osteonecrosis with empty lacunae (hematoxylin and eosin, original magnification ×100).

**Table 3** Summary of the histologic findings

| *Patient number* | *Actinomyces* | *Inflammatory infiltrate* | *OCL* | *PH* | *Vessel obliteration* |
|---|---|---|---|---|---|
| 1 | + | GRA | – | – | – |
| 2 | + | GRA | – | – | – |
| 3 | + | GRA | – | – | – |
| 4 | + | MI | + | + | – |
| 5 | + | MI | + | + | – |
| 6 | + | MI | + | + | + |
| 7 | + | MI | + | + | – |
| 8 | + | MI | + | + | – |
| 9 | + | MI | + | + | + |
| 10 | + | GRA | – | + | – |
| 11 | + | MI | + | + | – |
| 12 | + | MI | + | + | – |
| 13 | + | GRA | – | – | – |
| 14 | + | GRA | – | – | – |
| 15 | + | MI | + | + | – |
| 16 | (+) | MI | + | – | + |
| 17 | (+) | MI | + | + | |
| 18 | + | MI | + | + | + |

GRA, neutrophilic granulocytes; MI, mixed inflammatory infiltrate; OCL, osteoclast; PH, pseudoepitheliomatous hyperplasia; –, no findings.

*Actinomyces* colonies were found in all cases studied. These bacteria typically formed numerous sulfur granules. They could be stained with gram, Grocott, and PAS reaction. The colonies were most commonly detected at the site of necrotic bone exhibiting remarkable signs of erosion: in contrast to the other bone regions, osseous tissue was not clearly demarcated, but showed numerous irregularly shaped contours (Fig. 3a,b). Sometimes, the bacterial filaments were interspersed with a few cells, mainly with neutrophilic granulocytes. However, sulfur granules predominantly lacked the above-mentioned inflammatory. Further microorganisms were not detected except in one case of the bisphosphonate group with superficially localized fungal spores, most probably from *Candida* spp.

Numerous osteoclasts were detected in the majority of cases of both groups. These cells were localized in close

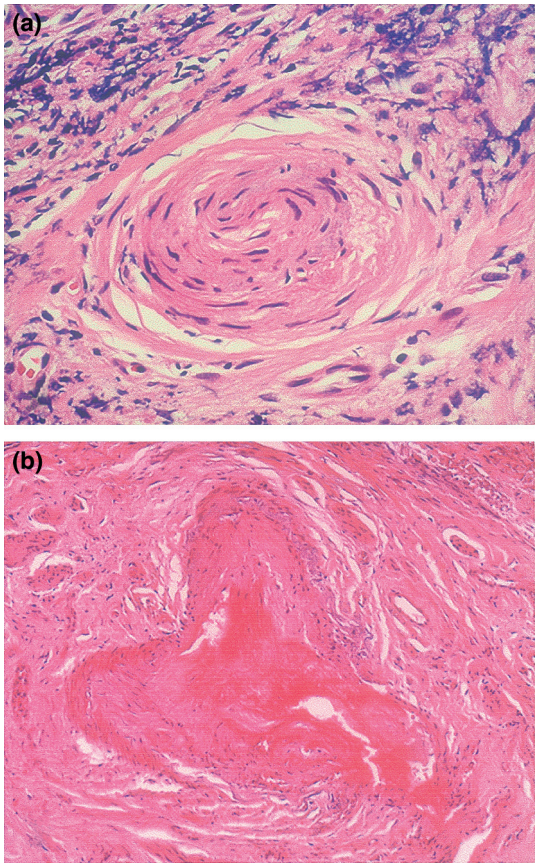


**Figure 2** (a) Tissue specimen from a patient with bisphosphonate treatment shows obliterated vessel with numerous spindle-shaped cells (hematoxylin and eosin, original magnification ×200). (b) Tissue from infected osteoradionecrosis (IORN) with a obliterated vessel reveals central hyalinosis and only a very few cells (hematoxylin and eosin, original magnification ×100).

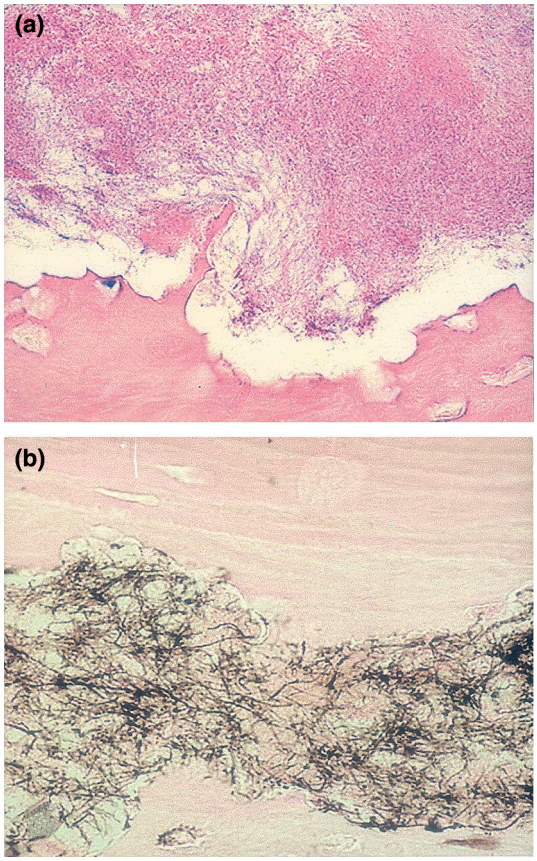


**Figure 3** (a) Tissue from a patient with bisphosphonate treatment reveals numerous *Actinomyces* colonies at the site of necrotic bone (hematoxylin and eosin, original magnification ×100). (b) Tissue specimen from the same patient as in Fig. 3a displays numerous *Actinomyces* filaments (black) with a strong positivity for Grocott's reaction (original magnification ×200).

contact to bone, and characteristically demonstrated lacunae as a sign of bone resorption. In these regions, the bone was not thoroughly necrotic, but also revealed areas with viable osteocytes (Fig. 4a). These findings were more prominently exhibited in the bisphosphonate group. In addition, the number of osteoclasts tended to be higher in these tissue specimens.

In five cases of the bisphosphonate group and in seven cases of the IORN group, epithelial proliferation occurred in the medullary spaces covering the bone trabeculae. The epithelium was non-keratinized and obtained an inflammatory infiltrate, mainly of neutrophilic granulocytes. Atypia could not be seen. These changes were diagnosed as pseudoepitheliomatous or pseudocarcinomatous hyperplasia. Interestingly, in several cases the epithelium was surrounded by *Actinomyces* colonies, which distinctly lied between the epithelium and the bone (Fig. 4b).

At the site of fistulas, mixed inflammatory infiltrates were a consistent finding. However, *Actinomyces* was not observed. Finally, we did not find any infiltrates of atypical plasma cells (no monoclonality detected by immunohistochemistry of the light chains – data not shown) or epithelial cells indicating infiltration by the known multiple myeloma or breast carcinoma.

## Discussion

There is growing evidence from several recently published reports that patients being treated with bisphosphonates because of osseous metastases can develop necrosis of the bone. Very interestingly, this disorder appears almost exclusively in the jaws. Several authors elucidated the clinical characteristics of these patients. In brief, patients commonly present with a non-healing extraction socket or exposed jawbone with progression to sequestration associated with purulent discharge (4–8). Together with the radiographical findings, the disorder has been described to be similar to osteoradionecrosis. In contrast to the clinical data, the histopathology of osteonecrosis in patients with bisphosphonate treatment has not been systematically analyzed. Therefore, we examined the light microscopical findings of these patients. In this context, osteomyelitis with the presence of *Actinomyces* has been described in two reports of patients treated with bisphosphonates (8, 12). This is of interest, as Støre et al. (10) and our group (11) recently reported on *A. israelii* in a high percentage of patients with IORN. In order to determine similarities and differences from IORN, tissues from both patient groups were compared.

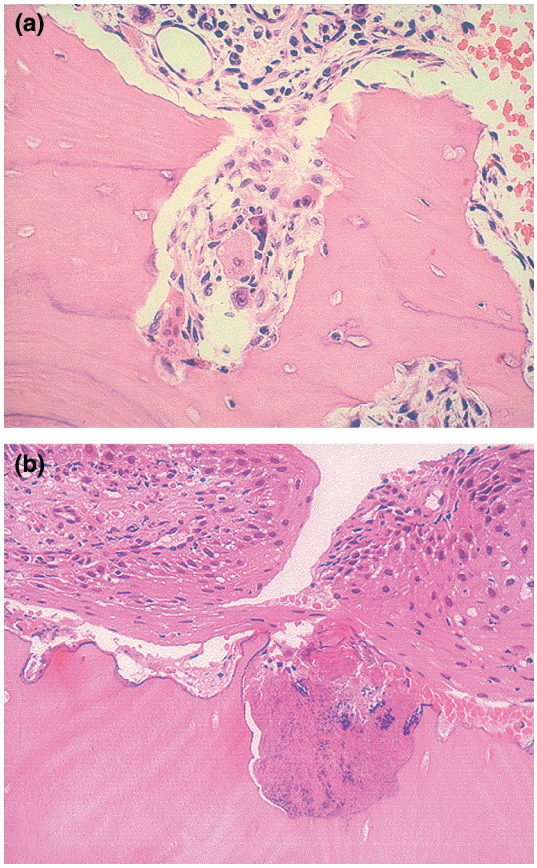


**Figure 4** (a) Numerous osteoclasts in a tissue specimen from a patient with bisphosphonate treatment, which demonstrates lacunae at the site of bone resorption. Note viable osteocytes in some areas of the bone (hematoxylin and eosin, original magnification ×200). (b) Pseudoepitheliomatous hyperplasia at the site of necrotic bone. Note centrally localized *Actinomyces* colonies, which are localized between the epithelium and the bone (hematoxylin and eosin, original magnification ×200).

In all cases, necrotic bone and inflammatory infiltrates were found. *Actinomyces* colonies forming characteristic sulfur granules occurred in all tissue specimens. They were mainly attached to the necrotic bone. Furthermore, the bone tissue showed remarkable signs of bone erosion. In all cases except one patient of the bisphosphonate group, they were the solely detectable microorganisms. In a recent study, *Actinomyces* were detected in about two-thirds of the IORN patients examined, and thus were remarkably more frequent than previously considered (11). Studying patients with bisphosphonate-associated osteonecrosis, Ruggiero et al. (4) reported that all of their specimens investigated (number of patients = 63) revealed bacterial debris in association with sequestrated bone. However, they did not mention whether *Actinomyces* was detected among these bacteria. From our study and from the reports of Lugassy et al. (8) and of Melo and Obeid (12), it can be concluded that *Actinomyces* in contact with a vital bone is a consistent histologic finding in osteonecrosis of the jaws of patients treated with bisphosphonates. From a clinical standpoint, these organisms might be involved in the chronic, non-healing inflammatory processes and the purulent discharge, which are mentioned above as a characteristic feature (4).

As a further histologic characteristic feature we observed pseudoepitheliomatous (also called pseudocarcinomatous) hyperplasia (PH) in five of eight bisphosphonate patients and in seven of 10 cases with IORN. This lesion commonly occurs as a rare complication of chronic osteomyelitis of long bones, most often in the tibia. But in the jaws, PH is unusual (16). Typically, this lesion is composed of squamous epithelium lacking signs of atypia and, of importance, exhibits a centrifugal type of involvement of medullary spaces, in contrast to a centro-medullary involvement as a general feature of squamous cell carcinoma. Warter et al. (16) report on three patients with PH of the jaws. Interestingly, in all cases bone was necrotic. Moreover, in one case *Actinomyces* was detectable. A topographic association between *Actinomyces* colonies and PH could also be demonstrated in the present study. These bacteria were found interposed between bone and epithelium in several cases. Thus, PH could play a role as a mechanism by which *Actinomyces* reaches the bone tissue.

In both IORN and osteonecrosis of patients treated with bisphosphonates, vessel obliteration is discussed as a major pathogenetic factor. While in IORN, this vessel obliteration is due to radiation-induced endarteritis followed by hyalinized narrowing of the vessels (17, 18), the mechanism in bisphosphonate-treated patients seems to be different. In particular, these drugs have been found to inhibit endothelial function *in vitro* and *in vivo* (19). Furthermore, in patients with bone metastases because of breast cancer, they diminish levels of vascular endothelial growth factor (20). These antiangiogenic effects are made responsible for the ischemia of the jawbone (4–6). The histologic changes of the vessels in bisphosphonate-associated osteonecrosis of the jaws have not yet been described. In a rat model, a decreased rate of capillary formation could be observed (19). In our study, we did not observe significant reduction of the capillaries, which is most probably due to the inflammation causing increased capillarization. However, we did observe obliteration of larger arterial vessels in one of eight patients, which was accompanied by an increased cellular proliferation of the intima and media. It remains to be further explored whether these findings are pathogenetically relevant.

Commonly, osteoclasts were detected in tissue specimens of both patients groups. They were surrounded by lacunae as sign of bone resorption. The presence of osteoclasts at the site of bone resorption strongly suggests that these cells are involved in the osteolysis mechanisms. It might be surprising that increased osteoclasts number and histologic evidence of osteoclastic activity is found in patients who are treated by drugs used to inhibit osteoclastic function and osteoclast recruitment. In this context, previous models of the interaction between osteoclasts, cytokines and bacterially induced bone destruction could be very intriguing (21, 22). However, the mechanisms of this osseous destruction are beyond the scope of this study and need further investigation.

As mentioned above, previous studies described the jaw findings in bisphosphonate-treated patients as being similar to IORN (4–6). From our study, it could be concluded that at least a few differences between both lesions do exist. Multifocality and occurrence of osteonecrosis in both the maxilla and the mandible can be observed in bisphosphonate-associated osteonecrosis, but these features are less frequent in IORN. The latter data are once more confirmed by a recent study of Bagan et al. (23), who found a relatively high percentage of patients treated with bisphosphonates (50%) presenting maxillary involvement. By means of histology, areas of osteonecrosis seemed to be patchier in tissue specimens of bisphosphonate-treated patients when compared with larger necrotic bone areas in IORN. However, further study containing larger number of patients should analyze the detailed clinical and histologic differences between both diseases.

In conclusion, this is the first study which describes the detailed histopathology of the osteonecrosis in patients treated with bisphosphonates. In all cases, we found *Actinomyces* colonies in close contact with the necrotic bone tissue, rendering it likely that these organisms may be involved in the chronic, non-healing inflammatory processes. Further study should elucidate, first, the role and origin of increased osteoclast numbers in bisphosphonate-associated osteonecrosis, secondly, the detailed histologic and clinical differences between IORN and bisphosphonate-associated osteonecrosis.

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