

**Osteomalacia: The Missing Link in the Pathogenesis of Bisphosphonate-Related
Osteonecrosis of the Jaws?**

Alberto Bedogni, Giorgia Saia, Giordana Bettini, Anita Tronchet, Andrea Totola,
Giorgio Bedogni, Paolo Tregnago, Maria Teresa Valenti, Francesco Bertoldo,
Giuseppe Ferronato, Pier Francesco Nocini, Stella Blandamura and Luca Dalle
Carbonare

The Oncologist published online June 20, 2012

The online version of this article, along with updated information and services, is
located on the World Wide Web at:
[http://theoncologist.alphamedpress.org/content/early/2012/06/19/theoncologist.2012-0
141](http://theoncologist.alphamedpress.org/content/early/2012/06/19/theoncologist.2012-0141)

Osteomalacia: The Missing Link in the Pathogenesis of Bisphosphonate-Related Osteonecrosis of the Jaws?

ALBERTO BEDOGNI,^a GIORGIA SAIA,^c GIORDANA BETTINI,^c ANITA TRONCHET,^c ANDREA TOTOLA,^a GIORGIO BEDOGNI,^{e,f} PAOLO TREGNAGO,^a MARIA TERESA VALENTI,^b FRANCESCO BERTOLDO,^b GIUSEPPE FERRONATO,^c PIER FRANCESCO NOCINI,^a STELLA BLANDAMURA,^d LUCA DALLE CARBONARE^b

^aUnit of Oral and Maxillofacial Surgery, Department of Surgery, and ^bDepartment of Medicine, Clinic of Internal Medicine, Azienda Ospedaliera Universitaria Integrata, Verona, Italy; ^cUnit of Maxillofacial Surgery, Department of Neurosciences, and ^dInstitute of Pathology, University of Padova, Padova, Italy; ^eClinical Epidemiology Unit, Liver Research Centre, Basovizza, Trieste, Italy; ^fDepartment of Maternal and Pediatric Sciences, University of Milan, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy

Key Words. Osteomalacia • Osteonecrosis • Jaw • Bisphosphonate • Risk factor

Disclosures: The authors indicated no financial relationships.

ABSTRACT

Background. Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a well-documented adverse event from treatment with nitrogen-containing bisphosphonates (NBPs). During a preliminary histomorphometric study aimed at assessing the rate of bone remodeling in the jaws of patient with surgically resected BRONJ, we found a defect of bone mineralization (unpublished data). We hypothesized that osteomalacia could be a risk factor for BRONJ in patients taking NBPs. Therefore, we looked for static and dynamic histomorphometric evidence of osteomalacia in biopsies from subjects with and without BRONJ.

Methods. This case-control study used histomorphometric analysis of bone specimens of patients using NBPs (22 patients with BRONJ and 21 patients without BRONJ) who required oral surgical interventions for the treatment/prevention of osteonecrosis. Patients were given tetracycline hydrochloride according to a standardized protocol

before taking bone biopsies from their jaws. Biopsies with evidence of osteomyelitis or necrosis at histology were excluded from the study. Osteomalacia was defined as a mineralization lag time >100 days, a corrected mean osteoid thickness >12.5 mm, and an osteoid volume >10%.

Results. In all, 77% of patients with BRONJ were osteomalacic compared with 5% of patients without BRONJ, according to histomorphometry. Because osteomalacia was found almost exclusively in NBP users with BRONJ, this is likely to be a generalized process in which the use of NBPs further deteriorates mechanisms of bone repair.

Conclusions. Osteomalacia represents a new and previously unreported risk factor for disease development. This finding may contribute to a better understanding of the pathogenesis of this disease and help with the development of strategies to increase the safety of NBP administration. *The Oncologist* 2012;17:000–000

INTRODUCTION

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a well-documented adverse event of treatment with nitrogen-containing bisphosphonates (NBPs) [1–3].

Several several risk factors for BRONJ have been identified, including type and cumulative dose of NBP, tooth extrac-

tion, and use of dentures [4]. However, its pathogenesis is not fully understood. Suppression of osteoclast-mediated bone remodelling and angiogenesis have been suggested to play a major pathogenetic role in BRONJ [5, 6].

During a preliminary histomorphometric study aimed at evaluating the rate of bone remodelling in the jaws of patients

Correspondence: Alberto Bedogni, M.D., Section of Oral and Maxillofacial Surgery, Department of Surgery, Azienda Ospedaliera Universitaria Integrata, Square L.A. Scuro 10, 37134 Verona, Italy. Telephone: +39 045 81 24 251; Fax: +39 045 80 27 437; e-mail: alberto.bedogni@univr.it. Received March 28, 2012; accepted for publication May 30, 2012. ©AlphaMed Press 1083-7159/2012/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2012-0141>

with surgically resected BRONJ, we found a defect of bone mineralization in all jaws (unpublished data). In agreement with a study reporting a higher frequency of BRONJ in zoledronate-treated patients with persistently low serum calcium and secondary hyperparathyroidism [7], we hypothesized that osteomalacia could be a risk factor for BRONJ in patients taking NBPs.

Because osteomalacia is essentially a histological diagnosis and may exist in the absence of biochemical and radiological abnormalities [8], we performed a case-control study to evaluate the static and dynamic histomorphometric evidence of osteomalacia in biopsies from subjects treated with NBP with or without BRONJ.

PATIENTS AND METHODS

A case-control study was performed between July 2007 and July 2009 by enrolling cases and controls from two cohort studies running at the maxillofacial units of Verona and Padova Universities [9, 10]. Patients in the BRONJ group were treated with NBP for a confirmed diagnosis of osteonecrosis of the mandible or maxilla [3]. They were candidates for surgical resection of the diseased jaw or for tooth extraction in the diseased jawbone without signs of BRONJ as a part of a preventive protocol of BRONJ [10]. Patients in the control group were treated with NBP without clinical and radiological signs of BRONJ [3]. They were candidates for tooth extraction as a part of a preventive protocol of BRONJ [10]. Vitamin D supplementation during NBP treatment was a reason for exclusion. The study protocol was approved by the local ethical committee (resolution 1428–07), and all patients gave written informed consent.

Clinical and Radiological Assessment

NBP usage was recorded in terms of type, dosage, and duration. Images of the oral cavity were taken using a digital camera (Nikon Finepix S1 Pro; Nikon, Tokyo, Japan). All patients underwent panoramic radiography before bone sampling. Cases underwent also spiral computed tomography and magnetic resonance imaging to define surgical resection margins [9].

Bone Sampling

Patients were given tetracycline hydrochloride, a fluorochrome incorporated at the bone mineralization front, at a standard dose of 500 mg twice daily for 2 days [11]. The same dose was repeated 12 days after the first dose. Surgery and bone sampling were performed from 5 to 7 days after administration of the second dose of tetracycline. In cases, bone sampling was performed on each resection margin to include the entire cross-sectional area of the mandible or maxilla [12]. Alternatively, 4-mm width core bone biopsies containing buccal and lingual cortical plates with cancellous bone were obtained from the alveolar process of the nondiseased jaw using a trephine dental drill under saline irrigation. In the control group, 4-mm width core bone biopsies containing buccal and lingual cortical plates with cancellous bone were obtained as described for the BRONJ group.

Bone samples were fixed in 70% ethanol and embedded in methyl-methacrylate resin (Merck 800590; Merck, Darmstadt, Germany). Bone sections were cut with a microtome (Polycut S; Leica Microsystems, Wetzlar, Germany) equipped with a carbide-tungsten blade. Serial sections of 5- μ m were cut from each specimen and stained with hematoxylin-eosin (HE) for histological examination. Resin-embedded bone blocks were then cut to obtain 8- μ m section, which were stained with Goldner's trichrome and mounted on microscope slides for histomorphometric evaluation. Such 8- μ m sections were obtained from three different levels of methyl-methacrylate block and were separated by a thickness of 250 μ m. A total of three to five sections from each level were left unstained for the measurement of fluorescent labelling.

Histological Examination

Histological examination was performed using an optical microscope (Leica DFC 280; Leica Microsystems, Wetzlar, Germany). A single experienced pathologist (B.S.), who was blinded to the clinical status of the patients, examined HE sections looking for signs of osteomyelitis and/or osteonecrosis. Bone was considered normal when its architecture was maintained and necrosis and inflammation were absent. Bone samples with findings of osteomyelitis or osteonecrosis were excluded from the analysis because inflammation and necrosis can interfere with bone mineralization [13].

Histomorphometry

Measurements were performed by a single experienced operator (D.C.L.), who was blinded to the clinical status of the patients. The system for image analysis included an epifluorescent microscope (Leica DM 2500; Leica Microsystems, Wetzlar, Germany), a digital camera (Leica DFC 420 C; Leica Microsystems, Wetzlar, Germany), and a computer equipped with a software for histomorphometric analysis (Bone version 3.5; Explora Nova, La Rochelle, France).

The histomorphometric values employed for analysis are the mean of three measurements obtained to approximate a three-dimensional evaluation. Histomorphometric analysis and reporting were performed following standard guidelines [14]. The full range of static and dynamic parameters of bone remodelling was measured, but only those pertinent to test the study hypothesis are reported in this paper. In particular, the abnormal deposition of unmineralized osteoid associated with an impaired (delayed) mineralization process was calculated using the following parameters: (a) osteoid volume (i.e., the percentage of unmineralized bone inside a given volume of bone); (b) corrected osteoid thickness (i.e., the mean corrected thickness of osteoid seams); and (c) mineralization lag time (i.e., the average time between osteoid formation and its mineralization). An estimate of mineralization lag time per day was obtained by dividing the osteoid thickness by the adjusted apposition rate. The adjusted apposition rate was obtained by multiplying the distance between the two tetracycline labels (divided by the time between the two tetracycline administrations) with the extent of the labeled surfaces averaged over the entire osteoid surface (mineralizing surfaces/osteoid surfaces).

Overall, osteomalacia was diagnosed in the presence of a mineralization lag time >100 days, a corrected osteoid thickness >12.5 mm, and an osteoid volume >10%, according to the internationally accepted histomorphometric definition of osteomalacia [14]. All thickness and depth measurements were corrected for obliquity by multiplying for $\pi/4$.

Statistical Analysis

Continuous variables are reported as 25th, 50th, and 75th percentiles and minimum and maximum values because of skewed distributions. Categorical variables are given as the number or percentage of patients with the characteristic of interest. Between-group comparisons of continuous variables were performed using the exact Wilcoxon-Mann-Whitney test and those of categorical variable using Fisher's exact test. Exact logistic regression was used to evaluate the association between BRONJ (yes vs. no), mineralization lag time (>100 vs. ≤ 100 days), corrected mean osteoid thickness (>12.5% vs. $\leq 12.5\%$), and osteoid volume (>10% vs. $\leq 10\%$).

Odds ratios (OR) and 95% confidence intervals (CI) were calculated before and after correction for sex (0 = female, 1 = male), age (years/10, continuous) and cancer (0 = no, 1 = yes). We adjusted for these factors because they could independently influence bone metabolism and mineralization.

Statistical analysis was performed on one bone specimen for each patient. If two specimens per patient were available, one was randomly chosen for statistical analysis. Even if we used exact logistic regression, the low number of subjects did not allow us to adequately model the possibility that one subject could contribute more than one bone to the analysis (i.e., a problem of correlated observations) [10]. Statistical analysis was performed using Stata 12.0 (Stata, College Station, TX) and StatXact 9 and LogXact 9 (Cytel, Cambridge, MA).

RESULTS

Figure 1 describes the enrollment and selection of cases and controls for bone histomorphometry. Of 25 potentially eligible patients, 2 patients were being supplemented with vitamin D and were excluded from the study. The remaining 23 patients underwent bone biopsy. One patient who underwent maxillary resection had histological evidence of osteomyelitis at the margin of bone resection where the biopsy was taken and was not considered further. Thus, 22 patients were left for bone histomorphometry. Of these, 18 had had their jaws resected and 4 patients had an alveolar bone biopsy from the unaffected jaw. Of 25 eligible control subjects, 4 were not included because their bone biopsies showed osteomyelitis. Therefore, 21 controls underwent bone histomorphometry.

Table 1 provides the clinical and histomorphometric measurements of the patients. In the BRONJ group, 18 patients were women and 4 were men. In the control group, 13 patients were women and 8 were men. Patients in the BRONJ group were older than control subjects. The cumulative dose of zoledronate but not of pamidronate was higher in the BRONJ group.

Table 2 reports the frequency of osteomalacia as detected by bone histomorphometry. Two bone specimens, all without

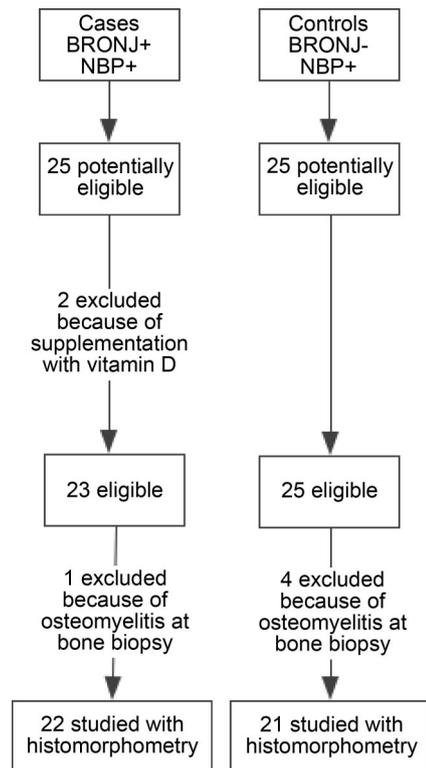


Figure 1. Enrollment and selection of cases and controls for bone histomorphometry. Abbreviations: BRONJ, bisphosphonate-related osteonecrosis of the jaw; NBP, nitrogen-containing bisphosphonate.

signs of osteomyelitis or osteonecrosis, were available for 14 patients, but only one of them was randomly chosen for statistical analysis. However, the bone specimens not considered for analysis showed signs of osteomalacia on histomorphometry.

In all, 77% of patients with BRONJ had a mineralization lag time >100 days as compared with 5% of patients without BRONJ. In addition, 64% of patients with BRONJ had corrected osteoid thickness >12.5% compared with 5% of patients without BRONJ. A total of 45% of patients with BRONJ had osteoid volume >10% compared with 5% of patients without BRONJ (Fig. 2).

As determined by exact logistic regression, the odds of having BRONJ were nearly 60 times higher for patients with a mineralization lag time >100 days than for those without it (OR = 59, 95% CI: 6–2,977, $p < .001$). These odds were still very high after correction for sex, age, and cancer (adjusted OR = 62, 95% CI: 8– ∞ , $p < .001$). Likewise, the odds of having BRONJ were much higher for patients with an osteoid thickness >12.5% (crude OR = 32, 95% CI: 4–1,551, $p < .001$; adjusted OR = 36, 95% CI: 3–2,657, $p < .001$) and for those with an osteoid volume >10% (crude OR = 16, 95% CI: 2–758, $p = .004$; adjusted OR = 55, 95% CI: 2–7,423, $p = .003$).

Control subjects who had been followed up as part of a cohort study did not develop any sign of BRONJ up to 1 year after bone sampling. The four patients who were excluded from the study because of osteomyelitis were all osteomalacic at histo-

Table 1. Clinical and histomorphometric characteristics of patients

	BRONJ group (n = 22)				Control group (n = 21)				p-value
	Percentile				Percentile				
	50th	25th	50th	Range	50th	25th	75th	Range	
Age (yrs)	66	65	71	55–84	61	56	64	17–75	.004
Mineralization lag time (days)	157	115	277	7–1370	15	9	21	5–115	<.001
Corrected osteoid thickness (mm)	13	12	14	3–15	3	2	4	2–13	<.001
Osteoid volume (%)	10	9	10	1–12	3	2	3	1–10	<.001
Cumulative dose (mg)									
Zoledronate	60	24	100	0–144	8	0	36	0–160	.00
Pamidronate	0	0	2160	0–6120	40	0	1620	0–6,480	.638
Alendronate	0	0	0	0–16,800	0	0	0	0–17,640	NA
Neridronate	0	0	0	0	0	0		0–1,200	NA
Risedronate	0	0	0	0	0	0		0–8,700	NA

p-values obtained by Wilcoxon-Mann-Whitney test.

Abbreviations: BRONJ, bisphosphonate-related osteonecrosis of the jaws; NA, not available.

Table 2. Frequency of osteomalacia

	BRONJ group (n = 22)	Control group (n = 21)	p-value
Mineralization lag time >100 days			<.001
No	5 (22.7)	20 (95.2)	
Yes	17 (77.3)	1 (4.8)	
Corrected osteoid thickness >12.5 mm			<.001
No	8 (36.4)	20 (95.2)	
Yes	14 (63.6)	1 (4.8)	
Osteoid volume >10%			.004
No	12 (54.5)	20 (95.2)	
Yes	10 (45.5)	1 (4.8)	

Data are n (%). p-values obtained by Fisher's exact test. Abbreviation: BRONJ, bisphosphonate-related osteonecrosis of the jaws.

morphometry and developed clinical and/or radiological signs of BRONJ within 6 months.

DISCUSSION

NBPs are a mainstay in the treatment of many diseases—especially metastatic cancer, for which they can have a substantial impact on a patient's quality of life. However, the use of these drugs has been questioned because of their association with BRONJ. BRONJ is most common in patients treated with high-dose intravenous cancer medications [3]; it also is associated with oral surgical procedures, such as tooth extraction [15]. However, the mechanisms of BRONJ are in-

completely understood, so it is difficult to explain why this disease affects only a small number of patients who use bisphosphonates [16].

Although a cause-effect relationship cannot be inferred from a single case-control study, our results support the association between osteomalacia and BRONJ development in patients treated with NBPs. Osteomalacia is characterized by an impairment of bone mineralization and is commonly caused by a decrease of the serum calcium-phosphate product, which has multiple causes. Bone mineralization defects are best quantified by histomorphometry, but the necessary harvesting of bone cannot be routinely performed. The availability of a sufficient number of bone biopsies to test the study hypothesis is a strength of this study. The fact that patients and control subjects were not strictly comparable in terms of age and type of NBP treatment was due to the limited number of patients that could be enrolled for the study—not only because BRONJ is infrequent but also because jawbone surgery in patients with BRONJ was considered to be unpredictable at the time of the study [17].

Vitamin D deficiency can lead to osteomalacia in adults [18, 19]. A recent study has found that vitamin D–depleted rats taking zoledronate are at greater risk of developing BRONJ than rats taking zoledronate alone [20]. Thus, it is tempting to speculate that hypovitaminosis D may also be a risk factor for BRONJ in humans because it was strongly associated with osteomalacia in our study. BRONJ is more frequent in patients with cancer than in patients with metabolic bone disease [3]. Also, hypovitaminosis D is common in such patients [21]; because of the well-known association between vitamin D depletion and osteomalacia, vitamin D deficiency may be an explanation for the high frequency of osteomalacia found in patients with cancer in our study. Routine use of vitamin D and calcium supplements during NBP treatment is currently rec-

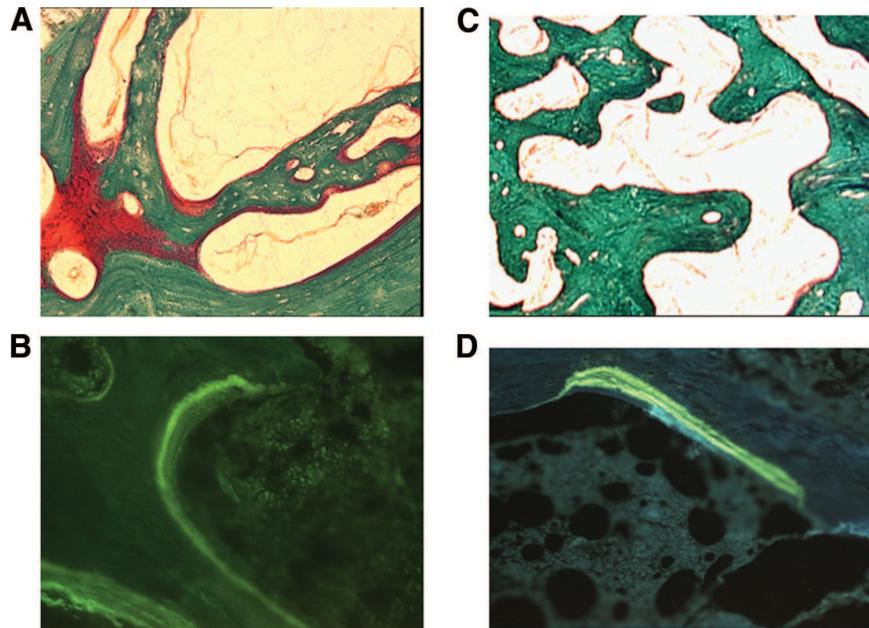


Figure 2. Histomorphometric evidence of osteomalacia. Goldner's trichrome-stained (A, C) and tetracycline double-labelled (B, D) bone specimens obtained from one patient with bisphosphonate-related osteonecrosis of the jaw (BRONJ) (A, B) and one patient without BRONJ (C, D). (A): The distribution of osteoid within the trabecular surface of a patient with BRONJ in terms of total seams, thickness, and volume is shown. A great amount of unmineralized osteoid (red) is evenly distributed within the trabeculae (green), which is suggestive of defective bone mineralization. (B): The absent double labelling and the poor, diffuse uptake of the tetracycline labels are shown. (C): The distribution of osteoid in the trabecular surface of a patient without BRONJ is shown, in terms of total seams, thickness, and volume. No areas of unmineralized osteoid can be seen within the trabeculae. (D): The characteristic double tetracycline labelling of normal bone is shown in a patient without BRONJ. Magnification, $\times 200$.

ommended [19]. However, the use of supplements was not consistently recommended in Italy when our study was performed [22]. This fact helped the recruitment of patients and allowed us to build up a unique study population, in which we could measure the association between osteomalacia and BRONJ.

Our results are at issue with the currently accepted pathogenetic theory of BRONJ, in which the type of NBP, cumulative dosage, and route of administration are considered to be the main risk factors [15, 23]. The risk of BRONJ has been primarily linked with the duration of NBP therapy, with an incidence up to 11% after 4 years of intravenous treatment in patients with cancer [24]. However, other factors may be related to BRONJ development. The recent observation of BRONJ in patients treated with denosumab [25], a non-bisphosphonate bone resorption inhibitor, suggests that the development of BRONJ is not specific to NBPs but common to drugs inhibiting osteoclast activity. In addition, undiagnosed mineralization defects in patients taking drugs that inhibit osteoclast activity may have a preminent role in promoting bone necrosis. Our results are in line with this finding because osteomalacia was a strong predictor of BRONJ in NBP users.

The fact that a combination of osteomalacia and NBP treatment may be needed to confer a substantial risk of BRONJ could explain why BRONJ affects only a small portion of NBP users and why some cancer patients may develop BRONJ even after one or few doses of NBP [26]. Also, high-dose NBPs can induce bone mineralization de-

fects [27, 28]. Of course, it is impossible to say whether osteomalacia was caused by or simply associated with NBP treatment in our patients. It should be nonetheless noted that our patients without BRONJ were not osteomalacic. This adds some plausibility to the hypothesis that NBPs affect bone mineralization in patients with established osteomalacia. A recent animal study has shown a negative impact of NBP on bone mineralization after tooth extraction [29]. Overall, the use of NBPs in subjects with a mineralization defect could compromise bone repair after traumatic injuries to the oral cavity and facilitate BRONJ.

In one study, patients treated with zoledronate had a normal bone mineralization and had not developed osteonecrosis after a median follow-up of 2 years [30]. However, they were mostly osteoporotic, received yearly infusions of zoledronate, and were given vitamin D and calcium supplements. It is possible that the combination of NBPs with undiagnosed osteomalacia may increase the risk of BRONJ in patients with cancer. If our hypothesis of a role for osteomalacia in the pathogenesis of BRONJ is correct, the role of NBP in the pathogenesis of BRONJ would be redefined.

CONCLUSION

Osteomalacia is common in patients with BRONJ. This finding may contribute to a better understanding of the pathogenesis of this disease and help in the development of strategies to increase the safety of NBPs.

ACKNOWLEDGMENT

This work was supported by the Italian Public Health Ministry (PRIN 2007LT5X87_004).

AUTHOR CONTRIBUTIONS

Conception/Design: Alberto Bedogni, Giorgia Saia, Giordana Bettini, Giorgio Bedogni, Francesco Bertoldo, Stella Blandamura, Luca Dalle Carbonare
Provision of study materials or patients: Giordana Bettini, Anita Tronchet, Andrea Totola, Giorgio Bedogni, Paolo Tregnago, Maria Teresa Valenti, Giuseppe Ferronato, Pier Francesco Nocini

Collection and/or assembly of data: Alberto Bedogni, Giordana Bettini, Anita Tronchet, Andrea Totola, Paolo Tregnago, Maria Teresa Valenti, Stella Blandamura, Luca Dalle Carbonare

Data analysis and interpretation: Alberto Bedogni, Giorgia Saia, Giordana Bettini, Giorgio Bedogni, Francesco Bertoldo, Stella Blandamura, Luca Dalle Carbonare

Manuscript writing: Alberto Bedogni, Giorgia Saia, Giorgio Bedogni, Luca Dalle Carbonare

Final approval of manuscript: Alberto Bedogni, Giorgia Saia, Giordana Bettini, Anita Tronchet, Andrea Totola, Giorgio Bedogni, Paolo Tregnago, Maria Teresa Valenti, Francesco Bertoldo, Giuseppe Ferronato, Pier Francesco Nocini, Stella Blandamura, Luca Dalle Carbonare

REFERENCES

- Filleul O, Crompot E, Saussez S. Bisphosphonate-induced osteonecrosis of the jaw: A review of 2,400 patient cases. *J Cancer Res Clin Oncol* 2010;136:1117–1124.
- Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: A growing epidemic. *J Oral Maxillofac Surg* 2003;61:1115–1117.
- Ruggiero SL, Dodson TB, Assael LA et al. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws—2009 update. *J Oral Maxillofac Surg* 2009;67:2–12.
- Vahsevanos K, Kyrgidis A, Verrou E et al. Longitudinal cohort study of risk factors in cancer patients of bisphosphonate-related osteonecrosis of the jaw. *J Clin Oncol* 2009;27:5356–5362.
- Allen MR, Burr DB. The pathogenesis of bisphosphonate-related osteonecrosis of the jaw: So many hypotheses, so few data. *J Oral Maxillofac Surg* 2009;67:61–70.
- Bi Y, Gao Y, Ehrhichou D et al. Bisphosphonates cause osteonecrosis of the jaw-like disease in mice. *Am J Pathol* 2010;177:280–290.
- Ardine M, Generali D, Donadio M et al. Could the long-term persistence of low serum calcium levels and high serum parathyroid hormone levels during bisphosphonate treatment predispose metastatic breast cancer patients to undergo osteonecrosis of the jaw? *Ann Oncol* 2006;17:1336–1337.
- Peach H, Compston JE, Vedi S et al. Value of plasma calcium, phosphate, and alkaline phosphatase measurements in the diagnosis of histological osteomalacia. *J Clin Pathol* 1982;35:625–630.
- Bedogni A, Saia G, Bettini G et al. Long-term outcomes of surgical resection of the jaws in cancer patients with bisphosphonate-related osteonecrosis. *Oral Oncol* 2011;47:420–424.
- Saia G, Blandamura S, Bettini G et al. Occurrence of bisphosphonate-related osteonecrosis of the jaw after surgical tooth extraction. *J Oral Maxillofac Surg* 2010;68:797–804.
- Frost HM. Measurement of human bone formation by means of tetracycline labelling. *Can J Biochem Physiol* 1963;41:31–42.
- Bedogni A, Blandamura S, Lokmic Z et al. Bisphosphonate-associated jawbone osteonecrosis: A correlation between imaging techniques and histopathology. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:358–364.
- Kröger H, Arnala I, Rehnberg V et al. Histomorphometry of periarticular bone in rheumatoid arthritis. *Ann Chir Gynaecol* 1994;83:56–62.
- Parfitt AM, Drezner MK, Glorieux FH et al. Bone histomorphometry: Standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res* 1987;2:595–610.
- Hoff AO, Toth B, Hu M et al. Epidemiology and risk factors for osteonecrosis of the jaw in cancer patients. *Ann N Y Acad Sci* 2011;1218:47–54.
- Migliorati CA, Epstein JB, Abt E et al. Osteonecrosis of the jaw and bisphosphonates in cancer: A narrative review. *Nat Rev Endocrinol* 2011;7:34–42.
- Khosla S, Burr D, Cauley J et al. Bisphosphonate-associated osteonecrosis of the jaw: Report of a task force of the American Society for Bone and Mineral Research. *J Bone Miner Res* 2007;22:1479–1491.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–281.
- Priemel M, von Domarus C, Klatt TO et al. Bone mineralization defects and vitamin D deficiency: Histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. *J Bone Miner Res* 2010;25:305–312.
- Hokugo A, Christensen R, Chung EM et al. Increased prevalence of bisphosphonate-related osteonecrosis of the jaw with vitamin D deficiency in rats. *J Bone Miner Res* 2010;25:1337–1349.
- Crew KD, Shane E, Cremers S et al. High prevalence of vitamin D deficiency despite supplementation in premenopausal women with breast cancer undergoing adjuvant chemotherapy. *J Clin Oncol* 2009;27:2151–2156.
- Adami S, Giannini S, Bianchi G et al. Vitamin D status and response to treatment in post-menopausal osteoporosis. *Osteoporosis International* 2009;20:239–244.
- Jung TI, Hoffmann F, Glaeske G et al. Disease-specific risk for an osteonecrosis of the jaw under bisphosphonate therapy. *J Cancer Res Clin Oncol* 2010;136:363–370.
- Bamias A, Kastritis E, Bamia C et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: Incidence and risk factors. *J Clin Oncol* 2005;23:8580–8587.
- Smith MR, Saad F, Coleman R et al. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: Results of a phase 3, randomised, placebo-controlled trial. *Lancet* 2012;379:39–46.
- Abu-Id MH, Warnke PH, Gottschalk J et al. “Bisphosphonate-induced osteonecrosis of the jaw. *J Craniomaxillofac Surg* 2008;36:95–103.
- Adamson BB, Gallacher SJ, Byars J et al. Mineralisation defects with pamidronate therapy for Paget’s disease. *Lancet* 1993;342:1459–1460.
- Recker RR, Delmas PD, Halse J et al. Effects of intravenous zoledronic acid once yearly on bone remodeling and bone structure. *J Bone Min Res* 2008;23:6–16.
- Huja SS, Mason A, Fenell CE et al. Effects of short-term zoledronic acid treatment on bone remodeling and healing at surgical sites in the maxilla and mandible of aged dogs. *J Oral Maxillofac Surg* 2011;69:418–427.
- Lyles KW, Colón-Emeric CS, Magaziner JS et al. Zoledronic acid and clinical fractures and mortality after hip fracture. *N Engl J Med* 2007;357:1799–1809.