RESEARCH ARTICLE

Interrelationship of clinical, radiographic and haematological features in patients under bisphosphonate therapy

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Objectives: To analyze the clinical, radiographic and haematological aspects of patients under bisphosphonate therapy.

Methods: A retrospective study was conducted where the records of patients taking bisphosphonates were analyzed considering the occurrence of bisphosphonate-related osteonecrosis of the jaw (BRONJ). Evaluation included panoramic and periapical radiographies, haematological examinations and clinical features. Radiographies were analyzed determining the presence or absence of bone sclerosis, osteolysis, persisting alveolar socket, narrowing of the mandibular canal, widening of the periodontal ligament space, periradicular radiolucency, sequestrum and thickening of the lamina dura. Laboratory tests consisted of complete blood count, fasting serum glucose, erythrocyte sedimentation rate (ESR) and serum levels of calcium, phosphorus, alkaline phosphatase, parathormone (PTH) and C-terminal telopeptide of collagen I (CTX).

Results: Alkaline phosphatase and ESR were significantly higher in the BRONJ group, whereas fasting serum glucose, CTX, PTH, calcium and phosphorus did not significantly differ. BRONJ showed association with smoking, tooth extraction, anaemia and leukocytosis. On radiographic analysis, persisting alveolar socket, osteolysis, bone sclerosis and narrowing of the mandibular canal were significantly more prevalent in the BRONJ group. Thickening of the lamina dura, periapical radiolucencies, widening of the periodontal ligament space and sequestrum did not significantly differ between the groups.

Conclusions: BRONJ is a multifactorial disease with high morbidity, which requires experimental studies to clarify the role of the reported risk factors and clinical radiographic signs to improve its diagnosis.

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Introduction

Since 2003, bisphosphonate therapy has been associated with osteonecrosis of the jaw [bisphosphonate-related osteonecrosis of the jaw (BRONJ)], a challenging side effect,¹ characterized by substantial morbidity and difficulty in treating. It significantly compromises the quality of life of patients, causing infection, necrotic bone exposure, pain, halitosis, tooth mobility, impaired

mastication and difficulty in speaking.^{2–4} The definition of BRONJ has required necrotic bone exposure in the oral cavity as a diagnostic criterion.⁵ However, recently, the occurrence of this disease without bone exposure has been extensively discussed.^{6–9} Taking into account the refractory behaviour of BRONJ, the sooner it is diagnosed, the greater the chances are of complete healing of the lesion. To improve the clinical evaluation of patients under bisphosphonate therapy and to make early diagnosis possible, associated aetiological and risk

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factors as well as imaging signs and laboratory markers have been investigated.

The diagnosis of BRONJ requires clinical signs and symptoms associated with radiographic findings.^{5,9,10} Even though BRONJ definition from American association of oral and maxillofacial surgeons (AAOMS)⁵ still requires bone exposure, at least through an intraoral or extraoral fistula, many authors believe that in case of symptoms such as pain, swelling, suppuration and paraesthesia, patients must be subjected to treatment.^{7-9,11} According to these authors, BRONJ diagnosis based on bone exposure is a late diagnosis, which is responsible for the refractory behaviour of the disease. In the meantime, studies have analyzed imaging modalities such as radiography, helical CT, CBCT, MR and bone scintigraphy, in search of imaging signs to confirm BRONJ,¹²⁻¹⁴ where persisting alveolar socket, bone sclerosis, osteolysis, thickening of the lamina dura, narrowing of the mandibular canal, widening of the periodontal ligament space and sequestrum have been reported as the most significant ones.^{15,16} Although helpful in determining the extension of the lesion, none of these examinations alone is capable of diagnosing BRONJ, without clinical signs and symptoms.

Moreover, serum biomarkers such as C-terminal telopeptide of collagen I (CTX), N-terminal telopeptide, osteocalcin, bone-specific alkaline phosphatase, parathormone (PTH) and deoxypyridinoline have been studied as possible tools for prediction of BRONJ development. However, except for CTX, these biomarkers have not shown satisfactory performance. Serum CTX, in turn, has been reported as a reliable predictor of BRONJ, indicating risk for developing the disease when its levels are lower than 150 pg ml^{-1} .¹⁷⁻²⁰ Nevertheless, this is a controversial issue, since several authors have raised important criticisms concerning the use of CTX for estimating BRONJ risk.^{21,22}

Salivary markers have also been tested in patients under bisphosphonate therapy. An increase in interleukin-1 and matrix metalloproteinase-9 and a decrease in desmoplakin were observed in saliva samples of patients with BRONJ compared with controls.^{23,24} Likewise, oxidative stress has been investigated in these patients, and preliminary results showed the increase of both serum and salivary oxidized glutathione/reduced glutathione ratio (GSSG/GSH) as the most significant predictive factor for BRONJ development.²⁵

Figure 1 Analysis of panoramic radiography in Photoshop CS6 v. 13.0 software (Adobe Systems Inc., San Jose, CA): the image was divided into six segments, with the paramedian region as reference.

Even though finding an alternative tool for early BRONJ diagnosis seems promising, most studies involve small samples, still requiring additional investigation. In fact, there is no single specific and accurate test capable of diagnosing BRONJ in patients who are asymptomatic without clinical evidence of necrotic bone exposure.²⁶ Considering the morbidity and refractory behaviour of the disease, development of new diagnostic methods to enable early detection of BRONJ is crucial. Accordingly, the present work aimed to analyze data from clinical, radiographic and haematological tests of patients under bisphosphonate therapy in search of interrelationships between them and BRONJ.

Methods and materials

This study was approved by the Research Ethics Committee of Pontifical Catholic University of Rio Grande do Sul (protocol number 834.129). The sample comprised 135 patients on oral or i.v. bisphosphonate, who had been seen at the Stomatology Service of São Lucas Hospital since 2003. Patients were allocated into two groups, according to the presence or absence of BRONJ: (1) BRONJ group, $n = 27$; and (2) non-BRONJ group, $n = 108$. Exclusion criteria were: (1) patients previously subjected to radiation therapy to the head and neck; (2) incomplete records; (3) metastasis in the jaws; and (4) history of bisphosphonate use withdrawal longer than 6 months. The records were

reviewed, and data from clinical, radiographic and laboratory tests were collected, analyzed and used for staging BRONJ.⁵ The criterion for diagnosing BRONJ considered bone exposure in oral cavity for more than 8 weeks or the association of clinical signs and symptoms (pain, swelling, suppuration, fistula tracts and paraesthesia)^{5,7-9} with radiographic features.^{5,9}

Clinical and laboratory analysis

Clinical data consisted of age and sex of the patients, primary disease in treatment, type of bisphosphonate used, risk factors and systemic comorbidities. The laboratory tests consisted of complete blood count, fasting serum glucose, erythrocyte sedimentation rate (ESR) and serum levels of calcium, phosphorus, alkaline phosphatase, PTH and CTX.

Radiographic analysis

The radiographic examinations were panoramic as well as periapical of upper and lower incisors and canines. Radiographs were digitized on an HP Scanjet G4050 photo scanner (Hewlett-Packard, São Bernardo do Campo, Brazil) and stored as uncompressed tagged image file format at 300 dpi. The images were analyzed twice, at different moments, by one observer, by using Photoshop CS6 v. 13.0 software (Adobe Systems Inc., San Jose, CA) and a colour antiglare 14-inch, 1366 × 768 light-emitting diode Samsung monitor (Samsung C&T do Brazil S/C Ltda, São Paulo, Brazil).

Panoramic radiography was divided into six segments, three mandibular and three maxillary, where the

Figure 2 Radiographic analysis: periapical radiographs were used to analyze Segments 2 and 5.

Table 1 Sample distribution according to bisphosphonate-related osteonecrosis of the jaw (BRONJ) occurrence, sex and age of the patients, primary disease and type of bisphosphonate

Feature	BRONJ	Non-BRONJ	Total	p-value ^d
Age (mean ± SD) (years)	69.93 ± 11.50	68.06 ± 10.28	68.44 ± 10.52	0.764 ^b
Sex				
Male	n 6	4	10	0.005^c
	% 22.2	3.7	7.05	
Female	n 21	104	125	
	% 77.8	96.3	92.6	
Total	n 27	108	135	
	% 100	100	100	
Disease				
Osteoporosis	n 14	99	113	0.000^c
	% 51.9	91.7	83.7	
Metastasis	n 12	5	17	0.000^c
	% 44.4	4.6	12.6	
Multiple myeloma	n 1	3	4	0.595 ^c
	% 3.7	2.8	2.96	
Prevention of metastasis	n –	1	1	0.200 ^c
	% –	0.9	0.74	
Total	n 27	108	135	
	% 100	100	100	
Type of bisphosphonate				
Oral				0.000^c
Alendronate	n 12	84	96	
	% 85.8	88.42	88.1	
Ibandronate	n 1	1	2	
	% 7.14	1.05	1.8	
Risedronate	n 1	10	11	
	% 7.14	10.53	10.1	
Total	n 14	95	109	
	% 100	100	100	
i.v.				
Zoledronic acid	n 12	1	13	
	% 92.3	14.29	65.0	
Pamidronate	n 1	4	5	
	% 7.7	57.14	25.0	
Clodronate	n –	2	2	
	% –	28.57	10.0	
Total	n 13	7	20	
	% 100	100	100	
Combinations				
Alendronate/ibandronate	n 1	1	2	
	% 100	16.67	28.57	
Alendronate/zoledronic acid	n –	1	1	
	% –	16.67	14.29	
Alendronate/risedronate	n –	4	4	
	% –	66.66	57.14	
Total	n 1	6	7	
	% 100	100	100	

n, number; SD, standard deviation.

^ap-value, $\alpha = 0.05$.

^bStudent's *t*-test.

^cFisher's exact test, adjusted residual analysis.

Bold values show significant difference.

Table 2 Sample distribution according to bisphosphonate-related osteonecrosis of the jaw staging and number of oral sites affected

Stage	Sample		Number of sites affected					
	n	%	1	2	3 or more	n	%	n
At risk	108	80	–	–	–	–	–	–
Stage 0	1	0.7	1	0.7	–	–	–	–
Stage 1	5	3.7	3	2.2	1	0.7	1	0.7
Stage 2	17	12.6	8	5.9	5	3.7	4	3
Stage 3	4	3	1	0.7	1	0.7	2	1.5
Total	135	100	13	9.5	7	5.1	7	5.2

n, number.

paramedian region was used as reference: maxilla from tuberos to paramedian region at right (Segment 1) and left (Segment 3); mandible from the angle to paramedian region at right (Segment 6) and left (Segment 4); anterior region of the maxilla (Segment 2); and anterior region of the mandible (Segment 5) (Figure 1). Each segment was analyzed determining the presence or absence of the following variables: bone sclerosis, osteolysis, persisting alveolar socket, narrowing of the mandibular canal, widening of the periodontal ligament space, periradicular radiolucency, sequestrum and thickening of the lamina dura. Segments 1, 3, 4 and 6 were analyzed only in panoramic imaging, while the analysis of Segments 2 and 5 was complemented by periapical radiographs of the anterior teeth to provide a better visualization, avoiding artefacts caused by spine superposition (Figure 2). The number of segments where each variable occurred in each patient was recorded. Variables that were not properly visualized in the available images were excluded from the respective analysis.

Statistical analysis

Data were analyzed by means of descriptive (mean, standard deviation, median, absolute and relative frequency) and inferential statistics. Fisher's exact test complemented by adjusted residual analysis as well as Mann–Whitney and Student's *t*-test were used to compare the variables between the groups, and Spearman correlation coefficient was used to compare the variables with each other. Data were processed in SPSS® v. 17.0 (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL) at a significance level of 5%.

Results

Sample distribution according to bisphosphonate-related osteonecrosis of the jaw occurrence, age, sex, primary disease and type of bisphosphonate

There was no significant difference in the age of patients between the groups (Student's *t*-test, $p = 0.764$). The non-BRONJ group showed association with females ($p = 0.005$), osteoporosis ($p = 0.000$) and use of oral bisphosphonate ($p = 0.000$), whereas the BRONJ group was associated with males ($p = 0.005$), metastasis ($p = 0.000$) and i.v. bisphosphonate use ($p = 0.000$, Fisher's exact test, adjusted residual analysis) (Table 1).

Bisphosphonate-related osteonecrosis of the jaw staging

The distribution of the sample according to BRONJ staging⁵ and number of affected sites is presented in Table 2. Most of the sample was at risk (without BRONJ), while most patients presenting with BRONJ had Stage 2 disease.

Serum markers

The BRONJ group showed significantly higher levels of alkaline phosphatase ($p = 0.005$) and ESR ($p = 0.041$) than the non-BRONJ group, whereas fasting serum glucose ($p = 0.331$), CTX ($p = 0.689$), PTH ($p = 0.719$), calcium ($p = 0.892$) and phosphorus ($p = 0.859$) did not show any significant difference between the groups (Mann–Whitney test, $\alpha = 0.05$) (Table 3).

Local and systemic risk factors and comorbidities

The BRONJ group showed association with smoking ($p = 0.003$), tooth extraction ($p = 0.000$), anaemia ($p = 0.001$) and leukocytosis ($p = 0.004$), whereas gastric ($p = 0.010$) and autoimmune ($p = 0.045$) disorders were significantly more prevalent in the non-BRONJ group. The other variables in this analysis did not show any significant difference between the groups (Fisher's exact test, $\alpha = 0.05$) (Table 4).

Radiographic analysis

Persisting alveolar socket ($p = 0.000$), osteolysis ($p = 0.000$), bone sclerosis ($p = 0.003$) and narrowing of the mandibular canal ($p = 0.041$) were significantly more prevalent in the BRONJ group. Widening of the

Table 3 Levels of serum markers in bisphosphonate-related osteonecrosis of the jaw (BRONJ) and non-BRONJ groups

Marker	BRONJ				Non-BRONJ				p-value ^a
	Mean	SD	Median	Mean rank	Mean	SD	Median	Mean rank	
Alkaline phosphatase (U/L)	97.21	57.10	82.00	71.63	69.21	25.80	63.00	51.00	0.005
ESR (mm/l h)	39.04	32.77	33.00	70.68	23.51	18.68	18.00	55.15	0.041
Fasting serum glucose (mg dl ⁻¹)	96.54	30.58	88.00	52.56	94.24	17.99	90.00	60.05	0.331
CTX (pg ml ⁻¹)	176.22	138.61	159.00	14.06	191.70	116.86	165.50	15.43	0.689
PTH (pg ml ⁻¹)	77.39	72.07	54.00	53.57	61.14	37.15	54.00	50.96	0.719
Calcium (mg dl ⁻¹)	9.26	0.67	9.40	56.59	9.27	0.99	9.30	57.72	0.892
Phosphorus (mg dl ⁻¹)	3.78	0.70	3.90	56.44	3.86	0.56	3.80	57.78	0.859

CTX, C-terminal telopeptide of collagen I; ESR, erythrocyte sedimentation rate; PTH, parathormone; SD, standard deviation; U/L, units per liter.

^ap-value for Mann–Whitney test, $\alpha = 0.05$.

Bold values show significant difference between the groups.

Table 4 Distribution of the sample in bisphosphonate-related osteonecrosis of the jaw (BRONJ) and non-BRONJ groups according to risk factors and local and systemic comorbidities

Risk factor comorbidities	BRONJ				Non-BRONJ				p-value ^a
	Presence		Absence		Presence		Absence		
	n	%	n	%	n	%	n	%	
Smoking	14	51.9	13	48.1	24	22.2	84	77.8	0.003
Tooth extraction	15	55.6	12	44.4	6	5.6	102	94.4	0.000
Dental implant	3	11.1	24	88.9	14	13.0	94	87.0	0.546
Torus	0	0	27	100	3	2.8	105	97.2	0.509
Periodontitis	2	7.4	25	92.6	7	6.5	101	93.5	0.571
Dental prosthesis	6	22.2	21	77.8	24	22.2	84	77.8	0.591
Anaemia	11	40.7	16	59.3	12	11.2	95	88.8	0.001
Leukocytosis	5	18.5	22	81.5	2	1.9	105	98.1	0.004
Thrombocytosis	1	3.7	26	96.3	2	1.9	105	98.1	0.491
Gastric disorders	0	0	27	100	19	17.6	89	82.4	0.010
Autoimmune disorder	3	11.1	24	88.9	31	28.7	77	71.3	0.045
Dyslipidemia	2	7.4	25	92.6	23	21.3	85	78.7	0.076
Vascular disorder	13	48.1	14	51.9	46	42.6	62	57.4	0.359
Endocrine disorder	6	22.2	21	77.8	19	17.6	89	82.4	0.379
Heart disease	4	14.8	23	85.2	13	12	95	88.0	0.454
Neurologic disorder	0	0	27	100	3	2.8	105	97.2	0.509
Pulmonary disorder	2	7.4	25	92.6	7	6.5	101	93.5	0.571

^ap-value for Fisher's exact test, $\alpha = 0.05$.

Bold values show significant difference.

periodontal ligament space ($p = 0.087$), sequestrum ($p = 0.103$), periradicular radiolucency ($p = 0.222$) and thickening of the lamina dura ($p = 0.603$) did not show any significant difference between the groups (Fisher's exact test, $\alpha = 0.05$) (Table 5) (Figures 3 and 4). To express the intensity of the variables, radiographic features were analyzed according to the number of segments (sum) in which they occurred in the whole sample. In this analysis, osteolysis ($p = 0.000$), persisting alveolar socket ($p = 0.000$), bone sclerosis ($p = 0.003$), sequestrum ($p = 0.043$) and narrowing of the mandibular canal ($p = 0.046$) showed a higher number of segments in the BRONJ group (Mann–Whitney test, $\alpha = 0.05$) (Table 6). All radiographic findings that showed a significantly higher prevalence in the BRONJ group occurred more in the mandible than in the maxilla: persisting alveolar socket (62.9%), in which Segments 4 (25.9%) and 5 (25.9%) were the most prevalent; osteolysis (81.4%), where Segment 4 (40.7%) was the most prevalent; and bone sclerosis (55.5%), where Segment 6 (29.6%) was the most prevalent.

Correlations

Positive correlation was observed between age and ESR ($r = 0.225$); ESR and widening of the periodontal ligament space ($r = 0.191$); and alkaline phosphatase and persisting alveolar socket ($r = 0.262$). Also, bone sclerosis was correlated with persisting alveolar socket ($r = 0.214$) and osteolysis ($r = 0.202$); and osteolysis with sequestrum ($r = 0.186$, Spearman correlation coefficient) (Table 7).

Discussion

Most of the sample in the present study was composed of females, whose age ranged from 41 to 95 years and, possibly because of that, osteoporosis was the most prevalent primary disease. There was no significant difference in age between the groups analyzed, which suggests that age is not associated with BRONJ.²⁷ Although higher than most reports in the literature,^{28–31} our data for BRONJ prevalence are similar to that found by Pelaz et al.³² Demographic specificities of the samples, the methods and the level of specialization of the centres

Table 5 Frequency of radiographic features in bisphosphonate-related osteonecrosis of the jaw (BRONJ) and non-BRONJ groups

Radiographic feature	BRONJ				Non-BRONJ				p-value ^a
	Presence		Absence		Presence		Absence		
	n	%	n	%	n	%	n	%	
Osteolysis	18	66.7	9	33.3	10	9.3	97	90.7	0.000
Persisting alveolar socket	14	51.9	13	48.1	13	12.1	94	87.9	0.000
Bone sclerosis	14	51.9	13	48.1	24	22.4	83	77.6	0.003
Narrowing of the mandibular canal	13	48.1	14	51.9	30	28.0	77	72.0	0.041
Widening of periodontal ligament space	13	48.1	14	51.9	34	31.8	73	68.2	0.087
Sequestrum	2	7.4	25	92.6	1	0.9	106	99.1	0.103
Periradicular radiolucency	8	29.6	19	70.4	22	20.6	85	79.4	0.222
Thickening of the lamina dura	6	22.2	21	77.8	24	22.4	83	77.6	0.603

^ap-value for Fisher's exact test, $\alpha = 0.05$.

Bold values show significant difference.

Figure 3 Radiographic features: (a) bone sclerosis and thickening of the lamina dura associated with widening of the periodontal ligament space; (b) diffuse osteolysis of the jaw with areas of bone sclerosis and sequestrum; (c) persisting alveolar socket; (d) periradicular radiolucency; (e) osteolysis; (f) periradicular radiolucency; (g) thickening of the lamina dura; (h) osteolysis; and (i) diffuse osteolysis of the jaw with areas of bone sclerosis.

where the different studies have been conducted as well as lack of consensus on the BRONJ case definition have been associated with such discrepancies of prevalence.

In this regard, some authors^{9,33} believe that many cases of BRONJ are undiagnosed and studies are biased because of the asymptomatic characteristic of the

Figure 4 Clinical and radiographic images of bisphosphonate-related osteonecrosis of the jaw in a patient subjected to zoledronic acid therapy for metastatic kidney cancer: (a) frank bone exposure in the mandible; site of probing bone through oral mucosa in the maxilla (arrows). (b) Panoramic radiography showing persisting alveolar sockets in the maxilla and mandible.

Table 6 Occurrence of radiographic features in bisphosphonate-related osteonecrosis of the jaw (BRONJ) and non-BRONJ groups according to the number of segments

Radiographic feature	Number of segments (sum)						p-value ^a
	BRONJ		Non-BRONJ		Total		
	n	%	n	%	n	%	
Osteolysis	30	18.5	14	2.2	44	32.6	0.000
Persisting alveolar socket	29	17.9	16	2.5	45	33.3	0.000
Bone sclerosis	19	11.7	30	4.6	49	36.3	0.003
Sequestrum	2	1.2	1	0.1	3	2.22	0.043
Narrowing of the mandibular canal	19	11.7	46	7.1	65	48.1	0.046
Widening of periodontal ligament space	15	9.3	42	6.5	57	42.2	0.112
Periradicular radiolucency	9	5.6	28	4.3	37	27.4	0.314
Thickening of the lamina dura	13	8.0	55	8.5	68	50.4	0.982

^ap-value for Mann–Whitney test, $\alpha = 0.05$.

Bold values show significant difference.

initial stage of the disease, where radiographic evaluation is crucial.

The BRONJ group was associated with i.v. bisphosphonates, whereas the non-BRONJ group was associated with the oral ones. This is in accordance with the fact that i.v. bisphosphonates have higher potency and cumulative dose and therefore higher risk for the disease.^{34,35} Also, the BRONJ group was associated with smoking, anaemia and leukocytosis. Anaemia has already been reported as a risk factor for BRONJ.^{36,37} Smoking, in turn, is a risk factor for not only BRONJ, but also cancer.^{27,31,34,36} In agreement with our results, zoledronic acid-induced leukocytosis has been reported in an *in vivo* study.³⁸ Moreover, considering that BRONJ is an infectious process, it could itself explain the occurrence of leukocytosis.³⁹

A local triggering factor was identified in 96.3% of our patients with BRONJ, where tooth extraction was the most prevalent one, as already reported.^{31,40,41} This finding corroborates the idea that all BRONJ cases develop from dentoalveolar causes⁴² or local comorbidities⁴³ and reinforces the hypothesis that BRONJ is exclusively a jaw disease because of the teeth, through which micro-organisms harboured in the oral cavity can reach the jaws.⁴⁴ Most individuals in the BRONJ group were classified as Stage 2, which is in accordance with the literature.^{45–47} On clinical evaluation, 52% of patients with BRONJ presented with at least two lesions in different sites of the oral cavity; and, on radiographic analysis, osteolysis and persisting alveolar socket were identified in at least two segments in each patient. These data suggest that, even though BRONJ presents with low incidence,^{28–31} its severity can be high.

Our radiographic data showed a significantly higher prevalence of persisting alveolar socket, osteolysis, bone sclerosis and narrowing of the mandibular canal in the BRONJ group, which corroborates the reported data.^{13,46,48,49} On the other hand, there was no significant difference for widening of the periodontal ligament space and thickening of the lamina dura between the groups. This finding disagrees with the literature, according to which these two variables would be early signs of BRONJ.^{18,40,43,50} Nevertheless, Sui⁴⁹

reported results similar to ours, finding that in patients with BRONJ, none had a widened periodontal ligament space and only 9% patients had thickening of the lamina dura. Actually, thickening of the lamina dura seems to be part of the whole-bone sclerosis process^{15,48,51} and accordingly, it should be evaluated within this feature. Another point to consider in the prevalence of these variables is their dependence on the presence and number of teeth in the oral cavity, which in turn can bias the results of the studies.

Bone sequestrum has been associated with BRONJ;^{13,48,49} but, in our study, it was the least prevalent radiographic feature. This could be explained by the transversal character of the study, where the images analyzed corresponded to the first radiographic examination to which the patient was subjected, and at this time it could be too early for sequestrum detection. Nevertheless, it is also important to recall here that a normal osteoclast function is necessary for formation of bone sequestrum, which does not happen under bisphosphonate effects, leading to the characteristic histopathological feature of continuity between non-vital and vital bones in the areas affected by BRONJ.^{52,53}

Tomography has been indicated to better evaluate jaw bones in bisphosphonate users.^{16,40,45} On the other hand, according to some authors,^{12,54} these patients should be routinely subjected to panoramic radiography and, only if needed for any reason, a more accurate examination should be requested. Radiography is not only easier to access but also exposes patients to less radiation.⁵⁵ On the basis of this premise, we used radiography in the imaging analysis.

The BRONJ group showed significantly higher levels of ESR than the non-BRONJ group. High values for this marker can indicate inflammatory activity,⁵⁶ and also malignancy.⁵⁷ Therefore, the high prevalence of patients in the BRONJ group showing soft-tissue infection, and cancer could explain the elevated ESR. Even though within the normal parameters, the BRONJ group had higher levels of alkaline phosphatase than the non-BRONJ group, and a positive correlation was identified between alkaline phosphatase and persisting alveolar socket. Taking into account that this is a

Table 7 “r” value for Spearman correlation coefficient

Variables	Age	AP	Ca	CTX	ESR	FSG	PHOS	PTH	PAS	OTL	BS	NMC	WPLS	SQ	PR	TLD
Age	1															
AP	0.016	1														
Ca	0.026	0.112	1													
CTX	0.207	0.228	−0.149	1												
ESR	0.225 ^a	0.203	0.082	−0.266	1											
FSG	0.137	0.065	0.089	−0.255	0.084	1										
PHOS	−0.012	0.001	0.054	0.454	−0.122	−0.109	1									
PTH	0.007	0.001	−0.161	−0.041	−0.065	0.081	−0.105	1								
PAS	0.052	0.262 ^b	0.007	−0.164	0.082	−0.038	−0.072	0.125	1							
OTL	−0.016	0.177	0.002	0.042	0.143	−0.069	0.020	−0.179	0.200	1						
BS	−0.129	0.086	−0.060	−0.147	0.115	0.046	0.121	0.174	0.214 ^a	0.202 ^a	1					
NMC	−0.119	0.187	0.000	−0.091	0.142	0.054	0.001	−0.018	0.097	0.065	0.045	1				
WPLS	−0.108	0.094	−0.051	0.079	0.191 ^a	0.065	−0.135	−0.032	0.128	0.025	0.137	0.015	1			
SQ	0.163	−0.180	0.021	−	0.011	0.195	0.025	0.130	−0.077	0.186 ^a	0.107	−0.010	−0.010	1		
PR	−0.002	−0.059	−0.104	0.193	0.037	−0.001	−0.203	0.025	0.121	0.172	0.029	0.054	0.142	−0.081	1	
TLD	−0.021	−0.098	0.034	−0.073	0.086	−0.014	0.110	0.017	0.153	0.013	0.139	−0.163	0.114	−0.082	0.044	1

AP, alkaline phosphatase; BS, bone sclerosis; Ca, calcium; CTX, C-terminal telopeptide of collagen I; ESR, erythrocyte sedimentation rate; FSG, fasting serum glucose; NMC, narrowing of mandibular canal; OTL, osteolysis; PAS, persisting alveolar socket; PHOS, phosphorus; PR, periradicular radiolucency; PTH, parathormone; SQ, sequestrum; TLD, thickening of lamina dura; WPLS, widening of periodontal ligament space.

^aSignificant correlation at the 0.05 level.

^bSignificant correlation at the 0.01 level.

radiographic sign of BRONJ,^{46,53,58} this finding would suggest that alkaline phosphatase increases with BRONJ occurrence and therefore, it could be a marker of the disease. Nevertheless, this enzyme lacks specificity, since changes in its levels are also attributed to other factors such as bone activity, cancer and impaired liver function.^{59,60} Other correlations were found, namely bone sclerosis with persisting alveolar socket and osteolysis, and osteolysis with sequestrum. These correlations seem plausible and corroborate the simultaneous occurrence of these radiographic variables in BRONJ lesions. CTX, which has been considered a predictor of BRONJ risk, showed no significant difference in levels between the BRONJ and non-BRONJ groups, or any correlations with any other variable.

Considering the results of the present work, the multifactorial behaviour of BRONJ and the complexity

of the clinical picture of patients suffering from this disease, it is clear that there are difficulties in clinical studies in isolating variables to confirm their association with BRONJ. This is probably the major factor responsible for disagreements between the studies. The effective role of each one of the postulated aetiologic risk factors and signs of this disease needs to be clarified, and experimental research with animal models would be of help.

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