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Bisphosphonate-related osteonecrosis of the human jaw: A combined 3D assessment of bone descriptors by histology and synchrotron radiation-based microtomography

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Bisphosphonate-related osteonecrosis of the jaw (BRONJ), as other bone diseases, is supposed to be associated with an unbalanced bone remodeling process: thus, the full comprehension of the pathophysiology is fundamental to advance its treatment.

Histological studies of morphometry and mineralization degree ofjaw sites have shown many histopathological aspects [1]; however, there is also the need for quantitative three-dimensional (3D) imagingat the nanoscale.

Recently, differences in lacunar morphology and peri-lacunar tissue properties at the submicrometer length scale were investigated by synchrotron radiation-based X-ray microtomography (SR-microCT) in human jaw bone samples obtained from both healthy subjects and patients suffering from BRONJ [2]. It was found no significant variation in mineralization after BP treatments, but a significant decreasein osteocyte-lacunar density in the BRONJ group compared to the control jaw, with no alteration of the osteocyte-lacunar volume distribution. However, beyond its innovative nature, this study presented some drawbacks that may have influenced the results. Indeed, the mean age of the control group (42 ± 14) was significantly lower than that of the BRONJ patient group (71 ± 12); there was a strong heterogeneity in gender, type of treatment (intravenous administration of Zoledronate and oral administration of Alendronate) and its duration. Thus, the standard deviations in the morphometric study of the lacunae were very high, perhaps preventing the detection of other significant differences, if any, between the BRONJ and the control group.

Therefore, we corrected, reducing the a priori heterogeneity of the samples, the experimental protocol previously proposed in Ref. [2], selecting 4 female patients with BRONJ at stage 2, treated with only zoledronic acid (dose of 4 mg) for comparable times (18 ± 2 months). Moreover, we improved the analysis by discrimination of specific jaw regions. In one patient, bone biopsiesfrom three different mandibular regions were extracted, in order to strictly evaluate possible region-dependent variability, as previously performed in femurs [3].

Two SR-microCT experiments were performed at the ELETTRA Synchrotron Facility (TS, Italy): in the first, the 3D morphology and the mass density distribution were investigated exploiting the phase contrast signal, in the second, at higher resolution, the imaging and the morphometric analysis of the lacunar network were achieved.

We used the reconstructed complex refractive index distribution, which is linearly related to the mass density, to compute the apparent mass density distribution (MDD^r) of each sample, following the Roschger approach [4] described in Fig. 1A.

Being the osteocytes actively involved in the bone remodeling processes, we performed the 3D analysis of the osteocyte lacunar morphology in all BRONJ jaws. Each biopsy was mapped in the entire volume, investigating the mean lacunar thickness (Lc.Th), the maximum lacunar thickness (Lc.MaxTh), the mean lacunar volume (Lc.V),the lacunar density (defined as the number of lacunae per total volume – Lc.Nr/TV).

SR-microCT analyses were supported by conventional histology, performed on each specimen, after staining with acid fuchsin and toluidine blue.

The morphometric analysis related to the trabecular structure produced values significantly different in the jaw 4 compared to the other BRONJ jaws (data not shown), with a morphometry much more compromised than the others from the pathology. Interestingly, the jaw 3 in the region B/46 presented a significantly higher specific surface thanthe same jaw but in the region C/36, suggesting a strong region-dependent variability also in the same patient.

The jaw 3 was also significantly ($p < 0.001$) more mineralized thanthe other jaws, with fewer areas under remodeling. Newsworthy and in analogy with the trabecular morphometric study, the jaw 3 showed a significant ($p < 0.001$) heterogeneity in terms of mineralization degree, signaling also in this case a region-dependent variability. See Movie 1 and Movie 2.

Significantly lower Lac.Th ($p < 0.001$), Lac.MaxTh ($p < 0.001$) and Lac.V ($P = 0.005$) were observed in the jaw 4 respect to jaw 1 and region C/36 of the jaw 3 (Fig. 1B). Interestingly, significantly different Lac.Th ($p < 0.001$) was found in two (B/46 and C/36) of the three examined regions of jaw 3, confirming that the osteonecrosis affects the jaws in a strong region-dependent way.

The SR-microCT results of the present study suggest that the osteocyte lacunar volume and distribution, as well as the mass density in the mineralized bone are not only dependent on the anatomical site, but also on the specific region of the jaw. A pronounced heterogeneity in terms of trabecular microarchitecture and osteocyte lacunar morphology was observed within the different jaw specimens, probably due to the variety of mechanical loading in the different regions.

The histological results confirmed these observations, showing different morphological features, also within the same sample, as mineralized and vital bone tissue close to demineralized tissue or bone side involved in a resorption process. Moreover, the osteocyte lacunae in some fields were colonized by osteocytes, in other fields were empty with margins not evident. One sample (Jaw 4 – Region 25) showed some fields where the lacunae appeared colonized by a collagen matrix, as shown in Fig. 2.

While the different mineralization degree did not seem to be correlated to trabecular microarchitecture mismatches, a good correlation between the MDD^r distribution and the morphometric parameters of osteocyte lacunae was observed. Indeed, mass density (mean, low, and high values) was found to be significantly higher in the jaws with smaller osteocyte lacunar dimensions (mean thickness and volume). These findings are in agreement with previous studies that showed an increased number of empty osteocyte lacunae [5,6] and some of them filled with minerals [7–9] in BP treated jaws.

The high affinity of BPs to hydroxyapatite [10] could lead to a higher deposition of BP in the jaws compared to other skeletal sites,because of the combination of prolonged BP exposure and the high remodeling rate of maxillary bones [11]. As BP becomes cytotoxic at high rates [12], this is expected to promote the osteocytes breakdown with the consequent emptying of the lacunae, which was confirmed by histology, and their filling with more BP-loaded mineral. This is in agreement with the higher mass density measured in jaws more affected by BRONJ, as showed in the present study.

Conflict of interest

The authors indicated no potential conflicts of interest.

Acknowledgments

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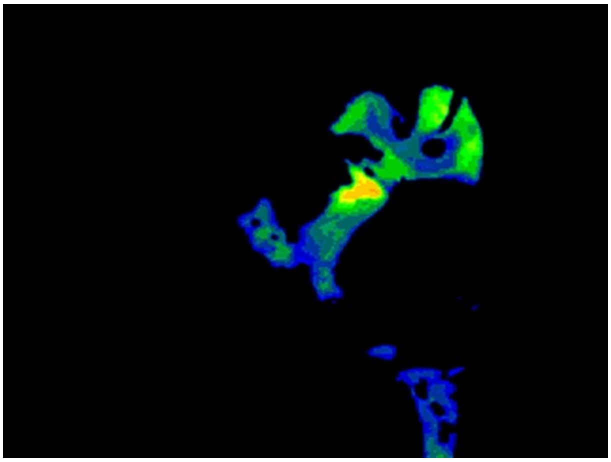
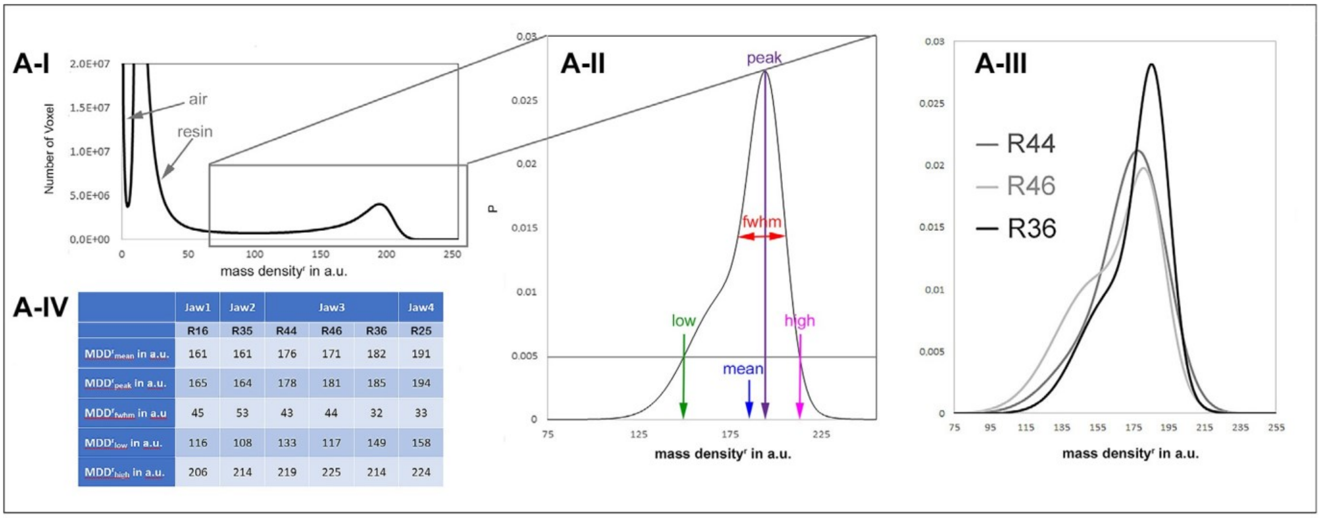


Movie 1. MicroCT 3D sequence of 2D axial slices of Jaw 3 - Region 46. The color maps are representative of the relative mass density distribution (MDD^r) (range 0–255 → 0 = blue-lowest bone mass density; 255 = red-highest bone mass density)



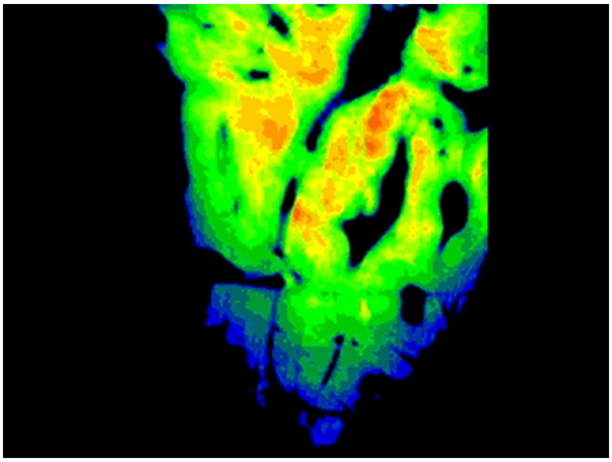
A-IV

	Jaw1	Jaw2	Jaw3			Jaw4
	R16	R35	R44	R46	R36	R25
MDD$^r_{mean}$ in a.u.	161	161	176	171	182	191
MDD$^r_{peak}$ in a.u.	165	164	178	181	185	194
MDD$^r_{fwhm}$ in a.u	45	53	43	44	32	33
MDD$^r_{low}$ in a.u.	116	108	133	117	149	158
MDD$^r_{high}$ in a.u.	206	214	219	225	214	224

B

	jaw 1 – R16	jaw 2 – R35	jaw 3A - R44	jaw 3B – R46	jaw 3C – R36	jaw 4 – R25	Significance
Lac.Th [µm]	4.5 (0.2)	4.0 (0.2)	4.2 (0.3)	4.0 (0.2)	4.4 (0.3)	3.9 (0.1)	4<1,3C; 3B<1,3C;
Lac.MaxTh [µm]	7.9 (0.6)	6.7 (1.0)	7.1 (1.6)	5.4 (1.1)	7.5 (0.7)	5.7 (0.7)	1>3B,4; 3C>3B,4;
Lac.V [µm³]	359 (62)	256 (48)	287 (46)	273 (88)	346 (87)	220 (21)	4<1,3C;
Lac.Nr/TV [× 10³ mm⁻³]	18 (2)	16 (3)	18 (5)	17 (8)	15 (3)	18 (5)	-

Fig. 1. (A) Relative Mass Density Distribution (MDD^r). (A-I) The peaks indicated by the arrows are referred to the air outside the sample and to the resin used to include the biopsy. (A-II) The parameters derived from the segmentation are indicated. (A-III) MDD^r are shown for the three regions, with region 44, region 46 and region 36 of the same jaw. The heterogeneity of the three distributions suggests a region-dependent behavior. A-IV: extracted MDD^r parameters for all the jaws. (B) Three-dimensional morphometric analysis of the retrieved jaw biopsies. Mean values (standard deviation in brackets). All statistical analyses were performed using the software SigmaStat (Systat Software, San Jose, CA). Differences between the samples were assessed by analyses of variance (ANOVA), followed by post hoc multiple comparison by Holm-Sidak tests. All statistical results were considered significant for $p < 0.05$.



Movie 2. MicroCT 3D sequence of 2D axial slices of Jaw 3 – Region 36. The color maps are representative of the relative mass density distribution (MDD^r) (range 0–255 → 0 = blue-lowest bone mass density; 255 = red-highest bone mass density). By comparison with the Movie 1, the mineralization hetero-geneity not only between different regions but also within the same region is here confirmed.

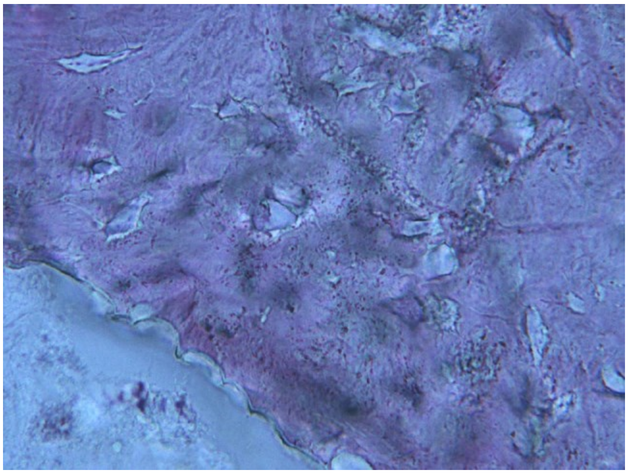


Fig. 2. At high magnification, bone trabeculae showed an indented appearance, typical of an osteoclasts-mediated resorption process. Osteocyte lacunae showed different sizes: some of them were empty, showing no presence of osteocytes, whilst the other ones were colonized by collagen matrix. Tolouidine blu and Acid fuchsin-toluidine blue. Original magnification 400×. A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM04927.

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