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PII: S1010-5182(18)30700-5

DOI: 10.1016/j.jcms.2018.08.011

Reference: YJCMS 3083

To appear in: *Journal of Cranio-Maxillo-Facial Surgery*

Received Date: 17 April 2018

Revised Date: 17 July 2018

Accepted Date: 20 August 2018

Please cite this article as: Bolette A, Lecloux G, Rompen E, Albert A, Kerckhofs G, Lambert F, Influence of induced infection in medication-related osteonecrosis of the jaw development after tooth extraction: a study in rats, *Journal of Cranio-Maxillofacial Surgery* (2018), doi: 10.1016/j.jcms.2018.08.011.

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Influence of induced infection in medication-related osteonecrosis of the jaw development after tooth extraction: a study in rats

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This research has not received funding support.

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Summary

Purpose: The mechanisms underlying the pathophysiology of medication-related osteonecrosis of the jaw (MRONJ) development have not yet been fully elucidated. MRONJ is described as a multi-factorial process in which bacterial infection seems to play an important role. The purpose of the present study was to investigate the influence of a primary installed infectious disease in the development of MRONJ after dental extraction.

Materials and Methods: Twenty-six rats underwent pulpal exposure of one upper and one lower first molar to induce periapical lesions. Thereafter, animals received zoledronate during a 4-week period. The day after the last injection, the four first molars from each quadrant were extracted. Eight weeks later, animals were sacrificed for macroscopic, radiological, and histological assessment.

Results: The incidence of MRONJ was highest in mandibular infected teeth (47.4%), while the histological evaluation showed the highest incidence of osteonecrosis (88.2%) and the largest mean value for extent of the necrotic bone area (1.22 ± 0.71 mm^2). Radiological findings confirmed the clinical and histological results. Statistical analysis showed that the combination of both factors, mandibular localization and periapical infection, significantly increased the incidence of MRONJ after extraction, considering clinical ($p = 0.0074$), radiological ($p = 0.026$), and histological ($p = 0.0022$) outcomes.

Conclusion: These findings support the potential implication of inflammatory/infectious dental pathology in initiating the osteonecrotic process before dental extraction. The possible partial role of the infectious process in MRONJ development emphasizes the importance of maintaining good oral health and dental care for preventing infectious pathology in the management of these patients.

Keywords: osteonecrosis, radiology, microscopy, nanotechnology, oncology, osteoporosis

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INTRODUCTION

Bisphosphonates (BPs) are antiresorptive drugs commonly used to treat diseases involving bone resorption, such as bone metastatic diseases, multiple myeloma, and osteoporosis (Russell et al., 2008; Ruggiero et al., 2009). Despite the significant benefits of antiresorptive drugs (ARD), medication-related osteonecrosis of the jaw (MRONJ) represents their main adverse side effect (Ruggiero et al., 2009; Marx, 2011). According to the latest definition of MRONJ established by the American Association of Oral and Maxillofacial Surgeons, patients may be considered to have MRONJ if each of the following characteristics is present: (1) current or previous treatment with antiresorptive or antiangiogenic agents; (2) exposed bone or bone that can be probed through an intraoral or extraoral fistula in the maxillofacial region that has persisted for more than 8 weeks; and (3) no history of radiation therapy to the jaw or obvious metastatic disease to the jaw (Ruggiero et al., 2014). This definition refers to bisphosphonates as well as other antiresorptive (denosumab) and antiangiogenic therapies. Globally, the most commonly triggering factor used for MRONJ occurrence is dental extraction. According to a recent systematic literature review, the risk of MRONJ after dental extraction in patients treated with ARD for oncological reasons reached 3.2%, and the occurrence may increase in the presence of risk indicators, such as concomitant chemotherapy (Gaudin et al., 2015). Other factors, such as corticosteroids or diabetes, are associated with an increased risk of MRONJ in humans and in animal studies (Saad et al., 2012; Tsao et al., 2013; Berti-Couto et al., 2014). Current knowledge of the physiopathology of MRONJ shows that this process is complex and multi-factorial. The main actors involved in the development of MRONJ are (1) the inhibition of osteoclastic bone resorption and remodeling (Ruggiero et al., 2014), (2) the inhibition of angiogenesis (Wood et al., 2002; Bezzi et al., 2003; Lopez-Jornet et al., 2010; Conte Neto et al., 2013), and (3) the infection/inflammation process (Hoff et al., 2008; Ripamonti et al., 2009; Otto et al., 2015), respectively. Tooth extraction in ARD patients is often associated with periodontal or periapical disease (Ficarra et al., 2005; Boonyapakorn et al., 2008;

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Ruggiero et al., 2009; Marx, 2011), and a clinical study concluded that the presence of osteomyelitis at the time of extraction might be associated with the occurrence of clinical and/or radiologic MRONJ in patients treated with intravenous (IV) BPs (Saia et al., 2010). The development of MRONJ may therefore be influenced by a previously or secondarily developed bone infectious disease, such as marginal or periapical periodontitis. Additionally, animal studies have shown that local inoculation with bacteria increases the risk of MRONJ development (Mawardi et al., 2011; Tsurushima et al., 2013; Sakaguchi et al., 2015). Other authors have demonstrated that both inflammation and bacterial infection combined with systemic ARD are sufficient to induce MRONJ in rodents (Aghaloo et al., 2011; Aguirre et al., 2012; Kang et al., 2013; Aghaloo et al., 2014; de Molon et al., 2014; Moreira et al., 2014). In a larger animal model, it was showed that MRONJ occurred not only after extraction but also in areas of periodontal infection (Otto et al., 2017). Therefore, infection/inflammation seems to play a role in the pathophysiology of MRONJ development, but the cause-and-effect relationship is not yet fully understood. The infectious process surrounding teeth could eventually initiate the bone necrotic process, but the incidence of MRONJ occurrence after extraction of healthy versus infected teeth has not been investigated in clinical or preclinical studies. The objective of the present study was to explore the pathophysiology of MRONJ development and to investigate the influence of an installed osteomyelitis in the development of MRONJ after dental extraction in rats. The hypothesis was that installed dental pathology, such as periapical periodontitis, is a risk factor for MRONJ after extraction. The effect of the localization (upper or lower jaw), the presence of primary infection or the combination of these two factors on MRONJ development was investigated.

MATERIALS AND METHODS

Animals and study design

The study involved 26 male Wistar rats aged 6 to 10 weeks and weighing 250 to 350 g. All experimental procedures and protocols used in this investigation were reviewed and approved by

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the Institutional Animal Care and Use Ethics Committee of the University of Liège, Belgium (Protocol #13-1639; Date:07/11/2014). The Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines were carefully followed, as well as national and European legislation.

Rats were housed in individual cages with filtered air, controlled humidity and temperature of 23 ± 2°C. A 12-hour light/dark cycle was maintained, and ad libitum access to diet and water was provided. At baseline (D0), all animals were subject to pulpal exposures in the first left maxillary molars and first right mandibular molars to induce periapical lesions. Thereafter, all animals started receiving zoledronic acid through intraperitoneal (IP) injections of 0.3 mg/kg 3 times per week for 4 weeks (Zometa, Novartis Pharma, Basel, Switzerland). Overall, 12 injections and a total dose of 3.6 mg/kg were administered to each rat. The day following the last injection (D0 + 4 weeks), all animals underwent extraction of the first molars in each quadrant. Eight weeks after teeth extraction (D0 + 12 weeks), the animals were euthanized by an overdose of pentobarbital, and samples were collected for assessment.

Experimental procedures

All intra-oral procedures were performed under general anesthesia. Animals were anesthetized with an IP injection of ketamine (70 mg/kg) and xylazine (30 mg/kg). Pulpal exposures were performed using a size 1/2 round bur, avoiding furcal perforation. Exposed teeth were left open throughout the experiment, in order to induce periapical lesions. After receiving ARD treatment (D0 + 4 weeks), all first molars (maxillary and mandibular, left and right) were extracted using a surgical technique as atraumatic as possible. Teeth were luxated using a periodontal scaler as an elevator. In most cases, the entire root or apical portion of the root broke off during the procedure. The remaining roots were extracted when possible, and broken apical portions were drilled using a size 1/2 round bur. Surgical procedures are illustrated in Figure 1.

Macroscopic analysis

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Following euthanasia, extraction sites were clinically examined for the presence of bone exposure and/or fistulae bone contact on probing (“macroscopic MRONJ”). Photographs of each site were taken for double checking the presence of bone exposure. Dissection of the maxillae and mandibles were performed, and the samples were fixed in 10% formaldehyde for 48 hours and then stored in sterile phosphate-buffered saline (PBS) at 4°C.

Radiological analysis

Samples were subjected to microfocus computed tomography (microCT) (NanoTom M, GE, Germany) to identify radiological evidence of MRONJ or the presence of residual dental roots (Figure 2). Based on radiological characteristics of MRONJ described in humans, extraction sites were defined as "positive radiological MRONJ" when at least two of the following criteria were present: (1) poor bone regeneration (maximum 30%), (2) disorganization of trabecular patterns, (3) cortical irregularity, (4) areas of osteolysis, (5) cortical disruption, (6) fragmentation aspect, and (7) sequestrum.

Histological analysis

Histological sections (hematoxylin and eosin stained) were first used to determine the presence of “histological MRONJ” based on the following criteria: (1) presence of mucosal ulceration, and/or (2) bone sequestrum associated with pseudoepitheliomatous hyperplasia (PEH), and (3) inflammatory infiltrate (Hokugo et al., 2010; Aguirre et al., 2012; Williams et al., 2014; Park et al., 2015) (Figure 3). Samples were defined as histological MRONJ (yes/no). Moreover, a 3 x 3-mm region of interest (ROI) was selected to perform histomorphometric measurements of the bone necrosis surface (Figure 4). Necrotic bone was defined as a region with empty osteocytic lacunae and was calculated using appropriate software (NDP.view 2.5.14 Hamamatsu Photonics, K.K., Japan).

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Statistical analysis

Results were presented as a frequency table for categorized variables and as mean, standard deviation (SD), median, interquartile range (IQR) and range for quantitative variables. Summary data were generally described according to the jaw (upper or lower) and the presence of infection (yes or no). In the statistical analysis, the fact that 4 teeth were available for each rat at each time point was considered. Thus, MRONJ occurrence at the clinical or histological level was studied in relation to localization and infection by logistic regression for repeated measures (GEE). GEE was also used for the radiological score. Finally, for the extent of necrotic areas, a generalized linear mixed model (GLMM) was applied. The interaction term between the two factors (localization and presence of infection) was included in the model. The degree of agreement between the clinical, histological, and radiological evaluation was assessed by the Cohen kappa (κ) coefficient and its 95% confidence interval (95% CI); the closer κ to 1, the better the agreement between the evaluations. Results were considered significant at the 5% level ($p < 0.05$). Analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Of the 26 rats used for the study, 19 rats survived until the end of the experiment. Therefore, 76 extraction sites were available for analysis. Quantification of necrotic areas and percentage rates of extraction sites defined as “macroscopic MRONJ”, “histological MRONJ” and “radiological MRONJ” are summarized in Table 1.

Macroscopic examination

Regarding the macroscopic evaluation, the incidence of MRONJ after extraction of healthy teeth was comparable in maxillae and mandibles (42.1% of extraction sites) (Figure 1). Considering the infected extracted teeth only, significantly more MRONJ development occurred in the mandible (47.4%) than in the maxilla (15.8%) ($p = 0.034$). Data also showed that when both factors (infection

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and mandibular localization) were present, the incidence of MRONJ was significantly increased (p = 0.0074).

Radiological examination

Detailed numerical data related to the radiological examination are displayed in Table 1. The lowest proportion of radiological MRONJ was shown for healthy teeth extracted from the maxilla (26.3%). MRONJ occurrence was higher considering other configurations (quadrants 2, 3, and 4) and comparable between them (63.2%). A statistically significant increased risk for MRONJ development was demonstrated when both factors were present (p = 0.026) (Figure 2).

Histological examination

Among the 76 samples, 6 could not be used for histological analysis for technical reasons. Occurrence of histological MRONJ after extraction of healthy teeth did not differ significantly between maxillae and mandibles (p = 0.75). However, occurrence of MRONJ after extraction of infected teeth was much higher in the mandible (88.2%) than in the maxillae (42.1%). As demonstrated for clinical findings, only the combination of both factors (mandibular localization and infection) significantly increased the risk of MRONJ occurrence (p = 0.0022).

Histomorphometry

Concerning the quantification of necrotic areas, the lowest mean value was found for non-infected sites in the maxilla, and the highest mean value was found for infected sites in the mandible (Table 1). A significant risk increase for MRONJ was evidenced when considering only mandibular localization (p = 0.005), while only a tendency was seen for infection. When combining both factors, none was significant. When comparing the extent of the necrotic area for sites clinically defined healthy and sites clinically defined as MRONJ, mean values were significantly higher in the latter group (p = 0.019).

Degree of agreement between methods of analysis

The degree of agreement between macroscopic and histological examination of MRONJ was satisfactory ($\kappa = 0.46$; 95% CI: 0.27–0.64), as was the degree of agreement between macroscopic and radiological examination of MRONJ ($\kappa = 0.25$; 95% CI: 0.05–0.46), indicating that criteria defined for histological and radiological confirmation of MRONJ are well associated with installed clinical MRONJ. These macroscopic, histological, and radiological results emphasize the aggravating role of MRONJ development when combining mandibular localization and installed infection.

DISCUSSION

Animal models are important to understanding the pathophysiological mechanisms involved in MRONJ and to develop evidence-based treatment options (Sharma et al., 2013). In this study, a high cumulative dose of zoledronate was chosen to induce lesions. This molecule is indeed associated with the highest rate of MRONJ in humans, and the incidence of MRONJ is known to be higher in patients treated for oncological reasons than in patients treated for osteoporosis (Gaudin et al., 2015). The objective of the present experimental study was to evaluate the impact of installed peri-apical infectious processes on MRONJ occurrence after tooth extraction. Macroscopic, histological, and radiological assessment revealed that MRONJ lesions were more frequently observed after extraction of periapically infected teeth at the mandible, in comparison with non-infected and/or maxillary teeth. However, the experimental data did not show a significant influence for factors (mandibular localization or periapical infection) taken individually, although they did for their combination. As demonstrated in human studies, MRONJ lesions occur more frequently at the mandible compared to the maxilla, and they are attributed to higher density and lower vascularity of mandibular bone (Ruggiero et al., 2014). Consequently, this type of bone displays less defensive potential and is more susceptible to MRONJ development. Considering pre-existing infection, our

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findings are in line with those of several clinical studies, suggesting that installed osteomyelitis before extraction was a risk factor for MRONJ development (Marx, 2003; Ficarra et al., 2005; Hoff et al., 2008; Ruggiero et al., 2009; Saia et al., 2010; Marx, 2011; Otto et al., 2012; Otto et al., 2015). However, the physiopathology of MRONJ is not completely understood. Although several animal studies have demonstrated a major role of periodontitis or peri-apical diseases in MRONJ pathogenesis, none of them has investigated the combination of installed infection and dental extraction (Aghaloo et al., 2011; Aguirre et al., 2012; Kang et al., 2013; Aghaloo et al., 2014; de Molon et al., 2014; Moreira et al., 2014). The present experimental study is the first to compare MRONJ occurrence after extraction of healthy versus infected teeth and to demonstrate that post-extraction MRONJ is enhanced in dense and poorly vascularized bone, such as mandibular bone, if pre-existing infection/inflammation is installed before extraction. These findings reinforce the hypothesis that a primary installed infectious disease leads to the initiation of the osteonecrosis, and secondary extraction in an infected site further exposes already necrotic bone and enhances the establishment of post-extractional MRONJ. According to this hypothesis, local infection/inflammation seems to trigger MRONJ development, before the extraction itself. Similar conclusions were found in clinical studies, reporting that baseline osteomyelitis was a risk factor for MRONJ development (Saia et al., 2010), and that the infectious conditions may be more determinant than tooth extractions in MRONJ development (Otto et al., 2015). Some pathogenesis theories state that local infections and consecutive acidosis could play a role in MRONJ development. Infectious inflammatory processes lead to local tissue acidosis, and BPs are known to be released from acidic milieu, resulting in high cytotoxicity (Otto et al., 2010). Our results showed that local infection seems to enhance MRONJ occurrence, but extraction remains obviously an additional risk as MRONJ were also observed after extraction of non-infected tooth.

In the present study, histological MRONJ (potentially compared to stage 0 in human patients) was more frequently observed than macroscopic lesions. Nevertheless, there is no evidence that

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extraction sites presenting with histological necrotic areas without clinical exposure could lead to established MRONJ lesions (stage 1, 2, or 3). Histological and radiological assessments were thus complementary to clinical evaluation, but the main criterion for defining MRONJ lesion was bone exposure.

Two limitations may be underlined in the present experimental study. The first concerns the high rate of root fractures. MicroCT scans revealed apical root remnants in 94% of extraction sites, despite the use of a size 1/2 round bur to drill broken apical portions during extraction procedures. Compared to the rate of root fractures provided by histological analysis (only 42%), we believe that the histological analysis underestimates these data. The upper first molars are composed of five divergent roots, and the lower first molars are composed of four roots, usually with a broadening of the most apical portion. These anatomical characteristics may explain the occurrence of fractures and why histological analysis underestimates their frequency. Considering the fact that most of the sites presented a complete mucosal healing, even with the remaining root fragments (infected or not), and that apical portions were present in the same proportion for each quadrant, then for each condition (infected tooth or not, upper or lower jaw), the risk of bias was limited. The second limitation concerns the study design. In the present study, the pulp opening needed to induce a peri-apical infection was created at the first ARD injection, indicating that the period for ARD administration and the period for periapical lesion development were grouped. Thus, during the earliest stage of periapical disease, resorptive function may still have been effective because animals were just starting to receive ARD. This context does not totally mimic the real situation found in patients. To mimic the clinical context, pulpal exposure should have been performed after a period of massive saturation of bone with ARD, making the resorptive function less competent. In this way, the effect of periapical infection on the prevalence of MRONJ development after extraction might have been greater, but this hypothesis needs to be confirmed by additional studies.

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Current guidelines recommend the avoidance of dental extraction in cancer patients treated with ARD because extraction is an important triggering risk factor for MRONJ occurrence. However, according to the present findings and the recent literature, compromised teeth affected by periodontal or periapical disease seem to increase the risk for MRONJ development, and their removal using an adjusted extraction protocol may therefore be recommended to prevent further MRONJ development. Additionally, since primary infection may be the starting point of necrosis, it may also be advised to remove necrotic bone, for example, by drilling, when extracting an infected tooth. This study enhances the importance of maintaining good oral health and dental care for preventing infectious disease in the management of ARD-treated patients. Our results also support human studies in which the authors concluded that the application of preventive measures, including elimination of infectious processes, could significantly reduce the incidence of MRONJ in cancer patients receiving ARD (Boonyapakorn et al., 2008; Dimopoulos et al., 2009; Ripamonti et al., 2009).

CONCLUSION

In the present study, we designed a rat model presenting with macroscopic, radiological, and histological characteristics of MRONJ comparable to those in patients. Our results support the potential role of inflammatory/infectious dental pathology in initiating the osteonecrotic process, leading to an increased risk for MRONJ development after extraction. These conclusions confirm the importance of maintaining good oral health and dental care for preventing infectious disease in the management of ARD-treated patients. Moreover, avoiding extraction of compromised teeth in patients at high risk for MRONJ development may not always be the best therapeutic option.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article. This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Acknowledgements

The authors wish to express their gratitude to Chantal Humblet, Pierre Drion, and their respective staffs for their input in the present study. The microCT images have been generated at the X-ray computed tomography facilities of the Department of Materials Engineering, financed by the Hercules Foundation (project AKUL 09/001).

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References

Aghaloo TL, Cheong S, Bezouglaia O, Kostenuik P, Atti E, Dry SM, Pirih FQ, Tetradis S: RANKL inhibitors induce osteonecrosis of the jaw in mice with periapical disease. J Bone Miner Res 29:843-854, 2014.

Aghaloo TL, Kang B, Sung EC, Shoff M, Ronconi M, Gotcher JE, Bezouglaia O, Dry SM, Tetradis S: Periodontal disease and bisphosphonates induce osteonecrosis of the jaws in the rat. J Bone Miner Res 26:1871-1882, 2011.

Aguirre JI, Akhter MP, Kimmel DB, Pingel JE, Williams A, Jorgensen M, Kesavalu L, Wronski TJ: Oncologic doses of zoledronic acid induce osteonecrosis of the jaw-like lesions in rice rats (Oryzomys palustris) with periodontitis. J Bone Miner Res 27:2130-2143, 2012.

Berti-Couto SA, Vasconcelos AC, Iglesias JE, Figueiredo MA, Salum FG, Cherubini K: Diabetes mellitus and corticotherapy as risk factors for alendronate-related osteonecrosis of the jaws: a study in Wistar rats. Head Neck 36:84-93, 2014.

Bezzi M, Hasmim M, Bieler G, Dormond O, Ruegg C: Zoledronate sensitizes endothelial cells to tumor necrosis factor-induced programmed cell death: evidence for the suppression of sustained activation of focal adhesion kinase and protein kinase B/Akt. J Biol Chem 278:43603-43614, 2003.

ACCEPTED MANUSCRIPT

Boonyapakorn T, Schirmer I, Reichart PA, Sturm I, Massenkeil G: Bisphosphonate-induced osteonecrosis of the jaws: prospective study of 80 patients with multiple myeloma and other malignancies. Oral Oncol 44:857-869, 2008.

Conte Neto N, Spolidorio LC, Andrade CR, A SB, Guimaraes M, Marcantonio E, Jr.: Experimental development of bisphosphonate-related osteonecrosis of the jaws in rodents. Int J Exp Pathol 94:65-73, 2013.

de Molon RS, Cheong S, Bezouglaia O, Dry SM, Pirih F, Cirelli JA, Aghaloo TL, Tetradis S: Spontaneous osteonecrosis of the jaws in the maxilla of mice on antiresorptive treatment: a novel ONJ mouse model. Bone 68:11-19, 2014.

Dimopoulos MA, Kastritis E, Bamia C, Melakopoulos I, Gika D, Roussou M, Migkou M, Eleftherakis-Papaiakovou E, Christoulas D, Terpos E, Bamias A: Reduction of osteonecrosis of the jaw (ONJ) after implementation of preventive measures in patients with multiple myeloma treated with zoledronic acid. Ann Oncol 20:117-120, 2009.

Ficarra G, Beninati F, Rubino I, Vannucchi A, Longo G, Tonelli P, Pini Prato G: Osteonecrosis of the jaws in periodontal patients with a history of bisphosphonates treatment. J Clin Periodontol 32:1123-1128, 2005.

Gaudin E, Seidel L, Bacevic M, Rompen E, Lambert F: Occurrence and risk indicators of medication-related osteonecrosis of the jaw after dental extraction: a systematic review and meta-analysis. J Clin Periodontol 42:922-932, 2015.

ACCEPTED MANUSCRIPT

Hoff AO, Toth BB, Altundag K, Johnson MM, Warneke CL, Hu M, Nooka A, Sayegh G, Guarneri V, Desrouleaux K, Cui J, Adamus A, Gagel RF, Hortobagyi GN: Frequency and risk factors associated with osteonecrosis of the jaw in cancer patients treated with intravenous bisphosphonates. J Bone Miner Res 23:826-836, 2008.

Hokugo A, Christensen R, Chung EM, Sung EC, Felsenfeld AL, Sayre JW, Garrett N, Adams JS, Nishimura I: Increased prevalence of bisphosphonate-related osteonecrosis of the jaw with vitamin D deficiency in rats. J Bone Miner Res 25:1337-1349, 2010.

Kang B, Cheong S, Chaichanasakul T, Bezouglaia O, Atti E, Dry SM, Pirih FQ, Aghaloo TL, Tetradis S: Periapical disease and bisphosphonates induce osteonecrosis of the jaws in mice. J Bone Miner Res 28:1631-1640, 2013.

Lopez-Jornet P, Camacho-Alonso F, Molina-Minano F, Gomez-Garcia F, Vicente-Ortega V: An experimental study of bisphosphonate-induced jaws osteonecrosis in Sprague-Dawley rats. J Oral Pathol Med 39:697-702, 2010.

Marx R: Oral and Intravenous Biphosphonate Induced Osteonecrosis of the Jaws: History, etiology, prevention, and treatment. Hanover Park: Quintessence Publishing

Marx RE: Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg 61:1115-1117, 2003.

Mawardi H, Giro G, Kajiya M, Ohta K, Almazrooa S, Alshwaimi E, Woo SB, Nishimura I, Kawai T: A role of oral bacteria in bisphosphonate-induced osteonecrosis of the jaw. J Dent Res 90:1339-1345, 2011.

Moreira MM, Bradaschia-Correa V, Marques ND, Ferreira LB, Arana-Chavez VE: Ultrastructural and immunohistochemical study of the effect of sodium alendronate in the progression of experimental periodontitis in rats. Microsc Res Tech 77:902-909, 2014.

Otto S, Pautke C, Martin Jurado O, Nehrbass D, Stoddart MJ, Ehrenfeld M, Zeiter S: Further development of the MRONJ minipig large animal model. J Craniomaxillofac Surg 45:1503-1514, 2017.

Otto S, Pautke C, Opelz C, Westphal I, Drosse I, Schwager J, Bauss F, Ehrenfeld M, Schieker M: Osteonecrosis of the jaw: effect of bisphosphonate type, local concentration, and acidic milieu on the pathomechanism. J Oral Maxillofac Surg 68:2837-2845, 2010.

Otto S, Schreyer C, Hafner S, Mast G, Ehrenfeld M, Sturzenbaum S, Pautke C: Bisphosphonate-related osteonecrosis of the jaws─characteristics, risk factors, clinical features, localization and impact on oncological treatment. J Craniomaxillofac Surg 40:303-309, 2012.

Otto S, Troltzsch M, Jambrovic V, Panya S, Probst F, Ristow O, Ehrenfeld M, Pautke C: Tooth extraction in patients receiving oral or intravenous bisphosphonate administration: a trigger for BRONJ development? J Craniomaxillofac Surg 43:847-854, 2015.

Park S, Kanayama K, Kaur K, Tseng HC, Banankhah S, Quje DT, Sayre JW, Jewett A, Nishimura I: Osteonecrosis of the Jaw Developed in Mice: Disease variants regulated by gammadelta T cells in oral mucosal barrier immunity. J Biol Chem 290:17349-17366, 2015.

ACCEPTED MANUSCRIPT

Ripamonti CI, Maniezzo M, Campa T, Fagnoni E, Brunelli C, Saibene G, Bareggi C, Ascani L, Cislaghi E: Decreased occurrence of osteonecrosis of the jaw after implementation of dental preventive measures in solid tumour patients with bone metastases treated with bisphosphonates. The experience of the National Cancer Institute of Milan. Ann Oncol 20:137-145, 2009.

Ruggiero SL, Dodson TB, Assael LA, Landesberg R, Marx RE, Mehrotra B, Task Force on Bisphosphonate-Related Osteonecrosis of the Jaws, American Association of Oral and Maxillofacial Surgeons: American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaw - 2009 update. Aust Endod J 35:119-130, 2009.

Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, O'Ryan F, American Association of Oral and Maxillofacial Surgeons: American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw—2014 update. J Oral Maxillofac Surg 72:1938-1956, 2014.

Russell RG, Watts NB, Ebetino FH, Rogers MJ: Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. Osteoporos Int 19:733-759, 2008.

Saad F, Brown JE, Van Poznak C, Ibrahim T, Stemmer SM, Stopeck AT, Diel IJ, Takahashi S, Shore N, Henry DH, Barrios CH, Facon T, Senecal F, Fizazi K, Zhou L, Daniels A, Carriere P, Dansey R: Incidence, risk factors, and outcomes of osteonecrosis of the jaw: integrated analysis from three blinded active-controlled phase III trials in cancer patients with bone metastases. Ann Oncol 23:1341-1347, 2012.

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Saia G, Blandamura S, Bettini G, Tronchet A, Totola A, Bedogni G, Ferronato G, Nocini PF, Bedogni A: Occurrence of bisphosphonate-related osteonecrosis of the jaw after surgical tooth extraction. J Oral Maxillofac Surg 68:797-804, 2010.

Sakaguchi O, Kokuryo S, Tsurushima H, Tanaka J, Habu M, Uehara M, Nishihara T, Tominaga K: Lipopolysaccharide aggravates bisphosphonate-induced osteonecrosis in rats. Int J Oral Maxillofac Surg 44:528-534, 2015.

Sharma D, Hamlet S, Petcu E, Ivanovski S: Animal models for bisphosphonate-related osteonecrosis of the jaws─an appraisal. Oral Dis 19:747-754, 2013.

Tsao C, Darby I, Ebeling PR, Walsh K, O'Brien-Simpson N, Reynolds E, Borromeo G: Oral health risk factors for bisphosphonate-associated jaw osteonecrosis. J Oral Maxillofac Surg 71:1360-1366, 2013.

Tsurushima H, Kokuryo S, Sakaguchi O, Tanaka J, Tominaga K: Bacterial promotion of bisphosphonate-induced osteonecrosis in Wistar rats. Int J Oral Maxillofac Surg 42:1481-1487, 2013.

Williams DW, Lee C, Kim T, Yagita H, Wu H, Park S, Yang P, Liu H, Shi S, Shin KH, Kang MK, Park NH, Kim RH: Impaired bone resorption and woven bone formation are associated with development of osteonecrosis of the jaw-like lesions by bisphosphonate and anti-receptor activator of NF-kappaB ligand antibody in mice. Am J Pathol 184:3084-3093, 2014.

ACCEPTED MANUSCRIPT

Wood J, Bonjean K, Ruetz S, Bellahcene A, Devy L, Foidart JM, Castronovo V, Green JR: Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. J Pharmacol Exp Ther 302:1055-1061, 2002.

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Table 1. Summary of Results

Site	Infection	Quadrant	Macroscopic MRONJ (%)	Histological MRONJ (%)	Radiological MRONJ (%)	Extent of necrotic area (mm^2 ± SD)
Maxilla	No	Q1	42.1 %	56.2 %	26.3 %	0.56 ± 0.28
	Yes	Q2	15.7 %	42.1 %	63.1 %	0.82 ± 0.50
Mandible	No	Q3	42.1 %	50.0 %	63.1 %	0.99 ± 0.66
	Yes	Q4	47.3 %	88.2 %	63.1 %	1.22 ± 0.71

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Fig 1. Surgical procedures. (A) Pulpal exposure confirmed by bleeding. (B) Luxation of the tooth using periodontal scalers. (C) Tooth extraction showing fracture of the mesial roots. (D) Use of a small round bur to drill remaining roots or apical fragments. (E) MRONJ clinically defined (bone exposure) 8 weeks after tooth extraction.

Fig 2. Alveolar bone healing assessment by micro-CT imaging. (A) Three-dimensional reconstruction of an extraction site defined as "radiological MRONJ" (score 4). (B) Axial section. (C) Coronal section. (D) Sagittal section. Radiological characteristics of osteonecrosis such as osteolysis, disorganization of trabecular patterns, cortical disruption and lack of newly bone formation are observable.

Fig 3. (A) Histological section showing typical signs of MRONJ. Invagination of pseudoepitheliomatous hyperplasia (PEH) (black arrows) surrounding necrotic bone (area outlined in red) and inflammatory infiltrate (green arrow). Apical fragment of distal root is outlined in yellow. (B) Histological section representing an extensive MRONJ lesion. Epithelial discontinuation, absence of newly bone formation and presence of bone sequestrum and larges area with empty osteocytic lacunae (outlined in red).

Fig 4. (A) Histological sections showing extraction site in defined region of interest (3 x 3 mm). Area outlined in red corresponds to necrotic bone area (with empty osteocytic lacunae). (B) Viable bone showing osteocytes with nuclei. (C) Necrotic bone with empty osteocytic lacunae.

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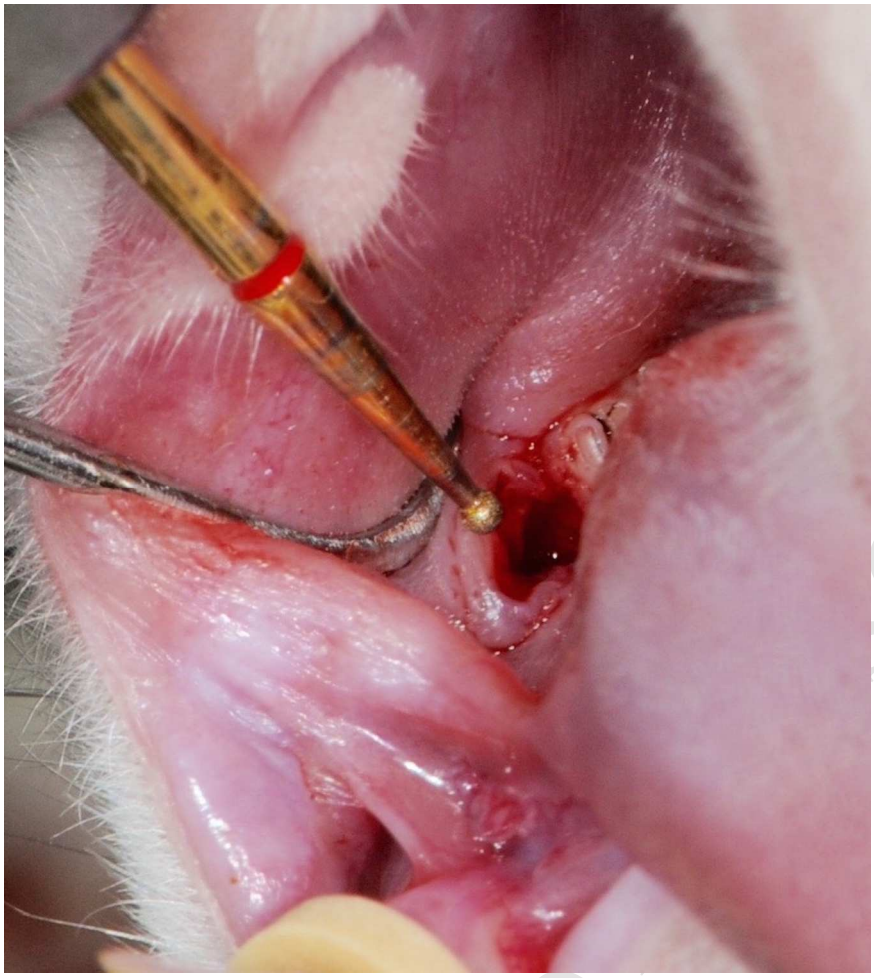
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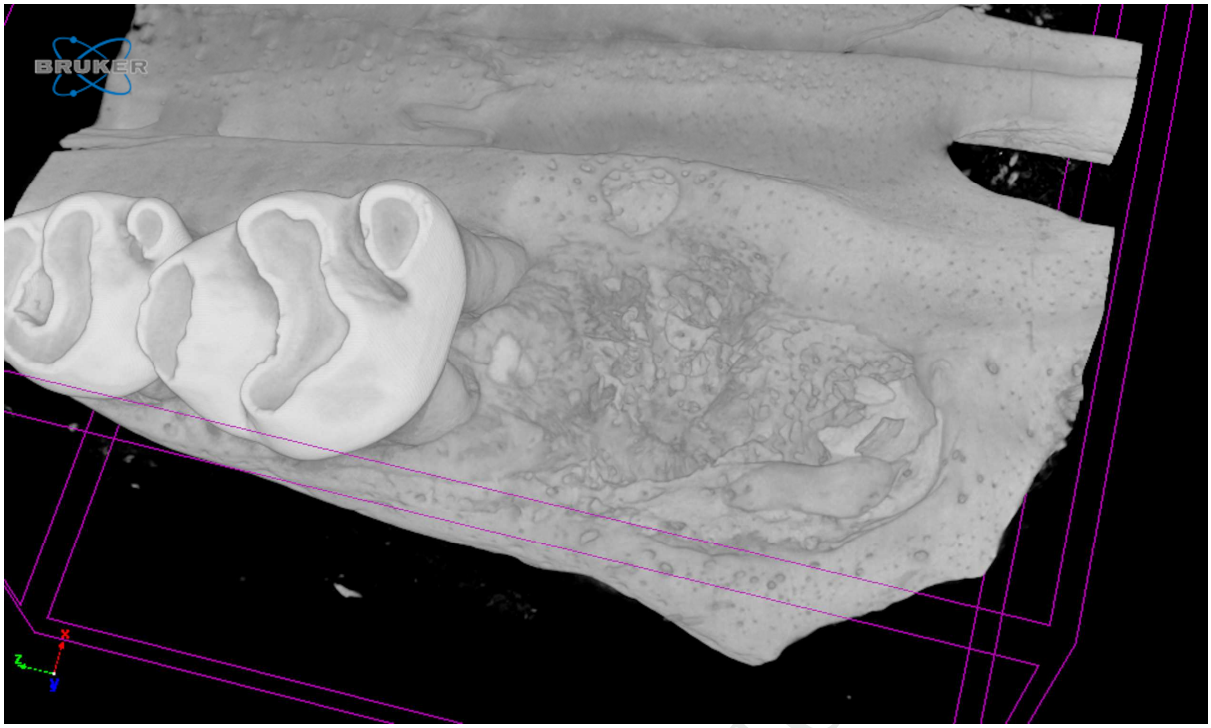
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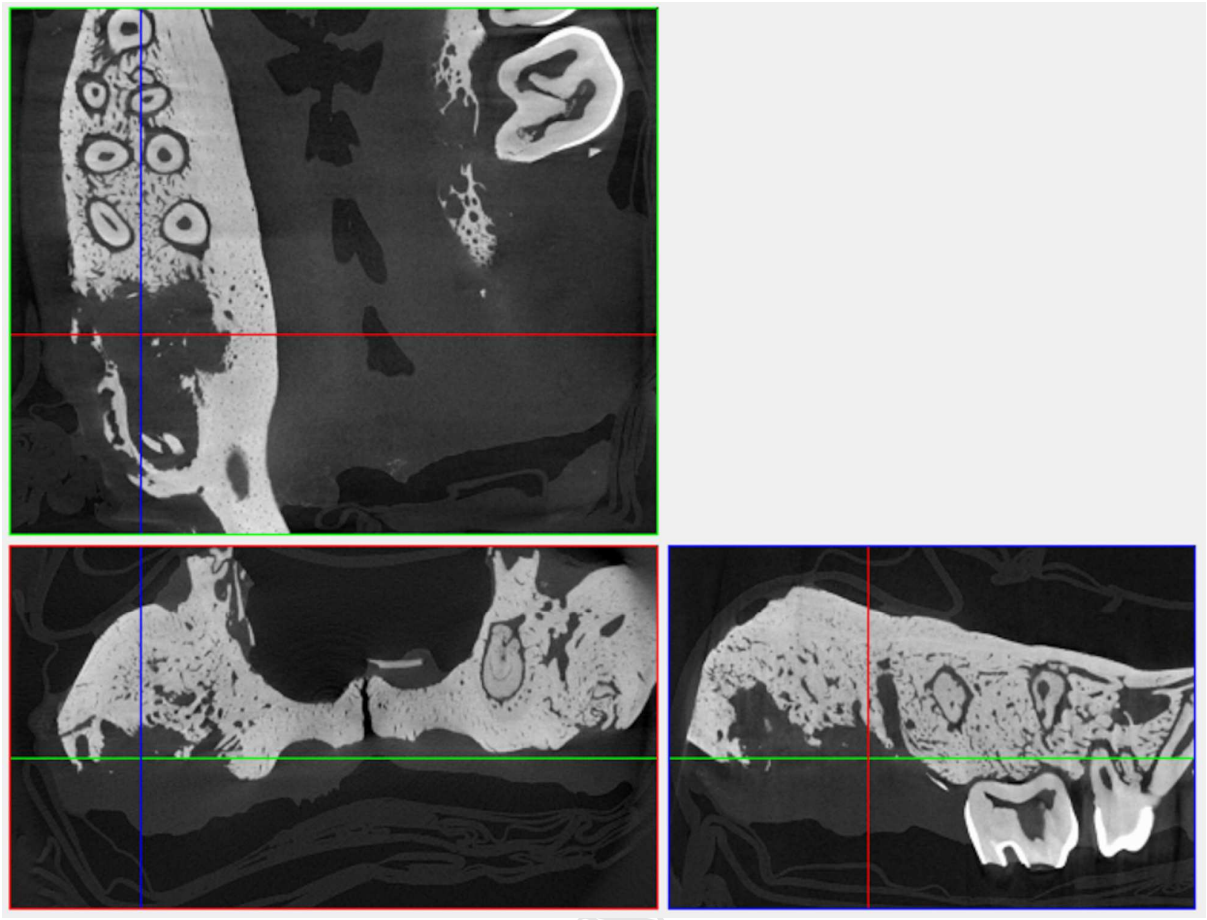
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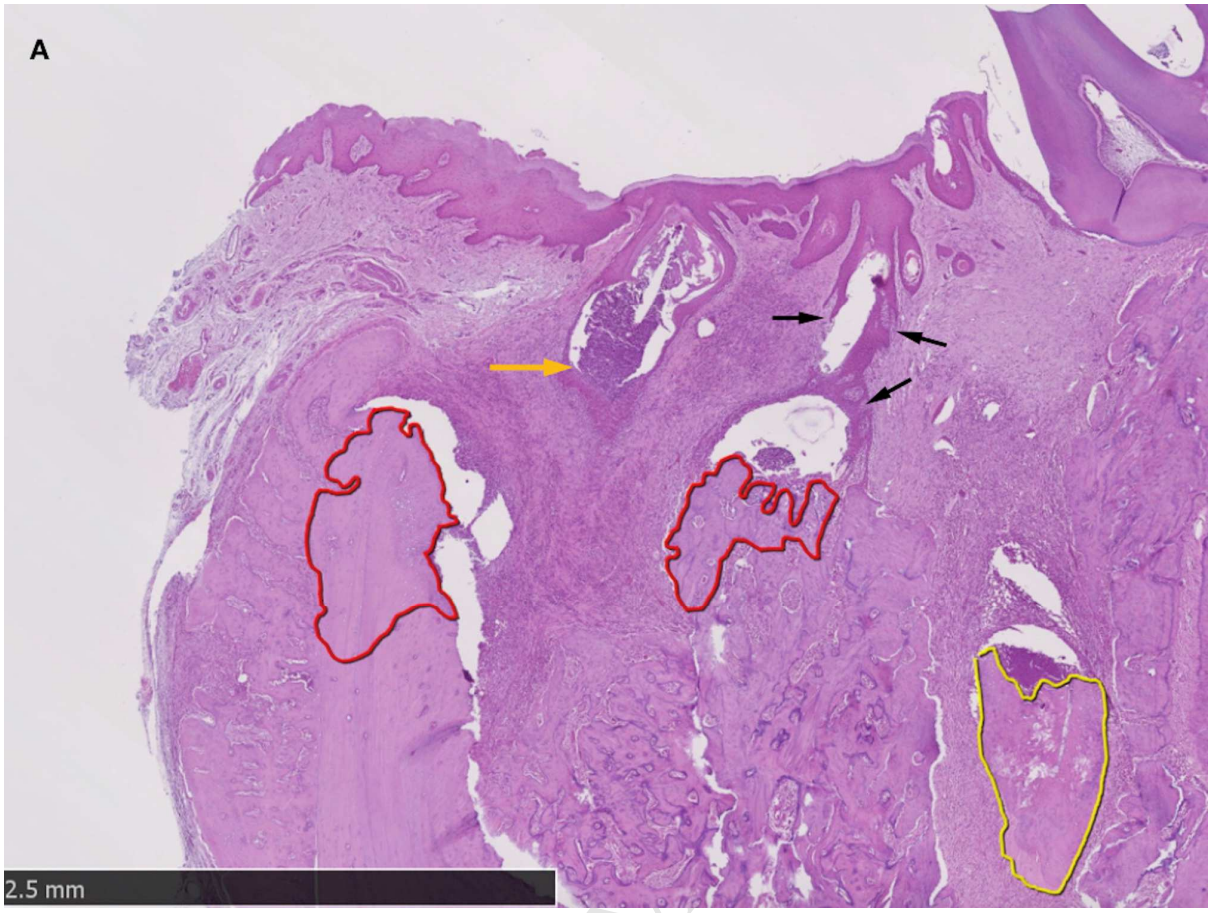
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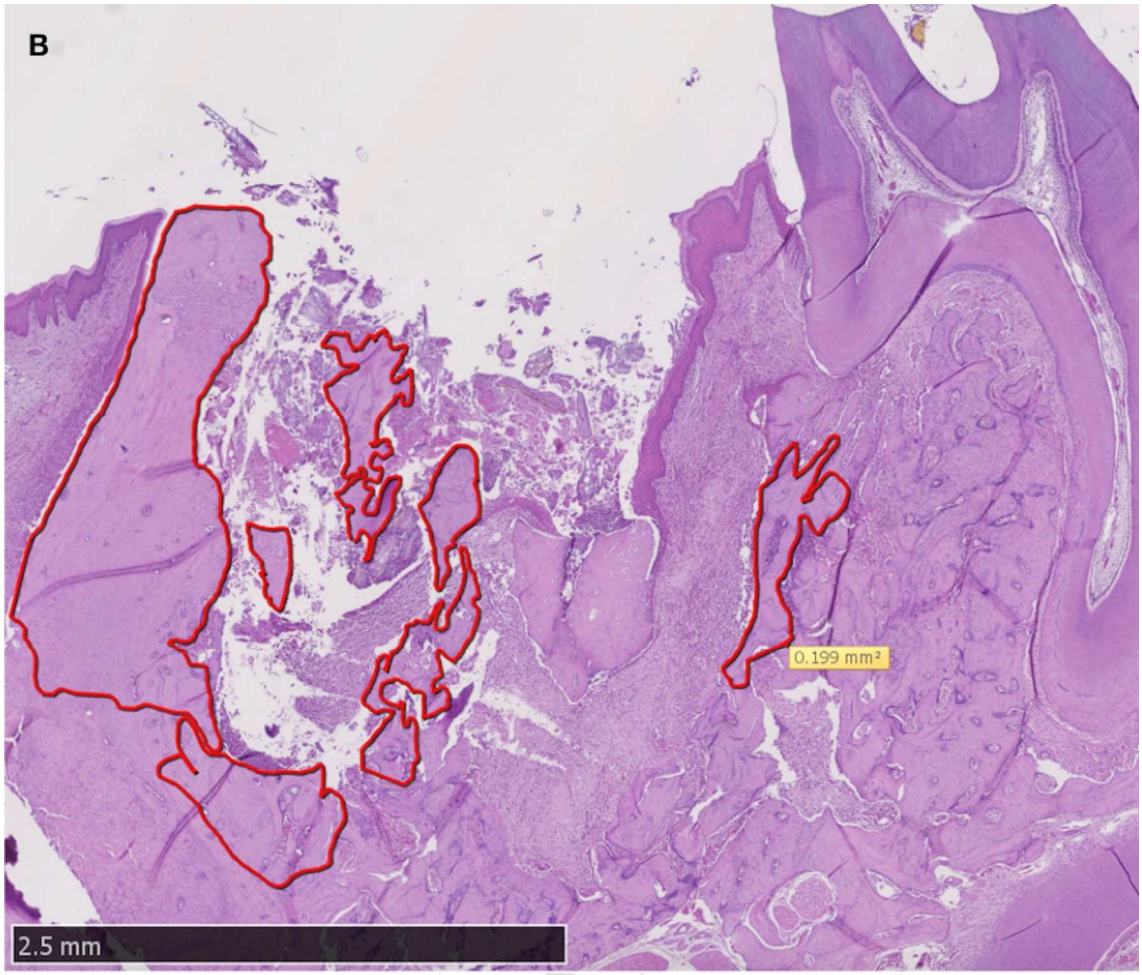
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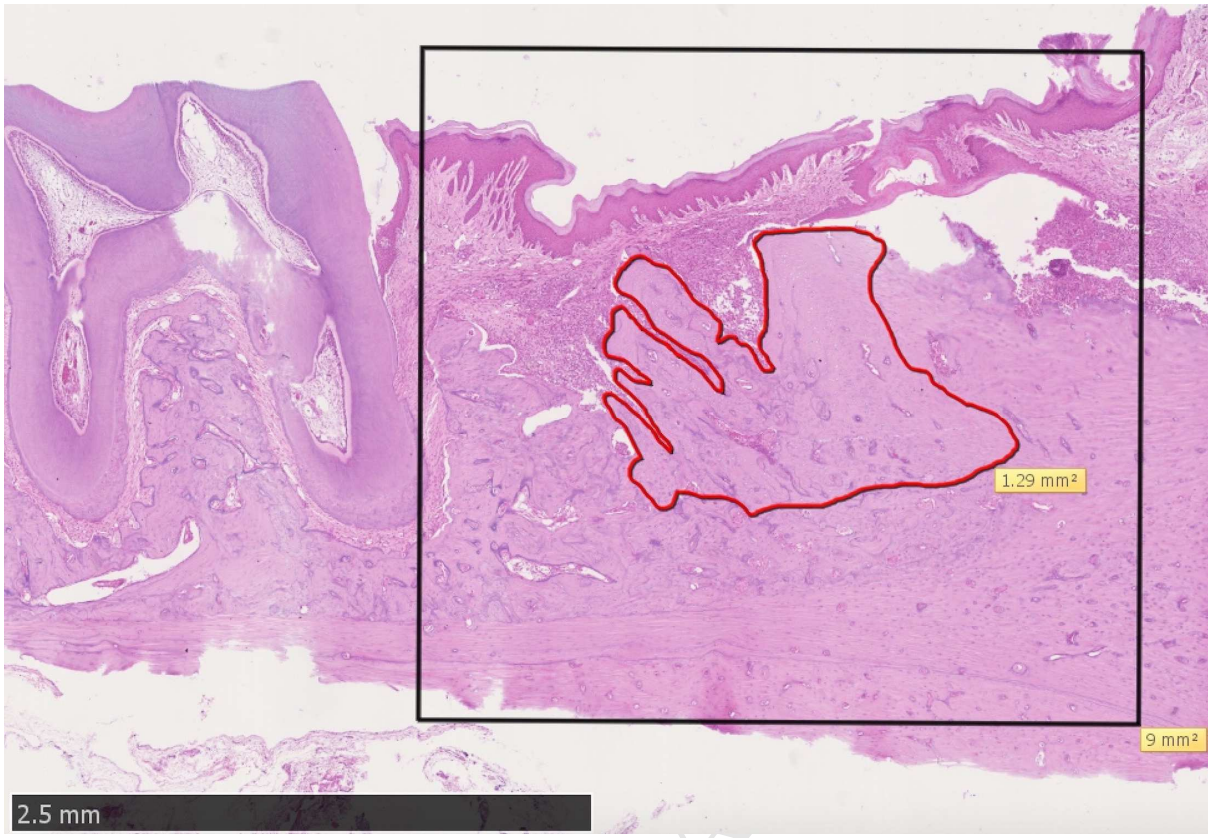
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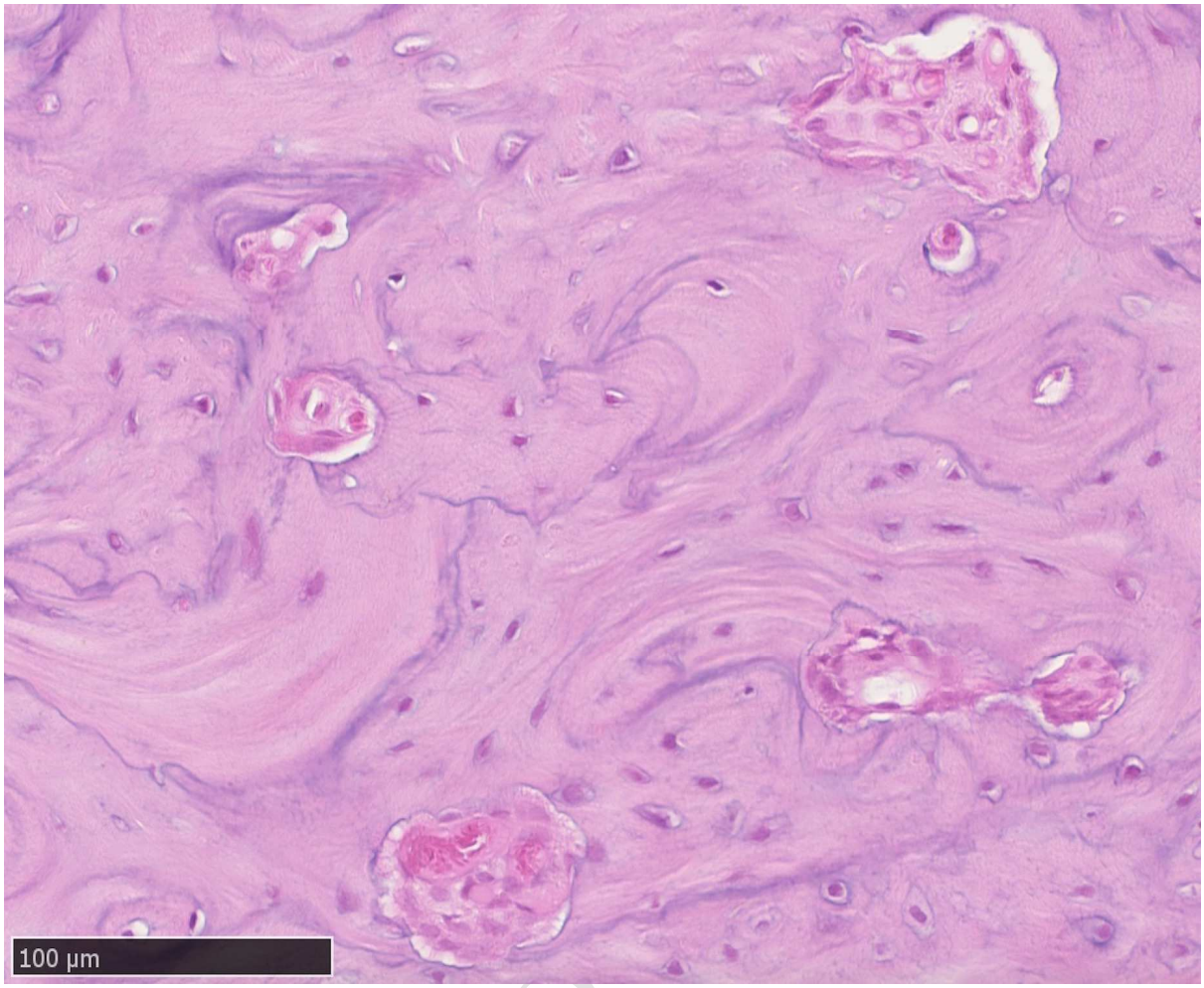
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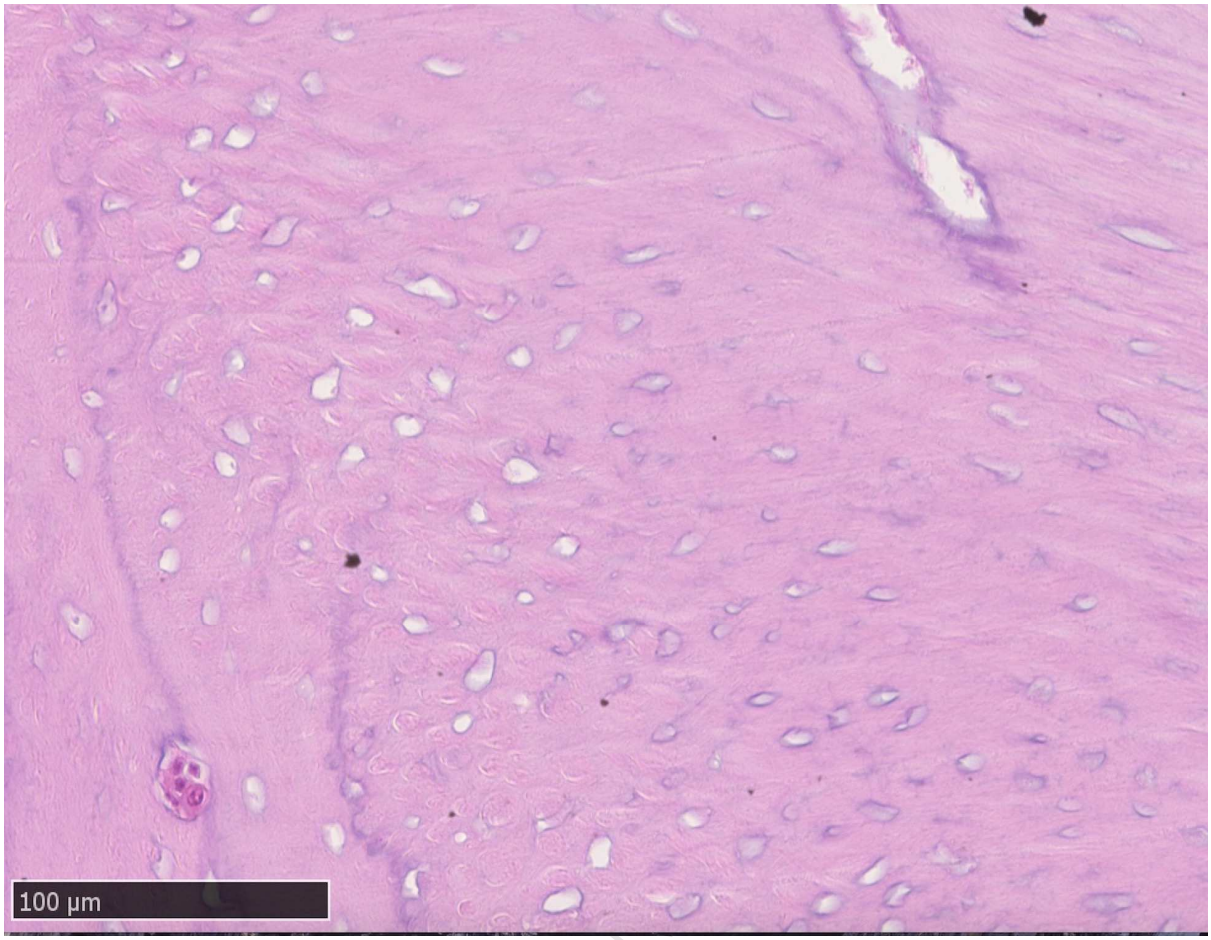
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