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MEDICATION-RELATED OSTEONECROSIS OF THE JAW (MRONJ): IMAGING ASSESSMENT OF RISK FACTORS

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PREFACE

This doctoral thesis consists of three parts, which in turn are made of six chapters. Additionally, this manuscript is framed by an introductory and a general discussion, conclusions, and future perspectives section. The introduction is partly based on a published systematic review of which parts of the results and discussion have been used. Furthermore, each chapter was based on one peer-reviewed publication which follows the standard IMRAD structure (introduction, material and methods, results, and discussion). Parts and chapters were arranged as follows:

Introduction

Moreno-Rabié C, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep;24(9):2973-2989. doi: 10.1007/s00784-020-03423-0.

Part 1: Risk Factors for MRONJ in Patients Undergoing Tooth Extractions.

Chapter 1: *Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, Jacobs R. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280. doi: 10.1038/s41598-022-15254-y.*

Chapter 2: *Moreno Rabie C, Cavalcante Fontenele R, Oliveira Santos N, Nogueira Reis F, Van den Wyngaert T, Jacobs R. Three-dimensional clinical assessment for MRONJ risk in oncologic patients following tooth extractions. Dentomaxillofac Radiol. 2023 Oct 24:20230238. doi: 10.1259/dmfr.20230238.*

Chapter 3: *Moreno-Rabié C, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, Jacobs R. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8. doi: 10.1111/odi.14496.*

Chapter 4: *Moreno-Rabié C, Fontenele RC, Oliveira Santos N, Nogueira-Reis F, Van den Wyngaert T, Jacobs R. Key Insights into Antiresorptive Drug Use and Osteonecrosis in Osteoporotic Patients Undergoing Tooth Extractions: a clinical and CBCT assessment. Under revision.*

Part 2: Therapeutic Prognostic Risk Factors for MRONJ.

Chapter 5: *Moreno Rabie C, García-Larraín S, Contreras Diez de Medina D, Cabello-Salazar I, Cavalcante Fontenele R, Van den Wyngaert T, Jacobs R. How does the clinical and tomographic appearance of MRONJ influence its treatment prognosis? Dentomaxillofac Radiol. 2023 Oct 23:20230304. doi: 10.1259/dmfr.20230304.*

Part 3: Artificial Intelligence as an Aid for MRONJ Diagnosis.

Chapter 6: *Baseri Saadi S, Moreno-Rabié C, van den Wyngaert T, Jacobs R. Convolutional neural network for automated classification of osteonecrosis and related mandibular trabecular patterns. Bone Rep. 2022 Oct 29;17:101632. doi: 10.1016/j.bonr.2022.101632. (Shared first authorship)*

LIST OF ABBREVIATIONS

AAOMS	American Association of Maxillofacial Surgeons
ABP	Abnormal bone pattern
anti-TKI	Tyrosine Kinase Inhibitors
anti-VEGF	Vascular Endothelial Growth Factor Inhibitors
APC	Autologous Platelet Concentrates
APIs	Application programming interfaces
ARDs	Antiresorptive drugs
ASBMR	American Society for Bone and Mineral Research
AUC	Area Under the Curve
BPs	Bisphosphonates
BRONJ	Bisphosphonate-related Osteonecrosis of the Jaws
CBCT	Cone-Beam Computed Tomography
CDC	Center for Disease Control and Prevention from the United States
CNNs	Convolutional Neural Networks
CRP	C-reactive protein
CT	Computed Tomography
DB	Denosumab
DMARDs	Disease-modifying antirheumatic drugs
DRONJ	Denosumab-related Osteonecrosis of the Jaws
FN	False Negative
FOV	Field of View
FP	False Positive
FPP	Farnesyl pyrophosphate
GLMM	Generalized Linear Mixed Model
Grad-CAM	Gradient-weighted Class Activation Mapping

L-PRF	Leukocyte- and platelet-rich fibrin
MCW	Mandibular Cortical Width
MRI	Magnetic Resonance Imaging
MRONJ	Medication-Related Osteonecrosis of the Jaws
NA	Not Applicable
N-BPs	Nitrogen-containing bisphosphonates
NS	Not specified
ONJ	Osteonecrosis of the Jaws
OPG	Osteoprotegerin
PPi	Pyrophosphate
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
ROC	Receiver operating characteristic curve
ROIs	Regions of Interest
SD	Standard Deviation
SICMF	Italian Society of Maxillofacial Surgery
SIPMO	Italian Society of Oral Pathology and Medicine
SIRT1	Sirtuin 1
SNPs	Single-nucleotide polymorphisms
SRE	Skeletal related events
STROBE	STrengthening the Reporting of OBservational studies in Epidemiology
TLD	Thickening of the Lamina Dura
TN	True Negative
TP	True Positive
XAI	Explainable artificial intelligence

GENERAL INTRODUCTION, AIMS, AND HYPOTHESIS

GENERAL INTRODUCTION, AIMS AND HYPOTHESIS

Moreno-Rabié C[1]

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This chapter was partly based on the following systematic review:

***Moreno-Rabié C**, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep;24(9):2973-2989. doi: 10.1007/s00784-020-03423-0.*

GENERAL INTRODUCTION

1. MRONJ and the clinical practice

Health professionals know the importance of a correct anamnesis in clinical practice. The knowledge of the patient's comorbidities and treating medications is of utmost importance to achieve safe and successful treatment results. Particularly in the oral and maxillofacial field, there is special concern regarding the use of antiresorptive drugs (ARDs) and one of their known side effects: medication-related osteonecrosis of the jaw (MRONJ).

2. What are antiresorptive drugs and how do they work?

Antiresorptive drugs are often used in the treatment of bone disease, such as osteoporosis, hypercalcemia, Paget's disease, metastatic bone disease, and multiple myeloma [1]. ARDs have various mechanisms of action, depending on the class of drugs they belong to: bisphosphonates, denosumab, calcitonin analogues, estrogen agonists, selective estrogen receptor modulators, and tissue-selective estrogen complexes [4,5]. Of these, denosumab and bisphosphonates (e.g., zoledronic acid, alendronate, ibandronate, pamidronate, and risedronate) have been associated with MRONJ. These drugs interfere with bone turnover by impeding osteoclastic activity through different pathways to prevent skeletal-related events (SRE) [1,2]. The latter is a group of distressing signs and symptoms, including hypercalcemia, fractures, spinal cord compression, pain, and reduced mobility [3].

Other drugs like anti-VEGF (Vascular Endothelial Growth Factor Inhibitors) and anti-TKI (Tyrosine Kinase Inhibitors), which have an antiangiogenic effect and are used as treatments for advanced cancer, have also been identified as contributors to the development of osteonecrosis of the jaws [4,5]. However, given that most reports arose from bisphosphonates and monoclonal antibodies (denosumab), only these drugs will be assessed in detail for the purpose of this thesis.

Bisphosphonates

Bisphosphonates (BPs) are synthetic analogues of pyrophosphate (PPi). PPi is a small inorganic molecule that is naturally present in our bodies and has as main effect to avoid soft tissue calcification [6,7]. Initially, it was thought that PPi could be used in a therapeutic setting to inhibit ectopic calcifications in blood vessels, skin, and kidneys. However, studies showed that PPi became inactive when given orally due to hydrolysis in the gastrointestinal tract [7]. Therefore, a hydrolysis-resistant PPi analogue was developed, which had a chemical structure with two geminal phosphonate groups sharing a carbon atom with high affinity for hydroxyapatite. Additionally, these molecules contained two side chains (R1 and R2) hanging from the carbon atom, which were responsible for their wide antiresorptive potency [7,8]. This led to what we know today as bisphosphonates. Interestingly, in these first experiments it was seen that BPs would not only prevent pathological soft tissue calcifications but also normal bone mineralization, which is how they started being investigated as antiresorptive drugs [7].

The first type of BPs used for human therapies included clodronate, medronate, etidronate, and tiludronate. This group was called simple or non-nitrogen containing BPs [9]. Later, complex side chains, including one or more nitrogen atoms, were added to the chemical structure, resulting in the drugs mostly used today and named nitrogen-containing bisphosphonates (N-BPs) [7,9]. Within the latter, pamidronate, alendronate, ibandronate, risedronate and zoledronic acid can be found [9]. Figure 1 shows an illustrative example of the mechanism of action of N-BPs.

Depending on the type, BPs can be taken by oral, subcutaneous, or intravenous routes. Concerning the treatment doses, osteoporosis is typically treated with alendronate 10mg orally every day or 70mg orally every week, risedronate 35mg orally every week, ibandronate 150mg orally every month, or zoledronic acid 5mg intravenously once a year. In the case of metastatic cancer, pamidronate 90mg, zoledronic acid 4mg, or ibandronate 6mg are administered intravenously every 3 to 4 weeks [12,13].

Once BPs enter circulation, the medication is only briefly available in the plasma. Part of it will be deposited in bone, while the remainder will be excreted through the kidneys, which is the only route of elimination. Yet, bone uptake is not

uniform since bones with a higher turnover rate accumulate greater amounts of BP, such as those in the maxillofacial region and the femur. Moreover, these molecules will be released only when osteoclasts resorb the bone where they are stored. This translates into a long half-life, which varies between 1 and 10 years in function of the type of BP and the turnover rate of the bone [14].

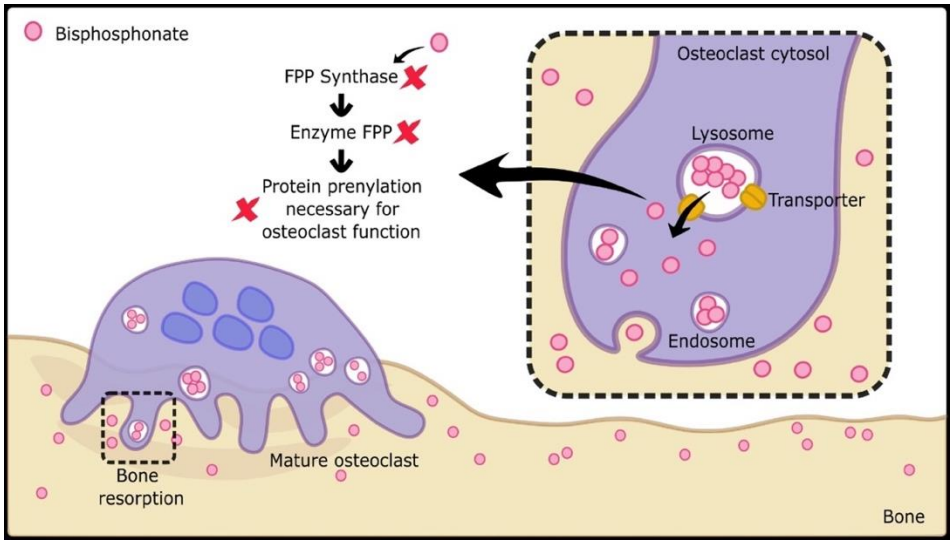

Figure 1. Mechanism of action of nitrogen-containing bisphosphonates (N-BPs). Image adapted from Rogers et al. 2020 [9]. N-BPs will bind to the calcium ions (hydroxyapatite) present on the surface of the bone after being transported in the blood. Once in the bone, the drug will be brought into the cytosol of the osteoclast via endosomal vesicles when the osteoclast performs bone resorption [7–9]. Intracellularly, these endosomes will mature into lysosomes, and membrane transporters will translocate the N-BP into the cytosol of the cell [9]. The main target of N-BP will be the enzyme farnesyl pyrophosphate (FPP) synthase, which is part of the mevalonate pathway [7,10]. When FPP synthase is inhibited, the formation of its end product, farnesyl diphosphate, will be prevented, and in turn, protein prenylation will be inhibited. The latter is an essential step for osteoclast function [7,11]. Thus, inhibiting FPP synthase will lead to a reduction of bone resorption due to the inhibition of osteoclast activity [7].

Denosumab

Denosumab is a fully human monoclonal antibody that interferes with the RANK/RANKL/OPG pathway [2]. This signalling sequence is essential to osteoclastogenesis, whose activation results in osteoclast maturation, bone shaping,

and remodelling. Among its key components are the receptor activator of nuclear factor kappa-B (RANK), receptor activator of nuclear factor kappa-B ligand (RANKL), and osteoprotegerin (OPG) [15,16]. RANK is a receptor expressed on the cell surface of osteoclasts and osteoclast precursors. In contrast, OPG and RANKL are molecules produced by osteoblasts, osteocytes, and bone marrow cells in response to endocrine, paracrine, and cytokine signals. As a result, when RANKL binds to RANK, differentiation of hematopoietic precursors into osteoclasts and bone resorption are favoured. When OPG binds to RANKL, differentiation of hematopoietic precursors into osteoclasts is inhibited [16]. Thus, denosumab was developed to mimic the function of the natural antagonist of RANKL, OPG, and prevent osteoclast differentiation [7,16]. An illustrative example of the mechanism of action of denosumab is depicted in Figure 2.

Denosumab is administered subcutaneously. The dose for osteoporosis treatment is 60mg every six months [13], while for oncological indications, it is 120mg monthly [16,17]. Given that denosumab is an antibody, it is metabolized in the reticuloendothelial system without impairing the renal function [17]. Furthermore, as the medication does not bind to bone, the reported half-life of this monoclonal antibody is 30 days [18] and its effect can be reversed within 6 months after drug discontinuation [2,19].

3. What is Medication-Related Osteonecrosis of the Jaws (MRONJ)?

The current most used definition, published by the American Association of Oral and Maxillofacial Surgeons (AAOMS) in 2007 [20], clinically describes MRONJ as exposed bone or bone that can be probed through an intraoral or extraoral fistula(e) in the maxillofacial region that has persisted for more than eight weeks in patients treated with antiresorptive or antiangiogenic drugs and who had no history of radiation therapy to the jaws or obvious metastatic disease [19–21]. Figure 3 shows clinical photographs of MRONJ.

Along with the definition, the AAOMS describes a staging system for all patients treated with antiresorptive drugs and who developed MRONJ [21]. The staging goes as follows:

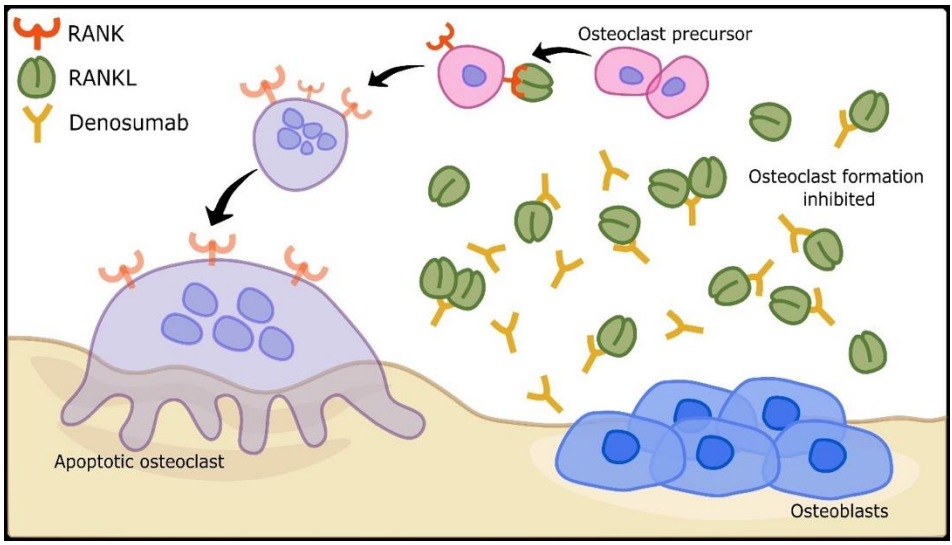

Figure 2. Mechanism of action of denosumab. Image adapted from Lipton et al. 2012 [17]. In a normal scenario, RANKL, which is produced by osteoblasts, osteocytes and stromal cells in the bone marrow, binds to RANK in the hematopoietic precursors from the monocyte-macrophage lineage and leads them to differentiate to multinucleated osteoclasts [2]. Once attached to the bone, the activated osteoclasts release acids and enzymes that enable bone resorption. When denosumab is administered, it will bind to RANKL inhibiting its attachment to RANK [17]. Consequently, there will be diminished bone resorption by preventing osteoclast differentiation.

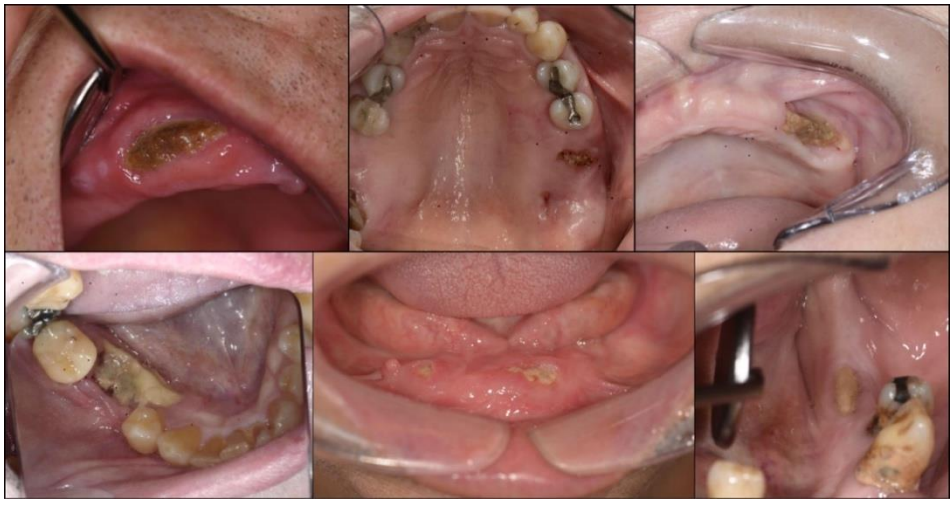

Figure 3. Clinical images of osteonecrosis of the jaws. All lesions correspond to stage 1 according to the classification of the AAOMS.

- At risk: these are all asymptomatic patients exposed to antiresorptive drugs, currently or in the past in absence of bone exposure.
- Stage 0: these patients also have absence of bone exposure but in presence of nonspecific symptoms or clinical and radiographic findings, such as:
    - Symptoms: odontalgia of non-dental origin, bone pain irradiating to the temporomandibular joint region, sinus pain associated to inflammation, and/or neurosensory dysfunction.
    - Clinical findings: unexplained teeth loosening and/or intraoral or extraoral swelling.
    - Radiographic findings: bone loss not attributable to periodontal disease, osteosclerosis, persistence of the alveolar socket, thickening of the mandibular cortical and/or lamina dura, and/or widening of the periodontal ligament space.
- Stage 1: describes the presence of exposed necrotic bone or fistula that probes to bone in patients who are asymptomatic and have no evidence of infection or inflammation. Further radiographic findings as described in Stage 0 may be present in the alveolar bone region.
- Stage 2: these are symptomatic patients who present exposed necrotic bone or fistula that probes to bone with evidence of infection or inflammation. Radiographic findings as described in Stage 0 may also be present in the alveolar bone region.
- Stage 3: these patients present the same case definition as for stage 2 together with one or more of the following, exposed necrotic bone extending beyond the alveolar bone region, pathologic fracture, extraoral fistula, oro-antral/oro-nasal communication, or osteolysis involving the inferior border of the mandible or sinus floor.

Alternatively, in 2012, the Italian Society of Maxillofacial Surgery (SICMF) and the Italian Society of Oral Pathology and Medicine (SIPMO) introduced a new diagnosis and classification [22,23]. Although, this classification has not been used extensively in the

literature. They define MRONJ as "*an adverse drug reaction characterized by progressive disruption and necrosis of the mandibular and/or maxillary bones, occurring in subjects who received antiresorptive drugs without previous radiotherapy*" [22]. In addition, the Italian Societies describe three clinical-radiographic stages, which can be further subclassified into asymptomatic or symptomatic stages if pain and infection are present. These stages are:

- Stage 1: Focal MRONJ. Where there is at least one minor clinical sign, including halitosis, abscess, asymmetry, bone exposure, fistula, mucous discharge, impaired healing after tooth extraction, unexplained tooth mobility, lip paraesthesia, purulent discharge, bone sequestrum formation, trismus, or soft tissue inflammation. In addition, osteosclerosis limited to the alveolar process can be observed in three-dimensional images.
- Stage 2: Diffuse MRONJ. This stage comprises at least one of the minor clinical signs previously described, together with three-dimensional images showing osteosclerosis extending to the basal bone.
- Stage 3: Severe MRONJ. Involving an extraoral fistula, fluid leakage through the nose, abnormal mobility of the jaws, or the radiographic presence of fracture, osteolysis of the maxillary walls, zygomatic bone, or hard palate.

During the course of this thesis, the definition and classification provided by the AAOMS shall be used as it is generally employed in the scientific literature.

4. How common is the development of MRONJ?

The incidence of MRONJ can vary depending on various factors such as the type of medication used, duration of treatment, and underlying medical conditions [21]. In this regard, patients receiving ARDs for malignancy are generally at a higher risk of developing MRONJ compared to those receiving ARDs for non-malignant conditions.

Hereafter, the incidences are presented according to the underlying condition and whether the drug used is a bisphosphonate or monoclonal antibody (denosumab).

a. Antiresorptive drugs for malignancy: As previously mentioned, oncologic patients are exposed to higher cumulative doses of ARDs, associated with higher incidences of MRONJ.
    I. Bisphosphonates: Phase III clinical trials have shown that the incidence of MRONJ among cancer patients receiving high-dose bisphosphonates varies between 0.5% [24] and 1.3% [25,26] during the first year of drug administration. The incidence increases with the duration of treatment reaching 1.4% after three years of medication [24,27]. Overall, it can be said that the incidence is less than 5%, with results ranging from 0% to 18% [21,28–31]. The variation in these estimates can be attributed to the differences in follow-up duration reported in various studies, ranging from 1 to 10 years [21], and the exposure to different risk factors.
    II. Denosumab: The same phase III clinical studies demonstrated that the incidence of MRONJ in cancer patients treated with denosumab varies between 0.8% [24] and 1.1% [25,26] during the first year of drug administration. This incidence also increases with treatment duration to 4.6% after three years of therapy [27,31]. Overall, the reported incidence is also less than 5%, ranging from 0% to 6.9% [21,28,31].
b. Antiresorptive drugs for non-malignant diseases: osteoporosis or other metabolic bone disease is treated with lower ARD doses, which results in lower incidences of MRONJ.
    I. Bisphosphonates: In patients exposed to intravenous zoledronic acid, the estimated risk of MRONJ is less than 0.02% [21,32], while for patients exposed to oral bisphosphonates, the estimated risk is less than 0.05% [33,34].
    II. Denosumab: The incidence of MRONJ after low dose denosumab was reported to be 0.3% [35].

Thus, based on the reported evidence, there appears to be no meaningful difference in the development of MRONJ between bisphosphonates and denosumab.

5. How can the development of MRONJ be explained?

The pathophysiology of MRONJ is not fully understood but experts agree that this rare pathology has a multifactorial nature [21]. In the next paragraphs, five hypotheses are described that may explain different angles of the development of osteonecrosis of the jaws, and where more than one hypothesis may contribute [21,36].

a. Bone remodelling inhibition: ARDs reduce osteoclast activity, which suppresses bone remodelling, and this is thought to be one of the key pathways to MRONJ. MRONJ occurs in similar proportions in BP and DB users, implying that the route by which osteoclasts are suppressed is less relevant to the development of osteonecrosis. Animal [37] and human [38] studies show that using ARDs lower osteoclast density in necrotic areas of the jawbones, indicating that osteoclast inhibition is crucial to the onset of MRONJ [21,36].
b. Inflammation and infection: Only a small percentage of patients on systemic ARDs develop ONJ, suggesting additional contributing factors besides the medications [36]. Tooth extraction is often associated with MRONJ, but pre-existing dental issues are usually present in these sites [21,36]. Research supports the importance of inflammation and infection in the development of MRONJ, given that preclinical studies have shown presence of inflammatory cytokines in MRONJ sites [39] and dental prevention therapies reducing its incidence [40].
c. Angiogenesis inhibition: ARDs have not only demonstrated to inhibit bone resorption, but also angiogenesis resulting in decreased blood flow to afflicted areas [41,42]. Animal models and human studies have shown decreased vascularity and micro vessel numbers during early stages of MRONJ and impaired healing of extraction sockets [43,44]. Moreover, antiangiogenic medications, such as anti-VEGF and anti-TKI, as well as

immunomodulatory drugs, have also been associated with MRONJ. Although, it is important to note that the incidence of MRONJ in patients taking antiangiogenic drugs is lower compared to those on ARDs [21].

d. Immunity dysfunction: Patients with medical conditions, such as diabetes, rheumatoid arthritis, or compromised immune systems are at a significant higher risk of developing MRONJ, even without exposure to antiresorptive agents [19,21]. Additionally, ARDs combined with chemotherapy, corticosteroids, or disease-modifying antirheumatic drugs (DMARDs) increase the severity or prevalence of MRONJ [45,46]. All prior situations result in a compromised immune system. Confirming this hypothesis, MRONJ affected bone samples have shown altered immune cell patterns [47].

e. Genetic factors: Several single-nucleotide polymorphisms (SNPs) associated with the development of MRONJ have been identified. These SNPs were found in genes related to bone turnover, collagen formation, metabolic bone diseases, angiogenesis, bone remodelling, and immune responses [48–50]. Particularly, SIRT1 is a bone remodelling regulator promoting bone formation, which may have a protective effect against MRONJ when upregulated [48].

6. Are there known risk factors for MRONJ?

Recognizing risk factors is an essential part of medical practice to best prepare for potential adversity. In the case of osteonecrosis, we can categorize the risk factors into three main groups [51]:

- Drug-related risk factors: These are explained by the pharmacokinetics and pharmacodynamics of the administered drugs. Here, the type of drug, its bioavailability, the cumulative dose, which is especially related to the half-life of the drug, and the duration of treatment are of main importance [51–53]. Specifically, it has been recognized that a higher risk exists in patients taking higher doses of ARDs in contrast to those receiving doses for non-

malignant reasons. In addition, regardless of the administered dose, a long treatment by itself is also a risk factor for MRONJ, suggesting the importance of cumulative exposure [21].

- Systemic risk factors: Chronic diseases, adjuvant pharmacological treatments, and habits can have an influence on bone health. Specifically, diabetes mellitus, the use of corticosteroids and smoking have been identified as potential risk factors [51,54].
- Local risk factors: As a rule, any condition that generates inflammation or infection in the dental support structures should be considered a risk factor [51]. These include endodontic and periodontal dental infections [55], as well as local trauma due to tooth extractions or a maladjusted prosthesis [19,52,56]. Against this background, all recommendations agree on the importance of maintaining adequate hygiene control in patients under treatment with bisphosphonates and denosumab and emphasize periodic visits to the dentist [21,51].

7. What is known about the early stages of MRONJ and what are the radiographic features of osteonecrosis?

In recent years, it has become increasingly recognized that MRONJ exhibits signs and symptoms even before the exposure of bone can be identified. To address this, the AAOMS introduced the concept of patients at risk and stage 0 in 2014 [19]. However, the classification of stage 0 remains somewhat controversial due to its ambiguity and lack of fulfilment with the definition [52]. These early stages of MRONJ may be accompanied by radiographic findings that indicate potential involvement of the jawbone. Consequently, the use of imaging techniques has been proposed to aid in the early diagnosis of this condition [52]. However, the existing literature on early radiographic findings is limited, highlighting the need for further investigation in this area.

Early Radiographic Findings

In order to address this knowledge gap, we conducted a systematic review to identify the early bone changes that occur in the jawbones as a result of ARD treatment [57]. Our

objective was to classify which changes are present in patients considered “at risk” or in stage 0 MRONJ. Additionally, we sought to explore secondary objectives, including: (a) reporting the timing of the occurrence of these bone changes; (b) investigating any potential relationship between the occurrence of bone changes and the type and dosage of the medication administered; and (c) determining if there is an association between the appearance of bone changes and the subsequent development of osteonecrosis of the jaw.

In this systematic review, we identified several radiographic findings, all of them reported in the tooth-bearing areas of the jaws and with osteosclerosis being the most common one. Table 1 summarizes the imaging findings for patients at risk and Stage 0, and Figure 4 displays examples of some of these features.

It seems reasonable to assume that different doses and medications would cause different degrees of osteosclerosis [60] or different frequency of radiographic findings. Unfortunately, the results in this review were not conclusive. No significant differences were seen when comparing the type and route of administration of the antiresorptive [85–87], nor when the treatment time was shorter or longer than 3 years [83]. Some authors indicate that the lack of significance is due to the absence of intervention [88,89], a short follow-up period [81,88], and drug dosage [81,88], because the cumulative use of more than one type of antiresorptive could aggravate their effects [81].

Relationship between early and late radiographic findings

In the systematic review different early radiographic findings are described but it is interesting to compare whether these features are also present in stages with bone exposure.

Radiographic findings revealed the presence of sclerotic areas across all stages, from stage 0 to stage 3. However, no significant differences were observed between the stages [90] nor between the at-risk area for MRONJ and the control group [63]. Diffuse sclerosis on computed tomography images was found in approximately half of the patients in stage 0 [68], emphasizing that bone changes can exist even in the absence of clinical bone exposure [68,87].

Table 1. Radiographic findings reported in the literature for patients at risk and stage 0 MRONJ.

Radiographic finding	At risk	Stage 0	R
Bone sclerosis [58–72]	+	+	
Osteolytic areas [60–62,64,67,70,72,73]	+	+	
Thickening of lamina dura [59,60,65,66,69,70,72–78]	+	+	
Visible/persisting alveolar socket [60,65,70,72,75,78,79]	+	+	
Periapical lesion (and osteitis) [58,65,74]	+	+	
Higher mandibular cortical thickness [66,80–83]	+	+	
Widening of periodontal ligament space [65,67,74,77,84]	+	+	
Periodontal bone loss [79,84]	+	+	
Enhancement of the mandibular canal [60,65,69,72]	+	+	
Narrowing of the mandibular canal [74]	-	+	
Changes in trabecular pattern [73,78]	-	+	
Bone sequestrum [67,70–73]	-	+*	
Cortical disruption [65,70–72]	-	+*	
Crater-like defect [71]	-	+*	
Periosteal reaction [58,60,71,73]	-	+*	

Radiographic findings indicated with a plus sign (+) have been reported for those groups of patients. *Findings observed in three-dimensional images (e.g., cone-beam computed tomography). R: strength of the recommendation based on the quality of the evidence. Orange: moderate. Red: low.

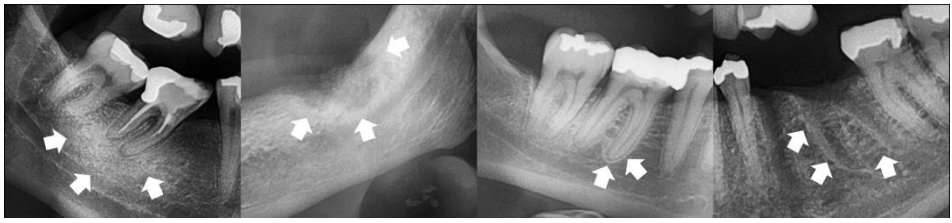

Figure 4. Examples of radiographic findings in patients treated with antiresorptive drugs. From left to right exhibiting osteosclerosis, osteolytic area, thickening of the lamina dura, and visibility of the extraction socket.

Significant differences were observed in the cortical bone between non-MRONJ and MRONJ groups, but no distinctions were seen between stage 0 and later stages. In addition, significant changes were seen in the same patient between the affected and non-affected side [87]. Hutchinson *et al*. added that the appearance of the bone changes is possibly a response to local dental issues or anatomic variations [65], suggesting that the most influencing factors are the proper characteristics of the bone [65,85], other than the systemic factors such as the dose and length of the treatment [87,91].

Once the bone changes take place, a number of these sites further develop MRONJ [85], but the precise mechanism is not yet clear [92]. For patients at stage 0, in addition to the reported radiographic findings for "at risk" patients, the following changes were also reported: narrowing of the mandibular canal, changes in the trabecular pattern, bone sequestrum, cortical disruption, crater-like defect and periosteal reaction. All these findings have also been seen in patients at stage 2 and 3 [66], which suggests that the development of MRONJ is not progressive when implementing the classification proposed by the AAOMS.

Bone sequestrum in Stage 0 has been considered a radiographic predictor of future bone exposure [71] and widening of the periodontal ligament space as an initial sign of necrosis, indicating that the alveolar socket could be the origin of a process that leads to MRONJ [93]. For Kubo *et al*. osteosclerosis is a radiographic finding that leads to the development of MRONJ, and thickening of the lamina dura is a change that occurs during the treatment with antiresorptive drugs [76]. Fedele *et al*. report that at least half of the patients that were diagnosed with stage 0 MRONJ progressed to bone exposure in a 0.5 to 14 months period [94], similar results were reported by Soundia *et al*. [71], while Hutchinson *et al*. mentioned that from 10 stage 0 patients, all with osteosclerosis in their symptomatic areas, none developed further bone exposure after a one-year follow-up [65].

To summarize, there seems to be no difference in the radiographic appearance between stage 0 and stages with bone exposure. Yet, in patients at risk, it is not clear which of these imaging signs are related to the development of osteonecrosis.

The Best Imaging Approach

There is no consensus regarding the choice of the imaging modality for the assessment of patients under antiresorptive therapy. Considering that the purpose of this examination would be risk identification and/or early detection of MRONJ. Some justify the use of panoramic images, by its lower costs, overall visibility of the maxilla and mandible structures, low radiation exposure [70] and due to its easier access than 3D imaging for dentists [70,76]. However, due to the superposition of structures in the maxilla, it has a greater diagnostic impact in the mandible [69]. Other authors indicate that two-dimensional images seem to have a limited diagnostic value for MRONJ [66,95].

Aghaloo *et al*. recommends a high resolution CBCT in any patient with unspecific symptomatology and in treatment with antiresorptive drugs [67], because conventional radiography tends to underestimate the bony changes [96] and is less sensitive in the trabecular bone, sinus, soft tissue involvement and lesion extent assessment [63,66,69,97,98]. It has been mentioned that CBCT has a greater value for detecting early asymptomatic lesions in the jaws, while the use of panoramic radiography can be used in later MRONJ stages [66]. Indeed, three-dimensional imaging showed more findings that were related to stage 0 patients, and that were not observed for patients at risk (Table 1). Despite its higher radiation dose, the benefit of its accurate evaluation surpasses the biological costs [81].

Finally, as a complementary measure suggested by Krishnan *et al.* in 2009 [93], it seems like a good first approach to observe the bone scintigraphy or brain magnetic resonance images (MRI) from the treated population, if available [93,99,100]. Since bone scintigraphy is a sensitive method that provides information over local metabolic or vascular changes, it should be useful to identify early bone changes [99,101]. Using bone scintigraphy, it has been noted a higher tracer uptake in areas that later developed MRONJ [93,99–103]. While with MRI, it has been observed low signal intensity in T1 and high signal intensity in T2 in areas without bone exposure in Stage 0 [86,93]. Given that these images are often taken first for oncologic reasons, the identification of abnormalities in the maxillary or mandibular regions would lead to early referral and the acquisition of a two- or three-dimensional radiograph as a complementary diagnostic method for MRONJ.

8. How can we treat osteonecrosis of the jaws?

The primary goals of prevention, in patient's "at risk", and treatment, in those affected by MRONJ, encompass several key aspects. These include prioritizing and facilitating the continuation of oncological or osteoporotic treatment, as well as preserving the quality of life through education, pain control, treatment of infection, and prevention of lesion extension and appearance of new necrotic areas [21]. Treatment options can be broadly classified into two main groups: conservative (non-operative) and surgical.

Prevention

Whilst this section is primarily focused on treating established MRONJ lesions and, therefore, with bone exposure, it is important to emphasize the recommended preventive measures. Prevention primarily encourages maintaining optimal oral health and minimizing the risk of complications. The most used approaches include regular dental check-ups, comprehensive dental assessments before starting antiresorptive therapy, and meticulous oral hygiene practices. All this is to avoid dental infections or invasive procedures such as tooth extractions. Concretely speaking, two to three dental control visits per year are recommended for patients treated with antiresorptive drugs [21,22].

Prevention also extends to measures that have shown a beneficial effect on reducing the risk of MRONJ when invasive procedures are to be performed. These measures include, among others, the use of pre- and post-operative antibiotics [104,105], closure by first intention [106], use of Leucocyte and Platelet-Rich Fibrin (L-PRF) [107,108], alveolectomy [104], and temporary cessation of antiresorptive drug administration (drug holiday) [109–113]. Although, this last measure is nowadays controversial, as it puts the patient at risk of complications associated with their underlying condition due to treatment cessation [21], especially when treated with denosumab.

Conservative Treatment

Conservative treatment focuses primarily on minimizing bacterial load and infection using broad-spectrum antibiotics and antiseptic rinses [22], accompanied by local wound care and pain management. Yet, evidence is inconclusive on the antibiotic of choice and

the duration of treatment [51]. Particularly, the Italian societies recommend limiting the indication of antibiotics to the presence of acute infection and relapse, aiming for a minimum duration of 7 days but not exceeding 21 days of intake [22].

Conservative treatment can be adopted at any clinical stage. In early stages, it has been shown to stabilize the lesions or even lead to their resolution [21]. Whereas, in advanced clinical stages, the purpose is to control infection and reduce inflammation, and if appropriate, to create a favourable environment for operative intervention [51]. Either way, non-operative therapy is associated with an improvement in the quality of life of affected patients by helping with pain control [21,114]. Additionally, other complementary non-invasive treatments have been investigated, including ozone, laser, and hyperbaric oxygen therapy. Although evidence is not conclusive of the beneficial effect of these therapies [51]. In terms of success rate, a meta-analysis described that 29% of patients treated conservatively reported healing of the lesions, with large variations and heterogeneity [115].

Surgical Treatment

In the early days of osteonecrosis treatment, surgery was reserved for refractory or extensive cases, as it was believed that bone manipulation was likely to worsen the lesion. However, current evidence has demonstrated that removing the affected bone leads to a higher success rate [116]. This is because advanced disease, such as metastatic cancer, should not preclude carrying out these interventions if patients are carefully selected. As such, clinical guidelines recommend evaluating on a case-by-case basis and determining the risk-benefit of surgery for the patient [21,22].

Once it has been decided to perform a surgical treatment, both minor and major invasive options are available. Some authors consider minimally invasive approaches as part of conservative treatment [51], but in the present manuscript, given that they still require an outpatient facility and local anaesthesia, we will consider them as a surgical option.

Minimally invasive surgical treatments include the removal of bone spikes, curettage, and sequestrectomy [117]. Whereas invasive procedures focus on partial or total removal of necrotic bone. Both approaches can be accompanied by laser surgery

and autologous platelet concentrates [115]. It is important to note that both approaches will have varying success rates [117]. Nonetheless, it is generally reported that 62% of surgically treated patients showed healing of the lesions [115]. Specifically, showing successful outcomes in at least 50% of patients treated with minimally invasive procedures and 84% of those treated with extensive surgeries [117]. Lastly, patients who have sequential conservative and surgical treatment, whether minor or major surgery, reported a healing rate of about 40% [118].

9. What are we missing?

Risk factors, influence of ARDs in the jawbones, and early identification of MRONJ

In conducting the systematic review, it was possible to find limitations of the studies published up to 2020. These included their retrospective nature and lack of follow-up, as reflected in the quality analysis [57]. A major challenge in the early detection of MRONJ is to determine the bone changes that are solely related to the antiresorptive drug mechanism, the precise timepoint in which those bone changes occur and become irreversible, and its potential to lead to the onset of necrosis. Those aspects of the disease had not yet been described in the literature. These research questions could not be answered in part because most studies lacked a positive and/or a negative control group. Determining the real effect of the antiresorptive drugs on the bone, would require a diseased group (i.e., treated with antiresorptive drug and with MRONJ), a positive control group, (i.e., treated with antiresorptive drugs and without MRONJ), and a negative control group (i.e., not exposed to the medication). Furthermore, most investigations were carried out in patients on antiresorptive drugs with and without bone exposure, where the main objective was not the identification of early radiographic findings.

Therapeutic prognostic risk factors for MRONJ

When it comes to the treatment of osteonecrosis, radiographic images have not been widely used to support the choice of the best treatment for MRONJ. Imaging has been used primarily to determine the extent or progression of the lesion and to avoid unnecessarily invasive procedures [21]. In 2018, Shin et al. demonstrated that lesions

compromising more than one-third of the mandible had a worse surgical prognosis than smaller lesions [119]. Similarly, Japanese researchers observed that periosteal reaction was an indicator of poor prognosis [120,121]. Yet, the radiographic appearance and its influence in the postoperative outcome of MRONJ is still needed [118,119]. Further research is necessary to understand the role of diagnostic images in the prognosis of conservative and surgical therapy for MRONJ.

Artificial intelligence as an aid for MRONJ diagnosis

With the rise of artificial intelligence and its potential beyond our imagination, it would be interesting to explore applications in patients treated with antiresorptive drugs and who developed osteonecrosis. For instance, a Korean research group has compared machine learning models with conventional statistical methods to predict with clinical information the occurrence of MRONJ after tooth extractions in patients treated with ARDs [122]. Apart from this study, until the start of this PhD, there was no model for predicting the occurrence of MRONJ in the maxillary bones. Similar convolutional networks have been published to predict the occurrence of osteonecrosis in the femoral head after surgery [123] and to diagnose osteonecrosis of the femoral head using MRI [124], but this approach has not been used yet for risk stratification in ARD-treated patients using panoramic radiographs nor cone beam computed tomography.

AIMS AND HYPOTHESIS

The main objective of this doctoral thesis was to use two- and three-dimensional radiographic images for the identification of risk factors for medication-related osteonecrosis of the jaws. It was hypothesized that the use of radiographic images is an essential diagnostic tool for early diagnosis and treatment selection of MRONJ. This general objective and hypothesis were subdivided into three parts and tested throughout the chapters of the present dissertation. The specific objective(s) are presented below.

Part 1: Risk factors for MRONJ in patients undergoing tooth extractions

Subobjectives: (1) to identify local radiographic features that act as a risk factor for MRONJ development in patients currently taking or with a history of antiresorptive drugs and undergoing tooth extractions. (2) to compare the radiographic findings between patients treated with antiresorptive drugs and a control group. These objectives were investigated in oncologic and osteoporotic patients and using panoramic radiographs and cone beam computed tomography (CBCT). The specific publications addressing these objectives are presented hereafter.

Hypothesis: We hypothesised that ARDs induce changes in the maxillary bones that are evidenced by clinical data and two- and three-dimensional images. In addition, bone changes visible on diagnostic images are indicators of local risk factors for MRONJ. Lastly, two- and three-dimensional imaging can provide early visualisation of the preclinical stages of MRONJ.

Oncologic Patients

Chapter 1: Two-dimensional Assessment in Oncologic Patients

Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, Jacobs R. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280. doi: 10.1038/s41598-022-15254-y.

Chapter 2: Three-dimensional Assessment in Oncologic Patients

Moreno Rabie C, Cavalcante Fontenele R, Oliveira Santos N, Nogueira Reis F, Van den Wyngaert T, Jacobs R. Three-dimensional clinical assessment for MRONJ risk in oncologic patients following tooth extractions. Dentomaxillofac Radiol. 2023 Oct 24:20230238. doi: 10.1259/dmfr.20230238.

Osteoporotic Patients

Chapter 3: Two-dimensional Assessment in Osteoporotic Patients

Moreno-Rabié C, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, Jacobs R. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8. doi: 10.1111/odi.14496.

Chapter 4: Three-dimensional Assessment in Osteoporotic Patients

Moreno-Rabié C, Fontenele RC, Oliveira Santos N, Nogueira-Reis F, Van den Wyngaert T, Jacobs R. Key Insights into Antiresorptive Drug Use and Osteonecrosis in Osteoporotic Patients Undergoing Tooth Extractions: a clinical and CBCT assessment. Under revision.

Part 2: Therapeutic prognostic risk factors for MRONJ

Subobjectives: (1) To identify clinical and tomographic prognostic factors for conservative and surgical treatment of MRONJ. (2) To investigate the imaging features associated with lesion relapse.

Hypothesis: It was hypothesised that the presence of certain radiographic features might be associated with a favourable prognosis in both conservative and surgical treatment. Moreover, the presence of some of these features would be associated with lesion relapse.

Chapter 5: Clinical and Tomographic Prognostic Risk Factors for MRONJ

Moreno Rabie C, García-Larraín S, Contreras Diez de Medina D, Cabello-Salazar I, Cavalcante Fontenele R, Van den Wyngaert T, Jacobs R. How does the clinical and tomographic appearance of MRONJ influence its treatment prognosis? Dentomaxillofac Radiol. 2023 Oct 23:20230304. doi: 10.1259/dmfr.20230304.

Part 3: Artificial intelligence as an aid for MRONJ diagnosis

Subobjective: To develop and validate a tool for the automated classification of normal, affected, and osteonecrosis mandibular trabecular bone patterns in panoramic images using convolutional neural networks.

Hypothesis: It was hypothesised that an artificial neural network would be able to successfully differentiate normal mandibular trabecular bone patterns from those affected by antiresorptive drugs and osteonecrosis.

Chapter 6: Automated Classification of Mandibular Trabecular Patterns

Baseri Saadi S, Moreno-Rabié C, van den Wyngaert T, Jacobs R. Convolutional neural network for automated classification of osteonecrosis and related mandibular trabecular patterns. Bone Rep. 2022 Oct 29;17:101632. (Shared first authoship)

References

1. Hamadeh IS, Ngwa BA, Gong Y. Drug induced osteonecrosis of the jaw. Cancer Treat Rev. 2015 May 1;41(5):455–64.

2. Baron R, Ferrari S, Russell RGG. Denosumab and bisphosphonates: Different mechanisms of action and effects. Bone. 2011 Apr 1;48(4):677–92.

3. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat Rev. 2001 Jun 1;27(3):165–76.

4. van Cann T, Loyson T, Verbiest A, Clement PM, Bechter O, Willems L, et al. Incidence of medication-related osteonecrosis of the jaw in patients treated with both bone resorption inhibitors and vascular endothelial growth factor receptor tyrosine kinase inhibitors. Supportive Care in Cancer. 2018 Mar 30;26(3):869–78.

5. Fantasia JE. The Role of Antiangiogenic Therapy in the Development of Osteonecrosis of the Jaw. Oral Maxillofac Surg Clin North Am. 2015 Nov 1;27(4):547–53.

6. Orimo H, Ohata M, Fujita T. Role of inorganic pyrophosphatase in the mechanism of action of parathyroid hormone and calcitonin. Endocrinology. 1971 Sep 1;89(3):852–8.

7. Russell RGG. Bisphosphonates: the first 40 years. Bone. 2011 Jul;49(1):2–19.

8. Russell RGG, Watts NB, Ebetino FH, Rogers MJ. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. Osteoporosis International. 2008 Jun 24;19(6):733–59.

9. Rogers MJ, Mönkkönen J, Munoz MA. Molecular mechanisms of action of bisphosphonates and new insights into their effects outside the skeleton. Bone. 2020 Oct 1;139:115493.

10. van Beek E, Pieterman E, Cohen L, Löwik C, Papapoulos S. Farnesyl Pyrophosphate Synthase Is the Molecular Target of Nitrogen-Containing Bisphosphonates. Biochem Biophys Res Commun. 1999 Oct 14;264(1):108–11.

11. Luckman SP, Hughes DE, Coxon FP, Russell RGG, Rogers MJ. Nitrogen-Containing Bisphosphonates Inhibit the Mevalonate Pathway and Prevent Post-Translational Prenylation of GTP-Binding Proteins, Including Ras. Journal of Bone and Mineral Research. 1998 Apr 1;13(4):581–9.

12. Vannala V, Palaian S, Shankar PR. Therapeutic Dimensions of Bisphosphonates: A Clinical Update. Int J Prev Med. 2020;11(1):166.

13. Hanley DA, Adachi JD, Bell A, Brown V. Denosumab: mechanism of action and clinical outcomes. Int J Clin Pract. 2012 Dec 12;66(12):1139–46.

14. Lin JH. Bisphosphonates: A review of their pharmacokinetic properties. Vol. 18, Bone. Bone; 1996. p. 75–85.

15. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003 May 15;423(6937):337–42.

16. Lacey DL, Boyle WJ, Simonet WS, Kostenuik PJ, Dougall WC, Sullivan JK, et al. Bench to bedside: elucidation of the OPG-RANK-RANKL pathway and the development of denosumab. Nat Rev Drug Discov. 2012 May;11(5):401–19.

17. Lipton A, Smith MR, Ellis GK, Goessl C. Treatment-Induced Bone Loss and Fractures in Cancer Patients Undergoing Hormone Ablation Therapy: Efficacy and Safety of Denosumab. Clin Med Insights Oncol. 2012 Jan 16;6:CMO.S8511.

18. Gibiansky L, Sutjandra L, Doshi S, Zheng J, Sohn W, Peterson MC, et al. Population Pharmacokinetic Analysis of Denosumab in Patients with Bone Metastases from Solid Tumours. Clin Pharmacokinet. 2012 Apr;51(4):247–60.

19. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw--2014 update. J Oral Maxillofac Surg. 2014 Oct;72(10):1938–56.

20. Advisory Task Force on Bisphosphonate-Related Ostenonecrosis of the Jaws AA of O and MS. American Association of Oral and Maxillofacial Surgeons Position Paper on Bisphosphonate-Related Osteonecrosis of the Jaws. Journal of Oral and Maxillofacial Surgery. 2007 Mar;65(3):369–76.

21. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022 May;80(5):920–43.

22. Campisi G, Bedogni A, Fusco V. Raccomandazioni clinico-terapeutiche sull'osteonecrosi delle ossa mascellari (ONJ) farmaco-relata e sua prevenzione. Versione 2. Palermo University Press; 2020. 1–234 p.

23. Bedogni A, Fusco V, Agrillo A, Campisi G. Learning from experience. Proposal of a refined definition and staging system for bisphosphonate-related osteonecrosis of the jaw (BRONJ). Oral Dis. 2012 Sep 22;18(6):621–3.

24. Stopeck AT, Lipton A, Body JJ, Steger GG, Tonkin K, de Boer RH, et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol. 2010 Dec 10;28(35):5132–9.

25. Henry DH, Costa L, Goldwasser F, Hirsh V, Hungria V, Prausova J, et al. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. J Clin Oncol. 2011 Mar 20;29(9):1125–32.

26. Fizazi K, Carducci M, Smith M, Damião R, Brown J, Karsh L, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. The Lancet. 2011 Mar;377(9768):813–22.

27. Nicolatou-Galitis O, Schiødt M, Mendes RA, Ripamonti C, Hope S, Drudge-Coates L, et al. Medication-related osteonecrosis of the jaw: definition and best practice for prevention, diagnosis, and treatment. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019 Feb 1;127(2):117–35.

28. Ng TL, Tu MM, Ibrahim MFK, Basulaiman B, McGee SF, Srikanthan A, et al. Long-term impact of bone-modifying agents for the treatment of bone metastases: a systematic review. Support Care Cancer. 2021 Feb 1;29(2):925–43.

29. Aguiar Bujanda D, Bohn Sarmiento U, Cabrera Suárez MA, Aguiar Morales J. Assessment of renal toxicity and osteonecrosis of the jaws in patients receiving zoledronic acid for bone metastasis. Ann Oncol. 2007 Mar 1;18(3):556–60.

30. Brufsky AM, Sereika SM, Mathew A, Tomifumi O, Singh V, Rosenzweig M. Long-term treatment with intravenous bisphosphonates in metastatic breast cancer: a retrospective study. Breast J. 2013 Sep 1;19(5):504–11.

31. Stopeck AT, Fizazi K, Body JJ, Brown JE, Carducci M, Diel I, et al. Safety of long-term denosumab therapy: results from the open label extension phase of two phase 3 studies in patients with metastatic breast and prostate cancer. Support Care Cancer. 2016 Jan 1;24(1):447–55.

32. Grbic JT, Black DM, Lyles KW, Reid DM, Orwoll E, McClung M, et al. The Incidence of Osteonecrosis of the Jaw in Patients Receiving 5 Milligrams of Zoledronic Acid. The Journal of the American Dental Association. 2010 Nov 1;141(11):1365–70.

33. Saag KG, Petersen J, Brandi ML, Karaplis AC, Lorentzon M, Thomas T, et al. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. N Engl J Med. 2017 Oct 12;377(15):1417–27.

34. Hallmer F, Andersson G, Götrick B, Warfvinge G, Anderud J, Bjørnland T. Prevalence, initiating factor, and treatment outcome of medication-related osteonecrosis of the jaw-a 4-year prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Dec 1;126(6):477–85.

35. Bone HG, Wagman RB, Brandi ML, Brown JP, Chapurlat R, Cummings SR, et al. 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. Lancet Diabetes Endocrinol. 2017 Jul 1;5(7):513–23.

36. Aghaloo T, Hazboun R, Tetradis S. Pathophysiology of Osteonecrosis of the Jaws. Oral Maxillofac Surg Clin North Am. 2015 Nov;27(4):489–96.

37. Soundia A, Hadaya D, Esfandi N, de Molon RS, Bezouglaia O, Dry SM, et al. Osteonecrosis of the jaws (ONJ) in mice after extraction of teeth with periradicular disease. Bone. 2016 Sep;90:133–41.

38. Wehrhan F, Gross C, Creutzburg K, Amann K, Ries J, Kesting M, et al. Osteoclastic expression of higher-level regulators NFATc1 and BCL6 in medication-related osteonecrosis of the jaw secondary to bisphosphonate therapy: a comparison with osteoradionecrosis and osteomyelitis. J Transl Med. 2019 Mar 4;17(1):69.

39. Soma T, Iwasaki R, Sato Y, Kobayashi T, Nakamura S, Kaneko Y, et al. Tooth extraction in mice administered zoledronate increases inflammatory cytokine levels and promotes osteonecrosis of the jaw. J Bone Miner Metab. 2021 May 17;39(3):372–84.

40. Ripamonti CI, Maniezzo M, Campa T, Fagnoni E, Brunelli C, Saibene G, et al. 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. 2009 Jan;20(1):137–45.

41. Wood J, Bonjean K, Ruetz S, Bellahcène A, Devy L, Foidart JM, et al. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. J Pharmacol Exp Ther. 2002 Sep;302(3):1055–61.

42. Santini D, Vincenzi B, Dicuonzo G, Avvisati G, Massacesi C, Battistoni F, et al. Zoledronic acid induces significant and long-lasting modifications of circulating

angiogenic factors in cancer patients. Clin Cancer Res. 2003 Aug 1;9(8):2893–7.

43. Gkouveris I, Hadaya D, Soundia A, Bezouglaia O, Chau Y, Dry SM, et al. Vasculature submucosal changes at early stages of osteonecrosis of the jaw (ONJ). Bone. 2019 Jun 1;123:234–45.

44. Bi Y, Gao Y, Ehirchiou D, Cao C, Kikuiri T, Le A, et al. Bisphosphonates cause osteonecrosis of the jaw-like disease in mice. Am J Pathol. 2010;177(1):280–90.

45. Rao NJ, Yu RQ, Wang JY, Helm A, Zheng LW. Effect of Periapical Diseases in Development of MRONJ in Immunocompromised Mouse Model. Biomed Res Int. 2019;2019:1271492.

46. Aghaloo TL, Tetradis S. Osteonecrosis of the Jaw in the Absence of Antiresorptive or Antiangiogenic Exposure: A Series of 6 Cases. J Oral Maxillofac Surg. 2017 Jan 1;75(1):129–42.

47. Qu X, Wang Z, Zhou T, Shan L. Determination of the molecular mechanism by which macrophages and γδ-T cells contribute to ZOL-induced ONJ. Aging. 2020 Oct 25;12(20):20743–52.

48. Yang G, Collins JM, Rafiee R, Singh S, Langaee T, McDonough CW, et al. SIRT1 Gene SNP rs932658 Is Associated With Medication-Related Osteonecrosis of the Jaw. J Bone Miner Res. 2021 Feb 23;36(2):347–56.

49. Kastritis E, Melea P, Bagratuni T, Melakopoulos I, Gavriatopoulou M, Roussou M, et al. Genetic factors related with early onset of osteonecrosis of the jaw in patients with multiple myeloma under zoledronic acid therapy. Leuk Lymphoma. 2017 Oct 3;58(10):2304–9.

50. Lee KH, Kim SH, Kim CH, Min BJ, Kim GJ, Lim Y, et al. Identifying genetic variants underlying medication-induced osteonecrosis of the jaw in cancer and osteoporosis: a case control study. J Transl Med. 2019 Nov 20;17(1):381.

51. De Cicco D, Boschetti CE, Santagata M, Colella G, Staglianò S, Gaggl A, et al. Medication-Related Osteonecrosis of the Jaws: A Comparison of SICMF–SIPMO and AAOMS Guidelines. Diagnostics. 2023 Jun 21;13(13):2137.

52. Schiodt M, Otto S, Fedele S, Bedogni A, Nicolatou-Galitis O, Guggenberger R, et al. Workshop of European task force on medication-related osteonecrosis of the jaw—Current challenges. Oral Dis. 2019 Oct 19;25(7):1815–21.

53. Otto S, Pautke C, Van den Wyngaert T, Niepel D, Schiødt M. Medication-related osteonecrosis of the jaw: Prevention, diagnosis and management in patients with cancer and bone metastases. Cancer Treat Rev. 2018 Sep 1;69:177–87.

54. Aljohani S, Fliefel R, Ihbe J, Kühnisch J, Ehrenfeld M, Otto S. What is the effect of anti-resorptive drugs (ARDs) on the development of medication-related osteonecrosis of the jaw (MRONJ) in osteoporosis patients: A systematic review. Journal of Cranio-Maxillofacial Surgery. 2017 Sep 1;45(9):1493–502.

55. Otto S, Tröltzsch M, Jambrovic V, Panya S, Probst F, Ristow O, et al. Tooth extraction in patients receiving oral or intravenous bisphosphonate administration: A trigger for BRONJ development? J Craniomaxillofac Surg. 2015 Jul 1;43(6):847–54.

56. Nicolatou-Galitis O, Papadopoulou E, Vardas E, Kouri M, Galiti D, Galitis E, et al. Alveolar bone histological necrosis observed prior to extractions in patients, who received bone-targeting agents. Oral Dis. 2020 Jul;26(5):955–66.

57. Moreno-Rabié C, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep 6;24(9):2973–89.

58. Sanna G, Preda L, Bruschini R, Cossu Rocca M, Ferretti S, Adamoli L, et al. Bisphosphonates and jaw osteonecrosis in patients with advanced breast cancer. Ann Oncol. 2006 Oct;17(10):1512–6.

59. Zaman MU, Nakamoto T, Tanimoto K. A retrospective study of digital subtraction technique to detect sclerotic changes in alveolar bone on intraoral radiographs of bisphosphonate-treated patients. Dentomaxillofacial Radiology. 2013 Dec;42(10):20130242.

60. Klingelhöffer C, Klingelhöffer M, Müller S, Ettl T, Wahlmann U. Can dental panoramic radiographic findings serve as indicators for the development of medication-related osteonecrosis of the jaw? Dentomaxillofac Radiol. 2016 May;45(5):20160065.

61. Matsuo A, Hamada H, Kaise H, Chikazu D, Yamada K, Kohno N. Characteristics of the early stages of intravenous bisphosphonate-related osteonecrosis of the jaw in patients with breast cancer. Acta Odontol Scand. 2014 Nov 12;72(8):656–63.

62. Bagan JV, Cibrian RM, Lopez J, Leopoldo-Rodado M, Carbonell E, Bagán L, et al. Sclerosis in bisphosphonate-related osteonecrosis of the jaws and its correlation with the clinical stages: study of 43 cases. British Journal of Oral and Maxillofacial Surgery. 2015 Mar 1;53(3):257–62.

63. Bagan L, Leopoldo-Rodado M, Poveda-Roda R, Murillo-Cortes J, Diaz-Fernández JM, Bagan J. Grade of sclerosis in the contralateral mandibular area in osteonecrosis of the jaws. Int J Oral Maxillofac Surg. 2017 Feb;46(2):167–72.

64. Fedele S, Porter SR, D'Aiuto F, Aljohani S, Vescovi P, Manfredi M, et al. Nonexposed variant of bisphosphonate-associated osteonecrosis of the jaw: a case series. Am J Med. 2010;123(11):1060–4.

65. Hutchinson M, O'Ryan F, Chavez V, Lathon P V., Sanchez G, Hatcher DC, et al. Radiographic findings in bisphosphonate-treated patients with stage 0 disease in the absence of bone exposure. J Oral Maxillofac Surg. 2010 Sep;68(9):2232–40.

66. Olutayo J, Agbaje JO, Jacobs R, Verhaeghe V, Velde F Vande, Vinckier F. Bisphosphonate-Related Osteonecrosis of the Jaw Bone: Radiological Pattern and the Potential Role of CBCT in Early Diagnosis. J Oral Maxillofac Res. 2010 Apr 23;1(2):e3.

67. Aghaloo TL, Dry SM, Mallya S, Tetradis S. Stage 0 osteonecrosis of the jaw in a patient on denosumab. J Oral Maxillofac Surg. 2014 Apr;72(4):702–16.

68. Bedogni A, Fedele S, Bedogni G, Scoletta M, Favia G, Colella G, et al. Staging of osteonecrosis of the jaw requires computed tomography for accurate definition of the extent of bony disease. British Journal of Oral and Maxillofacial Surgery. 2014 Sep;52(7):603–8.

69. Guo Y, Wang D, Wang Y, Peng X, Guo C. Imaging features of medicine-related osteonecrosis of the jaws: comparison between panoramic radiography and computed tomography. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016 Aug 1;122(2):e69–76.

70. Cardoso CL, Barros CA, Curra C, Fernandes LMP da SR, Franzolin S de OB, Júnior JSF, et al. Radiographic Findings in Patients with Medication-Related Osteonecrosis of the Jaw. Int J Dent. 2017;2017:1–6.

71. Soundia A, Hadaya D, Mallya SM, Aghaloo TL, Tetradis S. Radiographic predictors of bone exposure in patients with stage 0 medication-related osteonecrosis of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Dec;126(6):537–44.

72. Şahin O, Odabaşı O, Demiralp KOÖ, Kurşun-Çakmak EŞ, Aliyev T, Sahin O, et al. Comparison of findings of radiographic and fractal dimension analyses on panoramic radiographs of patients with early-stage and advanced-stage medication-related osteonecrosis of the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019 Jul;128(1):78–86.

73. Suei Y. Radiographic findings of bisphosphonate-related osteomyelitis of the jaw: investigation of the diagnostic points by comparison with radiation osteomyelitis, suppurative osteomyelitis, and diffuse sclerosing osteomyelitis. Oral Radiol. 2013 Jul 10;29(2):121–34.

74. Koth VS, Figueiredo MA, Salum FG, Cherubini K. Interrelationship of clinical, radiographic and haematological features in patients under bisphosphonate therapy. Dentomaxillofac Radiol. 2017 Apr;46(4):20160260.

75. Faria KM, Ribeiro ACP, Brandão TB, Silva WG, Lopes MA, Pereira J, et al. Radiographic patterns of multiple myeloma in the jawbones of patients treated with intravenous bisphosphonates. The Journal of the American Dental Association. 2018 May;149(5):382–91.

76. Kubo R, Ariji Y, Taniguchi T, Nozawa M, Katsumata A, Ariji E. Panoramic radiographic features that predict the development of bisphosphonate-related osteonecrosis of the jaw. Oral Radiol. 2018 May 12;34(2):151–60.

77. Rocha GCMA, Jaguar GC, Moreira CR, Neves EG, Fonseca FP, Pedreira EN. Radiographic evaluation of maxillofacial region in oncology patients treated with bisphosphonates. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Nov;114(5):S19–25.

78. Rugani P, Luschin G, Jakse N, Kirnbauer B, Lang U, Acham S. Prevalence of bisphosphonate-associated osteonecrosis of the jaw after intravenous zoledronate infusions in patients with early breast cancer. Clin Oral Investig. 2014 Mar 10;18(2):401–7.

79. Mawardi H, Treister N, Richardson P, Anderson K, Munshi N, Faiella RA, et al. Sinus Tracts—An Early Sign of Bisphosphonate-Associated Osteonecrosis of the Jaws? Journal of Oral and Maxillofacial Surgery. 2009 Mar;67(3):593–601.

80. Diniz-Freitas M, Fernández-Montenegro P, Fernández-Feijoo J, Limeres-Posse J, González-Mosquera A, Vázquez-García E, et al. Mandibular cortical indices on cone-beam computed tomography images in osteoporotic women on treatment with oral bisphosphonates. Gerodontology. 2016 Jun;33(2):155–60.

81. Feitosa ÉF, Vasconcellos MM, Magalhães RJP, Domingos-Vieira AC, Visconti MA, Guedes FR, et al. Bisphophonate alterations of the jaw bones in individuals with multiple myeloma. Dentomaxillofacial Radiology. 2020 Feb;49(2):20190155.

82. Torres SR, Chen CSK, Leroux BG, Lee PP, Hollender LG, Lloid M, et al. Mandibular inferior cortical bone thickness on panoramic radiographs in patients using bisphosphonates. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015 May;119(5):584–92.

83. Barngkgei I, Khattab R. Detecting the effect of bisphosphonates during osteoporosis treatment on jawbones using multidetector computed tomography: The OSTEOSYR project. J Investig Clin Dent. 2018 Aug 25;9(3):e12332.

84. Wazzan T, Kashtwari D, Almaden WF, Gong Y, Chen Y, Moreb J, et al. Radiographic bone loss and the risk of medication-related osteonecrosis of the jaw (MRONJ) in

multiple myeloma patients-A retrospective case control study. Special Care in Dentistry. 2018 Nov;38(6):356–61.

85. Matsuo A, Hamada H, Kaise H, Chikazu D, Yamada K, Kohno N. Characteristics of the early stages of intravenous bisphosphonate-related osteonecrosis of the jaw in patients with breast cancer. Acta Odontol Scand. 2014 Nov 12;72(8):656–63.

86. Ariji Y, Ariji E. Role of magnetic resonance imaging in diagnosis of bisphosphonate-related osteonecrosis of the jaw. Oral Radiol. 2013 Jul 26;29(2):111–20.

87. Hamada H, Matsuo A, Koizumi T, Satomi T, Chikazu D. A simple evaluation method for early detection of bisphosphonate-related osteonecrosis of the mandible using computed tomography. J Craniomaxillofac Surg. 2014 Sep;42(6):924–9.

88. Martelli SJR, Damian MF, Gomes APN, Schinestsck AR, Silva AER, Vasconcelos ACU. Comparison of effects of zoledronic acid and clodronate on the bone structure: imaginological and histomorphometrical study in vivo. Journal of Oral Pathology & Medicine. 2017 Sep;46(8):632–6.

89. Barba-Recreo P, Del Castillo Pardo de Vera JL, García-Arranz M, Yébenes L, Burgueño M. Zoledronic acid - related osteonecrosis of the jaws. Experimental model with dental extractions in rats. J Craniomaxillofac Surg. 2014 Sep;42(6):744–50.

90. Bagan JV, Cibrian RM, Lopez J, Leopoldo-Rodado M, Carbonell E, Bagán L, et al. Sclerosis in bisphosphonate-related osteonecrosis of the jaws and its correlation with the clinical stages: study of 43 cases. British Journal of Oral and Maxillofacial Surgery. 2015 Mar 1;53(3):257–62.

91. Iwata E, Akashi M, Kishimoto M, Kusumoto J, Hasegawa T, Furudoi S, et al. Meaning and Limitation of Cortical Bone Width Measurement with DentaScan in Medication-Related Osteonecrosis of the Jaws. Kobe J Med Sci. 2017 Feb 15;62(5):E114–9.

92. Mawardi H, Treister N, Richardson P, Anderson K, Munshi N, Faiella RA, et al. Sinus Tracts—An Early Sign of Bisphosphonate-Associated Osteonecrosis of the Jaws? Journal of Oral and Maxillofacial Surgery. 2009 Mar;67(3):593–601.

93. Krishnan A, Arslanoglu A, Yildirm N, Silbergleit R, Aygun N. Imaging Findings of Bisphosphonate-Related Osteonecrosis of the Jaw With Emphasis on Early Magnetic Resonance Imaging Findings. J Comput Assist Tomogr. 2009 Mar;33(2):298–304.

94. Fedele S, Porter SR, D'Aiuto F, Aljohani S, Vescovi P, Manfredi M, et al. Nonexposed variant of bisphosphonate-associated osteonecrosis of the jaw: a case series. Am J Med. 2010;123(11):1060–4.

95. Bianchi SD, Scoletta M, Cassione FB, Migliaretti G, Mozzati M. Computerized tomographic findings in bisphosphonate-associated osteonecrosis of the jaw in patients with cancer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Aug;104(2):249–58.

96. Arce K, Assael LA, Weissman JL, Markiewicz MR. Imaging findings in bisphosphonate-related osteonecrosis of jaws. J Oral Maxillofac Surg. 2009 May;67(5):75–84.

97. Imai Y, Hasegawa T, Takeda D, Kusumoto J, Akashi M, Ri S, et al. Evaluation and comparison of CT values in bisphosphonate-related osteonecrosis of the jaw. J Oral Maxillofac Surg Med Pathol. 2016 Jan;28(1):19–25.

98. Barngkgei I, Halboub E, Almashraqi A. Effect of bisphosphonate treatment on the jawbone: an exploratory study using periapical and panoramic radiographic evaluation. Oral Radiol. 2019 May 7;35(2):159–70.

99. O'Ryan FS, Khoury S, Liao W, Han MM, Hui RL, Baer D, et al. Intravenous Bisphosphonate-Related Osteonecrosis of the Jaw: Bone Scintigraphy as an Early Indicator. Journal of Oral and Maxillofacial Surgery. 2009 Jul;67(7):1363–72.

100. Thomas C, Spanidis M, Engel C, Roos FC, Frees S, Neisius A, et al. Bone scintigraphy predicts bisphosphonate-induced osteonecrosis of the jaw (BRONJ) in patients with metastatic castration-resistant prostate cancer (mCRPC). Clin Oral Investig. 2016 May 26;20(4):753–8.

101. Sanchez-Lopez J, Cariati P, Cambil-Martin J, Villegas-Calvo M, Moreno-Martin M. Use of bone scintigraphy in the early diagnosis of bisphosphonate related osteonecrosis of the jaw. Case report and review of the literature. J Clin Exp Dent. 2018 Dec;10(12):e1235–7.

102. Watanabe S, Nakajima K, Mizokami A, Yaegashi H, Noguchi N, Kawashiri S, et al. Bone scan index of the jaw: a new approach for evaluating early-stage anti-resorptive agents-related osteonecrosis. Ann Nucl Med. 2017 Apr 19;31(3):201–10.

103. Lesclous P, Abi Najm S, Carrel JP, Baroukh B, Lombardi T, Willi JP, et al. Bisphosphonate-associated osteonecrosis of the jaw: A key role of inflammation? Bone. 2009 Nov;45(5):843–52.

104. Ferlito S, Puzzo S, Liardo C. Preventive Protocol for Tooth Extractions in Patients Treated With Zoledronate: A Case Series. Journal of Oral and Maxillofacial Surgery. 2011 Jun;69(6):e1–4.

105. Montefusco V, Gay F, Spina F, Miceli R, Maniezzo M, Teresa Ambrosini M, et al. Antibiotic prophylaxis before dental procedures may reduce the incidence of osteonecrosis of the jaw in patients with multiple

myeloma treated with bisphosphonates. Leuk Lymphoma. 2008 Jan;49(11):2156–62.

106. Lodi G, Sardella A, Salis A, Demarosi F, Tarozzi M, Carrassi A. Tooth Extraction in Patients Taking Intravenous Bisphosphonates: A Preventive Protocol and Case Series. Journal of Oral and Maxillofacial Surgery. 2010 Jan 1;68(1):107–10.

107. Del Fabbro M, Gallesio G, Mozzati M. Autologous platelet concentrates for bisphosphonate-related osteonecrosis of the jaw treatment and prevention. A systematic review of the literature. Eur J Cancer. 2015 Jan;51(1):62–74.

108. Fortunato L, Bennardo F, Buffone C, Giudice A. Is the application of platelet concentrates effective in the prevention and treatment of medication-related osteonecrosis of the jaw? A systematic review. Journal of Cranio-Maxillofacial Surgery. 2020 Mar 1;48(3):268–85.

109. Fink HA, MacDonald R, Forte ML, Rosebush CE, Ensrud KE, Schousboe JT, et al. Long-Term Drug Therapy and Drug Discontinuations and Holidays for Osteoporosis Fracture Prevention. Ann Intern Med. 2019 Jul 2;171(1):37.

110. Kim YH, Lee HK, Song S Il, Lee JK. Drug holiday as a prognostic factor of medication-related osteonecrosis of the jaw. J Korean Assoc Oral Maxillofac Surg. 2014;40(5):206–10.

111. Hayashida S, Yanamoto S, Fujita S, Hasegawa T, Komori T, Kojima Y, et al. Drug holiday clinical relevance verification for antiresorptive agents in medication-related osteonecrosis cases of the jaw. J Bone Miner Metab. 2020 Jan 1;38(1):126–34.

112. Hayes KN, Winter EM, Cadarette SM, Burden AM. Duration of Bisphosphonate Drug Holidays in Osteoporosis Patients: A Narrative Review of the Evidence and Considerations for Decision-Making. J Clin Med. 2021 Mar 9;10(5):1140.

113. Ottesen C, Schiodt M, Gotfredsen K. Efficacy of a high-dose antiresorptive drug holiday to reduce the risk of medication-related osteonecrosis of the jaw (MRONJ): A systematic review. Heliyon. 2020 Apr 1;6(4):e03795.

114. Hadaya D, Soundia A, Freymiller E, Grogan T, Elashoff D, Tetradis S, et al. Nonsurgical Management of Medication-Related Osteonecrosis of the Jaws Using Local Wound Care. Journal of Oral and Maxillofacial Surgery. 2018 Nov 1;76(11):2332–9.

115. Lobekk OK, Dijkstra W, Pedersen TØ. Surgical vs conservative treatment of medication-related osteonecrosis of the jaw: A complex systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2021 Dec 1;132(6):671–9.

116. Bedogni A, Saia G, Bettini G, Tronchet A, Totola A, Bedogni G, et al. Long-term outcomes of surgical resection of the jaws in cancer patients with bisphosphonate-related osteonecrosis. Oral Oncol. 2011 May;47(5):420–4.

117. Rupel K, Ottaviani G, Gobbo M, Contardo L, Tirelli G, Vescovi P, et al. A systematic review of therapeutical approaches in bisphosphonates-related osteonecrosis of the jaw (BRONJ). Oral Oncol. 2014 Nov;50(11):1049–57.

118. Fliefel R, Tröltzsch M, Kühnisch J, Ehrenfeld M, Otto S. Treatment strategies and outcomes of bisphosphonate-related osteonecrosis of the jaw (BRONJ) with characterization of patients: a systematic review. Int J Oral Maxillofac Surg. 2015 May;44(5):568–85.

119. Shin WJ, Kim CH. Prognostic factors for outcome of surgical treatment in medication-related osteonecrosis of the jaw. J Korean Assoc Oral Maxillofac Surg. 2018 Aug 31;44(4):174.

120. Kojima Y, Kawaoka Y, Sawada S, Hayashida S, Okuyama K, Yutori H, et al. Clinical significance of periosteal reaction as a predictive factor for treatment outcome of medication-related osteonecrosis of the jaw. J Bone Miner Metab. 2019 Sep 4;37(5):913–9.

121. Kawaoka Y, Kojima Y, Sawada S, Funahara M, Hayashida S, Yutori H, et al. Periosteal reaction as a risk factor for poor outcomes after surgical treatment for medication-related osteonecrosis of the jaw: A retrospective analysis of 205 surgeries. J Oral Maxillofac Surg Med Pathol. 2020 Jan 1;32(1):8–13.

122. Kim DW, Kim H, Nam W, Kim HJ, Cha IH. Machine learning to predict the occurrence of bisphosphonate-related osteonecrosis of the jaw associated with dental extraction: A preliminary report. Bone. 2018 Nov;116:207–14.

123. Zhu W, Zhang X, Fang S, Wang B, Zhu C. Deep Learning Improves Osteonecrosis Prediction of Femoral Head After Internal Fixation Using Hybrid Patient and Radiograph Variables. Front Med (Lausanne). 2020 Oct 7;7.

124. Chee CG, Kim Y, Kang Y, Lee KJ, Chae HD, Cho J, et al. Performance of a Deep Learning Algorithm in Detecting Osteonecrosis of the Femoral Head on Digital Radiography: A Comparison With Assessments by Radiologists. American Journal of Roentgenology. 2019 Jul;213(1):155–62.

PART 1: RISK FACTORS FOR MRONJ IN PATIENTS UNDERGOING TOOTH EXTRACTIONS

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Oncologic Patients

CHAPTER 1: Panoramic Radiography

Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, Jacobs R. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280. doi: 10.1038/s41598-022-15254-y.

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CHAPTER 2: Cone Beam Computed Tomography

Moreno Rabie C, Cavalcante Fontenele R, Oliveira Santos N, Nogueira Reis F, Van den Wyngaert T, Jacobs R. Three-dimensional clinical assessment for MRONJ risk in oncologic patients following tooth extractions. Dentomaxillofac Radiol. 2023 Oct 24:20230238. doi: 10.1259/dmfr.20230238.

Osteoporotic Patients

CHAPTER 3: Panoramic Radiography

Moreno-Rabié C, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, Jacobs R. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8. doi: 10.1111/odi.14496.

CHAPTER 4: Cone Beam Computed Tomography

Moreno-Rabié C, Fontenele RC, Oliveira Santos N, Nogueira-Reis F, Van den Wyngaert T, Jacobs R. Key Insights into Antiresorptive Drug Use and Osteonecrosis in Osteoporotic Patients Undergoing Tooth Extractions: a clinical and CBCT assessment. Under revision.

CHAPTER 1: TWO-DIMENSIONAL ASSESSMENT IN ONCOLOGIC PATIENTS

Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction

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Abstract

Tooth extractions are a risk factor for the development of osteonecrosis of the jaw following treatment with antiresorptive drugs (ARDs), but not all extraction sites develop this pathology. Therefore, we aimed to identify local radiographic predictors of Medication-Related Osteonecrosis of the Jaw (MRONJ) in panoramic images of oncologic patients undergoing tooth extraction. Based on a retrospective longitudinal cohort study design, patients were included if undergoing one or more tooth extractions, with at least one administration of ARDs, and presence of pre- and post-operative panoramic radiographs. After data collection, blinded and independent observations were performed. Eleven distinct imaging-related parameters were assessed preoperatively and five postoperatively, at each extraction site. A case-control and subgroup analysis assessing MRONJ development was performed. Significance level is set to 0.05 (5%). A total of 77 oncologic patients were selected, undergoing 218 tooth extractions, from which 63 teeth (29%) in 39 patients (51%) developed MRONJ. Results showed that patients developed significantly more MRONJ with longer ARD treatment (p=0.057), teeth with absent and incomplete endodontic fillings with caries, widened periodontal ligament space and/or periapical lesions (p=0.005), and sclerotic and heterogenous bone patterns (p=0.005). In conclusion, tooth extraction sites presenting with infections and bone sclerosis are at higher risk to develop MRONJ.

Keywords: Diphosphonates, Denosumab, Antiresorptive Drugs, Tooth Extraction, Osteonecrosis, Panoramic Radiography

Introduction

Antiresorptive drugs (ARDs) are widely used as treatment of patients with osteoporosis and cancer, among other diseases [1]. Particularly in an oncologic setting, these drugs are used to effectively prevent skeletal morbidity in patients with metastatic bone disease or multiple myeloma [2], which may involve pain, nerve compression, and pathologic fractures [3]. Despite the benefits of ARDs, a common adverse effect is Medication-Related Osteonecrosis of the Jaws (MRONJ) [1,4].

MRONJ corresponds to exposed bone or bone that can be probed through an intraoral or extraoral fistula(e) in the maxillofacial region that has persisted for more than eight weeks, in patients treated with antiresorptive drugs and who have not received radiation therapy to the head and neck region nor have obvious metastatic disease in the jaws [5]. Besides the stages involving bone exposure, the American Association of Maxillofacial Surgeons (AAOMS) proposed two additional groups, namely "at risk" and stage 0. Whereas both refer to the absence of bone exposure, the first includes asymptomatic patients receiving ARD and the second one involves patients presenting with non-specific symptoms or clinical and radiographic findings [6].

Within the systemic risk factors associated to MRONJ development are the type and dose of these medications, but most importantly their cumulative dosage, in particular the higher doses administered for longer periods [7,8]. Some examples of bisphosphonates are zoledronic acid, alendronate, ibandronate, pamidronate, and risedronate. Other implicated drugs include monoclonal antibodies such as denosumab [9], which, like bisphosphonates, alter the bone resorption-apposition balance to prevent skeletal-related events.

Tooth extractions are often reported as an important triggering factor for this pathology. Though, experts suggest that underlying infections at the extraction site, such as periodontitis or periapical lesions, could play an even greater role in the onset of MRONJ [7,10], especially when analyzing patients who had multiple tooth extractions and in whom only some of these sites developed osteonecrosis [11], thus having the same systemic condition, but different local factors. Perhaps these sites could be masking an

unexposed form of the pathology that would only be revealed at the time of extraction[7], presenting in their radiographic appearance, sclerosis, thickening of the lamina dura, and persistence of the extraction socket [12]. In these cases, a radiographic evaluation is of great value to identify hidden lesions or abnormal bone patterns and recognize the high-risk sites for MRONJ.

Bearing the previous evidence in mind, the main objective of this study was to identify local radiographic characteristics in panoramic images that act as a risk factor for development of MRONJ in oncologic patients currently taking or with a history of antiresorptive drugs undergoing tooth extractions.

Material and Methods

Study design and settings

Prior to the start of this study, ethical approval was granted by the ethical committee of UZ/KU Leuven (S63934) and waived the need for informed consent. This study corresponds to a retrospective longitudinal cohort study, and to report the present information, the STROBE guidelines were followed [13]. In addition, ethical standards from the Institutional Review Board and the Helsinki Declaration were obeyed.

Participant selection

A retrospective search was carried out in the database of University Hospitals Leuven, where medical files of patients in treatment with ARD, who visited the Oral and Maxillofacial Surgery department between January 1st, 2010, and December 30th, 2019, were assessed. The inclusion criteria included, (1) patients in the category "at risk" according to the AAOMS [6], (2) at least one administration of ARD in oncologic doses, (3) had undergone one or more tooth extractions, (4) had a pre- and post-operative panoramic image, and (5) documented follow-up until mucosal healing within eight weeks to refute or exposed bone for at least eight weeks to confirm the clinical development of MRONJ. Exclusion criteria were, (1) history of radiation in the head and the neck region, (2) prior MRONJ diagnosis, (3) insufficient image quality (i.e., artefacts)

and/or objects in the extraction site (i.e. implants), (4) pre-operative radiographs acquired earlier than one year prior to tooth extraction, and (5) concomitant maxillofacial pathologies.

After including the study group, a control group of patients who were age-, gender-, tooth-, and sextant of extraction matched was selected from the same database. Inclusion criteria for the control group were, no intake of ARD and having panoramic radiographs from before and after a tooth extraction. Further exclusion criteria were as mentioned for the study patients.

Data selection

Along with the panoramic images, clinical information was collected from the patient's files. The following variables were included: age, gender, tobacco and alcohol use, oncologic diagnosis, previous chemotherapy and/or radiotherapy to other body regions than to the head and neck, antiresorptive drug, dosage, treatment duration, corticosteroids intake, date of the tooth extraction(s), number, site and extracted tooth, date of pre- and post-operative panoramic radiograph, development of MRONJ, date of diagnosis, staging according to the AAOMS[6], site of development, presence of drug holiday at least 60 days before the extraction, use of leukocyte- and platelet-rich fibrin (L-PRF), prophylactic antibiotics, and antiseptic mouthwash.

Radiographic assessment

Panoramic radiographs were acquired using VistaPano S or S Ceph (Dürr Dental SE, Bietigheim-Bissingen, Germany) at 73 kVp, 12 mA, and an exposure time of 7 seconds. Images of eligible study and control participants were anonymized, exported in DICOM format, and assigned an arbitrary participant number. All images were then transformed to TIFF files, and observations were done using Image J program version 1.53j (Wayne Rasband, https://imagej.nih.gov/ij/). Blinded and independent observations were performed by two dentomaxillofacial radiologists and a general dentist. Prior to the radiographic assessment, a calibration session was held to assess 20 panoramic images from ten before and after tooth extraction cases external to this study, to achieve baseline consensus in the diagnosis. The observations took place in a

room with dim light, using a high-resolution display (MD Barco MDRC-2221; Barco, Kortrijk, Belgium) at approximately 60cm. After evaluation, the result of the observation was calculated using the mode. For example, if two observers judged a parameter as present and one as absent, then it was considered as present. If the mode could not be applied because three different interpretations were assigned, the case was discussed individually until agreement was reached. One month after the first assessment, a second reading session was carried out with 10% of the sample to evaluate the intraobserver agreement.

The following parameters were assessed in the panoramic radiographs before the extraction at a tooth level, based on the description of Gaêta-Araujo et al.[11]:

1. Horizontal bone loss: absent/initial bone loss, if bone resorption was up to 1/3 cervical of the root, or moderate/severe bone loss, if bone resorption was more than 1/3 cervical of the root.
2. Angular bone loss: absent or present.
3. Furcation involvement: absent or present.
4. Periodontal ligament space: normal or widened.
5. Lamina dura: normal or thickened.
6. Root remnant: absent or present.
7. Periapical lesion: absent or present.
8. Endodontic treatment: absent, adequate endodontic filling in length or width, or inadequate endodontic filling (over and underfilling).
9. Prosthodontics: absence or presence of crowns, bridges, and/or fillings.
10. Caries: absent, dentin caries, or caries in contact with or overlapping the pulp cavity.
11. Bone pattern surrounding the tooth: normal, sclerotic (increased radio-opacity), radiolucent, or heterogeneous (mixed radiolucent and radiopaque).

Furthermore, parameters appraised in the extraction site on the respective post-operative panoramic image included:

1. Bone pattern: as described before.

2. Alveolar socket: not visible or visible.
3. Lamina dura: not visible or visible.
4. Sequestrum formation: absent or present.
5. Crater-like defect: with reference to an accentuated resorption in the form of a prominent concavity, absent or present.

Statistical analysis

The statistical analysis was done using RStudio Software version 4.0.4 (RStudio, Boston, MA US). A p-value ≤0.05 was considered statistically significant. Fleiss' Kappa test was used to calculate interobserver agreement and Cohen's Kappa test for intraobserver agreement. Agreement was considered fair when the test result was >0.21 - 0.40, moderate when >0.41 - 0.60, substantial when >0.61 - 0.80, and almost perfect when >0.81 - 0.99 [14].

Univariate analysis

The significance of differences in characteristics between control and study patients was tested before the start of the analysis for gender, age, and extracted tooth, using Chi-Square and Wilcoxon rank-sum test.

Observations and clinical data documented for each extracted tooth were tested for independence using the Chi-square/Fisher's exact test and the Wilcoxon rank-sum test for ordinal variables. Comparisons were made between the control and antiresorptive-treated group. In addition, the latter was divided into sites that developed MRONJ (MRONJ+) after tooth extraction and sites that did not (MRONJ-). The null hypothesis was that the assessed parameters are independent in the control and study group, and in the MRONJ+ and MRONJ- extraction sites. Lastly, the McNemar-Bowker test was used to compare pre- and post-operative appearance of bone patterns.

Considering that some patients had multiple tooth extractions, with some sites MRONJ+ and other sites MRONJ-, further analysis was carried out using a generalized linear mixed model, which tested the independency of the assessed variables while respecting the grouped character of the data.

Multivariate analysis of risk factors

A stepwise model selection was performed, through a generalized linear model for binary data using the logit link and with patient as random factor, to identify the combination of variables that had the best relationship with MRONJ development among the study group. The assessed variables were horizontal bone loss, angular bone defect, furcation involvement, periodontal ligament, lamina dura, root remnant, periapical lesion, endodontic treatment, presence of composite or crown, presence of caries, pre-operative bone pattern, type of extracted tooth, sextant, duration of ARD treatment, and presence of drug holiday. For those variables that were part of the selected model, a pairwise comparison between the group's variables was performed and corrected for simultaneous hypothesis testing according to Tukey.

Results

In this ten-year observational study, 1468 patients visited the Oral and Maxillofacial Surgery department and were currently or in the past treated with ARDs. From these patients, 927 had either prior diagnosis of MRONJ or only one panoramic image available, 219 patients did not have tooth extraction, 130 received ARD treatment for other reasons than oncology, 89 did not have either a pre- or post-operative panoramic image, 22 had images with bad quality, and 4 had panoramic images older than one year before their tooth extraction.

From the total, 77 patients, who underwent 218 tooth extractions complied with the inclusion criteria, and 88 patients with 238 tooth extractions were selected as controls. The study and control group showed no significant differences regarding age (W=3392, p=0.992), gender (X^2=0.0185, p=0.892), tooth (W=26859, p=0.514), sextant (W=25718, p=0.872), and number of extractions (W=3293, p=0.739). Demographic data can be found in Table 1, at a patient level, and in Table 2, at a tooth level.

All included patients had at least one administration of zoledronic acid 4mg or denosumab 120mg. Eleven patients had treatment with a combination of bisphosphonate and denosumab. The mean duration of the ARD treatment was 20.8

months (range 1 – 83). The mean time between pre-operative panoramic radiograph and tooth extraction was of 1.6 months (range 0 – 12) for the oncologic group and 1.1 months (range 0 – 9.6) for the control group. While the mean time interval between tooth extraction and post-operative panoramic was 9.6 months (range 0 – 61.2) for the study and 7.3 months (range 0 – 70.8) for the control group.

From the 77 oncologic patients, 39 developed MRONJ in 63 tooth extraction sites (50.6% of the study group, 95% CI 0.47 – 0.70; 28.9% of the extracted teeth, 95% CI 0.23 – 0.35). From the affected patients, 21 had from one to three tooth extractions and developed MRONJ in all sites, while 18 patients had in average 5.6 tooth extractions (range 2 – 24) and presented both MRONJ+ and MRONJ- sites. This last group had in average two MRONJ+ sites (range 1 – 8).

Among the antiresorptive-treated patients, MRONJ+ patients had a longer treatment duration with a mean of 24.9 months, than MRONJ- patients, who had a mean of 16.7 months. Despite this difference, the test result was borderline significant (W=555, p=0.057). Furthermore, no significant differences were found in the distribution of age (W=785, p=0.659), gender (X^2=0.659, p=0.417), history of chemotherapy and/or radiotherapy (p=0.679), type and number of ARDs (X^2=3.149, p=0.207; W=684, p=0.376), duration of corticosteroid use (X^2=0.624, p=0.429), alcohol consumption (W=500, p=0.394), and tobacco abuse (p=0.115), between those who did and did not develop the pathology.

Regarding the extracted teeth, no significant differences were observed in the type of extracted tooth (W=4695, p=0.633), region (W=4866, p=0.969), presence of drug holiday (X^2=1.538, p=0.215), use of L-PRF (X^2=1.762, p=0.184), antiseptic mouthwash (p=0.634), nor prophylactic antibiotics (p=0.239), between the MRONJ+ and MRONJ- sites.

The overall Kappa for interobserver agreement was moderate (0.66), ranging between a fair (0.34) and an almost perfect agreement (0.96) in the periodontal ligament assessment and the presence of endodontic treatment, respectively. The mean Kappa value for the intraobserver agreement was substantial (0.79) for the

overall assessment, ranging between a moderate agreement (0.45) in the assessment of lamina dura, and an almost perfect agreement (0.98) in the presence of endodontic treatment.

Pre- and post-operative parameters: control vs. study group.

Thickening of the lamina dura was significantly more present in the antiresorptive-treated patients (10%) than in the control group (3%, p=0.003). Significant lower caries and specifically pulpal caries prevalence was also seen in this group (34%) in comparison to the control group (45%, p=0.006).

When looking at the bone pattern surrounding the extraction site postoperatively, the oncologic group had a higher prevalence of a sclerotic (33%) and a heterogeneous pattern (6%, p<0.001), than the control group (20% and 0%, respectively). The visibility of the alveolar socket after the extraction (p<0.001), and when looking at those sites with images taken at least one year postoperatively (p<0.001), were also significantly more prevalent among the patients under ARD. The same was observed for the persistence of the lamina dura (p<0.001). Finally, the visibility of sequester formation was present only in the ARD group (3%, p=0.012).

Pre- and post-operative parameters: MRONJ+ vs. MRONJ-.

Teeth that were not treated endodontically developed MRONJ more frequently (35%), than teeth with endodontic treatments (17%). However, when present, an endodontic filling material insufficient in length and/or width increased the chance of the onset of MRONJ (p=0.005). Additionally, 82% of the extracted teeth had either caries, widened periodontal ligament space and/or periapical lesions. From these decayed teeth, 37% without endodontic treatment, 13% with adequate fillings, and 31% with inadequate fillings, developed MRONJ. There was no development of MRONJ in teeth without signs of endodontic infection and presence of endodontic treatment, whether adequate or inadequate. Lastly, a pre-operative bone pattern different than normal increased the likelihood of MRONJ (p=0.005), as 46% of the sclerotic and 67% of the heterogeneous sites developed the pathology. Detailed results are displayed in Table 3 and an illustrative example in Figure 1.

Table 1. Descriptive data from study group and control patients.

Characteristics		Oncologic group				Control
Number of patients, n		77				88
Development of osteonecrosis, n		**MRONJ +** 39		**MRONJ -** 38		NA
Age a tooth extraction (mean ± SD)		68.4 ± 11.3		67.2 ± 10.9		67.9 ± 11.2
Age (years)	30-45	1	50%	1	50%	3
	46-60	11	52%	10	48%	22
	61-75	12	40%	18	60%	43
	76-92	15	63%	9	38%	20
Sex, n	Female	19	45%	23	55%	50
	Male	20	57%	15	43%	38
Underlying disease, n	Breast cancer	17	49%	18	51%	NA
	Prostate cancer	9	60%	6	40%	NA
	Multiple Myeloma	6	33%	12	67%	NA
	Lung cancer	3	60%	2	40%	NA
	Gastrointestinal Cancer	1	100%	0	0%	NA
	Renal Cancer	3	100%	0	0%	NA
Chemo- and radiotherapy, n	None	4	50%	4	50%	NA
	Chemotherapy	6	67%	3	33%	NA
	Radiotherapy	7	58%	5	42%	NA
	Both	22	46%	26	54%	NA
Antiresorptive drug, n	Bisphosphonate	12	41%	17	59%	NA
	Denosumab	19	51%	18	49%	NA
	Both	8	73%	3	27%	NA
Number of ARDs, n	1	31	48%	33	52%	NA
	2	7	58%	5	42%	NA
	3	1	100%	0	0%	NA
Time on ARDs (months), n (%)	≤12	12	41%	17	59%	NA
	>12 - ≤24	10	40%	15	60%	NA
	>24 - ≤36	11	79%	3	21%	NA
	>36 - ≤48	1	33%	2	67%	NA
	>48 - ≤60	4	80%	1	20%	NA
	>60 - ≤120	1	100%	0	0%	NA
Corticoid use, n	No	25	56%	20	44%	78
	Yes	14	44%	18	56%	10
Alcohol consumption, n	No consumption	14	54%	12	46%	30
	1-2 units daily	15	45%	18	55%	38
	>2 units daily	0	0%	1	100%	6
	Unknown	10	59%	7	41%	14
Tobacco use, n	Previous user	13	68%	6	32%	28
	Active user	6	67%	3	33%	5
	Non-user	16	41%	23	59%	52
	Unknown	4	40%	6	60%	3

NA: not applicable.

Table 2. Descriptive data of the extracted teeth in the study and control group.

Characteristics		Oncologic group				Control
Number of extracted teeth, n		218				238
Development of osteonecrosis, n (%)		**MRONJ +**		**MRONJ -**		NA
		63	29%	155	71%	
Type of teeth, n (%)	Incisors and canines	18	30%	43	70%	75
	Premolars	14	24%	45	76%	59
	Molars	31	32%	67	68%	104
Region, n (%)	Anterior maxilla	8	27%	22	73%	34
	Posterior maxilla	22	26%	63	74%	87
	Anterior mandible	10	32%	21	68%	41
	Posterior mandible	23	32%	49	68%	76
Underlying dental disease, n (%) *	Nonapparent	3	12%	21	88%	23
	Periodontal disease	3	20%	12	80%	31
	Endodontic pathology	17	31%	38	69%	62
	Combined lesion	40	32%	84	68%	122
Drug holiday >60 days, n (%)	No	20	36%	35	64%	NA
	Yes	43	26%	120	74%	
Antibiotic prophylaxis, n (%)	No	4	50%	4	50%	225
	Yes	59	29%	146	71%	13
Antiseptic mouthwash, n (%)	No	2	40%	3	60%	9
	Yes	61	29%	147	71%	229
Use of L-PRF, n (%)	No	18	38%	29	62%	228
	Yes	45	27%	122	73%	10
MRONJ worse stage, n (%)	Stage 1	32	51%	NA		NA
	Stage 2	28	44%	NA		NA
	Stage 3	3	5%	NA		NA

Further description of the teeth from the study group that did (MRONJ+) and did not (MRONJ-) develop osteonecrosis is given. NA: not applicable. (*): based on the radiographic characteristics, teeth were classified into: periodontally diseased, which had horizontal bone loss, an angular bone defect, or furcation involvement; with endodontic pathology, which presented pulpal caries, widened periodontal ligament space, prosthodontic treatment and concomitant caries, or periapical lesion; and with endodontic-periodontal combined lesions, when presenting characteristics from both groups.

Table 3. Shows the distribution of extraction sites according to pre- and post-operative radiographic characteristics observed in the MRONJ+ and MRONJ- subgroups and in the control group.

Observed parameters		Oncologic group			Control group	
		MRONJ +	MRONJ -	p-value	n	p-value
Pre-operative assessment						
Horizontal bone loss	Absent/initial	23 (26%)	65 (74%)	0.557	99	0.864
	Moderate/severe	40 (31%)	90 (69%)		139	
Angular bone loss	Absent	58 (30%)	136 (70%)	0.493	224	0.070
	Present	5 (21%)	19 (79%)		14	
Furcation involvement	Absent	46 (28%)	117 (72%)	0.835	179	1.000
	Present	17 (31%)	38 (69%)		59	
Periodontal ligament space	Normal	19 (21%)	70 (79%)	0.059	109	0.329
	Widened	44 (34%)	85 (66%)		129	
Lamina dura	Normal	56 (29%)	140 (71%)	0.944	231	*0.003*
	Thickened	7 (32%)	15 (68%)		7	
Root remnant	No	50 (29%)	123 (71%)	1.000	198	0.352
	Yes	13 (29%)	32 (71%)		40	
Periapical lesion	Absent	41 (29%)	102 (71%)	1.000	153	0.846
	Present	22 (29%)	53 (71%)		85	
Endodontic treatment	Absent	51 (35%)	95 (65%)	*0.005*	145	0.539
	Adequate filling	4 (9%)	39 (91%)		54	
	Inadequate filling	8 (28%)	21 (72%)		39	
Prosthodontic treatment	Absent	30 (35%)	56 (65%)	0.155	107	0.274
	Present	33 (25%)	99 (75%)		131	
Caries depth	Absent	32 (26%)	91 (74%)	0.253*	104	*0.006**
	Reaches dentine	6 (29%)	15 (71%)		26	
	Reaches pulp	25 (34%)	49 (66%)		108	
Bone pattern preoperative	Normal	40 (24%)	128 (76%)	*0.005*	193	0.273
	Sclerotic	21 (46%)	25 (54%)		44	
	Radiolucent	0 (0%)	1 (100%)		1	
	Heterogenous	2 (67%)	1 (33%)		0	
Post-operative assessment						
Bone pattern postoperative	Normal	27 (21%)	104 (79%)	*<0.001*	187	*<0.001*
	Sclerotic	24 (33%)	48 (67%)		47	
	Radiolucent	2 (67%)	1 (33%)		3	
	Heterogenous	10 (83%)	2 (17%)		1	
Alveolar socket	Absent	24 (29%)	60 (71%)	1.000	168	*<0.001*
	Visible	39 (29%)	95 (71%)		70	
Lamina dura	Absent	31 (29%)	76 (71%)	1.000	183	*<0.001*
	Visible	32 (29%)	79 (71%)		55	
Sequestrum formation	Absent	57 (27%)	155 (73%)	*<0.001*	238	*0.012*
	Visible	6 (100%)	0 (0%)		0	
Crater-like defect	Absent	50 (25%)	147 (75%)	*0.001*	223	0.253
	Visible	13 (62%)	8 (38%)		15	

The p-value under "oncologic group" describes the results obtained from the comparison of MRONJ+ and MRONJ- sites, while the p-value under "control group" describe the results from the comparison of study and control sites. Italic text is marked in those assessments where differences are statistically significant. Results obtained using the exact chi-square/Fisher's exact test, except for (*), which used the Wilcoxon rank sum test.

A post-operative heterogeneous (83%) and radiolucent bone pattern (67%) was witnessed significantly more at MRONJ+ than MRONJ- sites ($p<0.001$), while 67% of the sclerotic and 79% of the normal bone patterns were seen in MRONJ- sites. Furthermore, sequester formation was exclusively seen in locations that developed MRONJ. These differences were thus significant ($p<0.001$). Moreover, crater-like defects were significantly more detected, and when present more extensive, in MRONJ+ sites (62%) in comparison to MRONJ- (<0.001).

Results of the paired test regarding the bone pattern before and after tooth extraction show a significant difference in the appearance among the oncologic patients ($X^2=36.77$, df=6, $p<0.001$) but not in the control group ($X^2=3.27$, df=6, $p=0.773$). In the study group, it was seen that 38 sites that had initially a normal trabecular pattern showed a sclerotic one after surgery, and four a heterogenous pattern. Moreover, eight sites with a pre-operative sclerotic pattern showed after tooth extraction a heterogeneous one.

Lastly, teeth were classified based on their radiographic characteristics into, periodontally diseased, with endodontic pathology, with endodontic-periodontal combined lesions, or no apparent disease, as described in Table 2. However, no significant differences were found between the control and study group ($p=0.161$) nor the subgroups MRONJ+ and MRONJ- ($p=0.219$).

Within patient analysis: MRONJ+ and MRONJ-.

When looking at ARD-patients that had multiple extractions and sites that did and did not develop MRONJ, the assessment of endodontic treatment status ($p=0.033$) and pre-operative surrounding bone pattern ($p<0.001$) showed significant results. More specifically, and as mentioned before, a sclerotic bone pattern and absent and inadequate endodontic treatments showed a higher predisposition for the pathology.

Multivariate analysis of risk factors

Additionally, the logistic regression model showed significant results in the pre-operative assessment for the variables, endodontic treatment ($p=0.019$), periapical lesion ($p=0.002$), surrounding bone pattern ($p=0.013$), and angular bone defect

(p=0.048). However, when performing pairwise comparisons and correcting for multiple testing, only the absence of periapical lesion (OR=1.78, 95% CI 1.459 – 2.175, p=0.002) and angular bone defect (OR=1.72, 95% CI 1.278 – 2.306, p=0.048), and the presence of a sclerotic bone pattern (OR=3.45, 95% CI 1.094 – 10.309, p=0.027), showed significant results (Figure 2).

Discussion

Radiographic bone changes in the maxilla and mandible, after the intake of antiresorptive drugs, have been widely reported in the literature even before clinical exposure of bone in the oral cavity. Although, it is unclear whether most of these findings are solely related to the antiresorptive therapy, indicate a potential progress to osteonecrosis, or represent different stages of the necrotic process [15]. In the present study, we aimed to confirm the associations we previously identified in a smaller and different patient cohort, and validate those pre-operative radiologic local risk factors to the development of MRONJ in patients treated with oncologic-related doses undergoing tooth extraction[11]. To our knowledge, this is the first large series to include a pre- and post-operative radiologic assessment of the extraction site.

Prevention in patients who belong to the category “at risk” according the AAOMS [6] is key to reduce the incidence of this pathology [16–18]. Yet, these strategies are not always timely and dental extractions may become inevitable. Given the need for extractions, it is worth questioning how many of these teeth with pain, associated radiographic changes and absence of bone exposure are classified as "at risk", when in fact they belong to "stage 0". This refers above all to sites that showed clinical post-operative bone exposure and suggests that the pain, initially considered of dental origin, might be due to an undiagnosed osteonecrosis. Validating this point, Nicolatou-Galitis et al. found in such patients the presence of necrotic bone in biopsies obtained at tooth extraction [10].

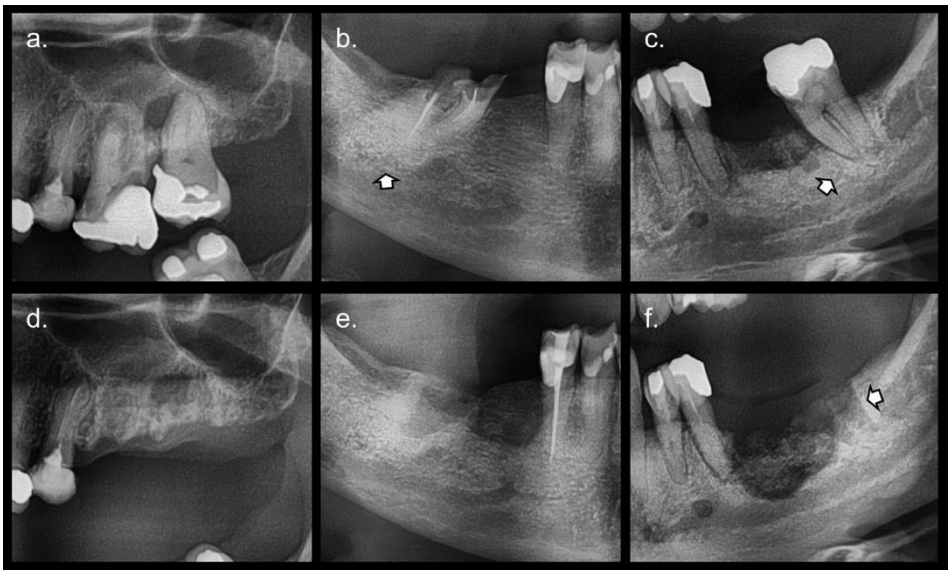

Figure 1. Cropped panoramic images showing pre-operative sites (a, b, c) and their respective post-operative evolution (d, e, f). Pre-operative images show presence of risk factors, teeth without endodontic treatment and with periapical lesion (a, c), incomplete endodontic filling in length and presence of caries (b), and sclerotic bone pattern (b, c; white arrow). All extraction sites developed osteonecrosis, showing a heterogenous bone pattern (d, f), a sclerotic bone pattern (e), visible extraction socket (e, f), persistence of the lamina dura (f; white arrow), and sequester formation (f).

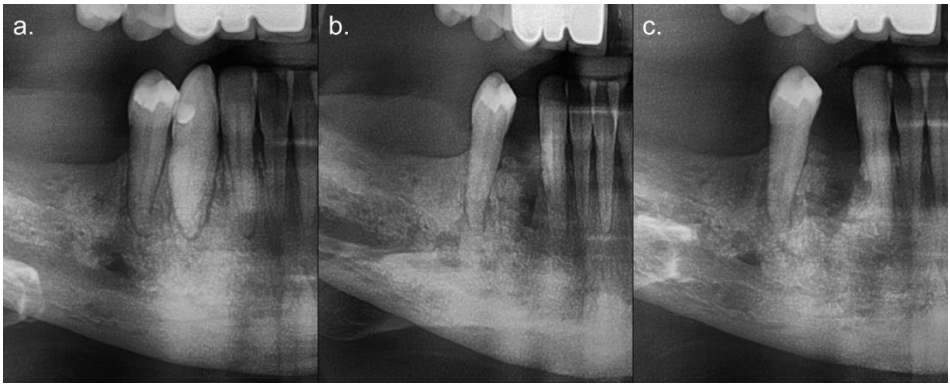

Figure 2. Cropped panoramic radiographs of a 67-year-old female in treatment with denosumab, showing tooth 43 two months before extraction (a), and ten (b) and eighteen months after (c). This site had absence of periapical lesion and angular bone defect, but presence of sclerosis and widening of the periodontal ligament space. Osteonecrosis was diagnosed eight weeks after surgery, as the site did not heal. Persistence of the alveolar socket and sclerotic pattern can be seen postoperatively.

In this light, the use of radiographs for diagnostics, treatment, and the identification of risk factors at the extraction site is of utmost importance to plan and understand possible complications. In that sense, panoramic radiographs are widely available in the dental practice environment [19,20], thus a good starting point for diagnosis and risk assessment. However, for a thorough diagnosis, a three-dimensional method such as computed tomography (CT) and Cone Beam CT is recommended [21]. These tools allow the assessment of the extension of the pathology [22,23] and to identify incipient lesions like those seen in patients "at risk" or in "stage 0" [15,21,23]. Consequently, new diagnostic staging systems have been proposed using a complement of clinical and radiographic signs [24,25].

Comparable to what has been described in other studies, patients under ARD, in contrast to the control group, showed significantly more thickening of the lamina dura [11,15,19]. Thickening of the lamina dura and the mandibular cortex, enhancement of the mandibular canal, and trabecular sclerosis are all examples of the sclerosing process that the bone undergoes secondary to antiresorptive treatment. These sclerotic forms have been reported by numerous authors [19,20,26–29]. In fact, Gaêta-Araujo et al. argued that such sclerotic changes in bone might have a better relationship with the antiresorptive therapy per se than a predisposing factor to MRONJ [11].

In the present sample, sites which initially showed sclerosis seem to have a larger chance of developing osteonecrosis than those with a normal pattern. Besides, bone remodeling led in eight initially sclerotic sites to a postoperative heterogeneous pattern, which in most cases (7/8) were associated to MRONJ, while bone remodeling from a normal to a heterogeneous pattern was less common. Albeit every extracted tooth in sclerotic sites had either periodontal, endodontic, or endodontic-periodontal disease. As discussed by other authors, these sclerotic changes can also be a bony response to local dental infection [11,30,31]. Thus, it is conceivable that sites with sclerotic changes represent a risk factor for MRONJ [32], but this higher risk could be also due to underlying dental disease, or a combination of both.

Although the results border on significance, oncologic patients with longer ARD treatments and undergoing tooth extractions have a higher chance of developing

osteonecrosis, since MRONJ+ patients had a mean treatment of 24 months and MRONJ- of 16 months. These findings align to what is reported in the literature [7–9]. Some authors debate that besides the length of the treatment, the type of drug, whether bisphosphonates or denosumab, differed in their radiographic appearance [22]. However, these findings are described in exposed variants of MRONJ and these differences were not studied in this cohort due to the restricted sample size.

Perhaps the most important findings of the present study are those related to the MRONJ+ group, being that the pathology presented itself significantly more in teeth with absent and incomplete endodontic fillings, and a sclerotic and heterogenous alveolar bone pattern. Furthermore, the most likely combination of radiological characteristics leading to MRONJ was a preoperative sclerotic bone pattern in absence of periapical lesion and angular bone defect. Nevertheless, teeth with these features were not exempt of dental disease, as they had periodontal ligament space widening (79%), radiographic signs of periodontitis (62%), caries (50%), or were root remnants (29%). Therefore, the results of the multivariate analysis may find an explanation in the sample distribution, rather than in the absence of chronic infection and its relationship with MRONJ.

Endodontic treatments have been strongly advocated in patients receiving ARD since the first appearances of the pathology, in order to promote conservative treatments and thus avoid bone trauma [33]. Yet, the present results reveal that the quality of endodontic treatment is an equally important aspect to consider, especially in teeth presenting with signs of infection such as caries, periapical lesions and widening of the periodontal ligament. We saw that decayed teeth with inadequate endodontic treatments exhibited more osteonecrosis than those in the same condition with adequate fillings. Supporting this finding, it has been described that the root-filling technique influences the success of the endodontic treatment[34]. Therefore, it seems a plausible explanation that infected teeth with inadequate endodontic fillings are a greater reservoir of chronic infections than its counterpart.

In terms of post-operative findings, persistence of the alveolar socket is associated with the development of osteonecrosis [19,35], which in turn is associated with

previous bone sclerosis at the site [35]. In addition, osteolytic changes are associated with progression to exposed osteonecrosis [36] and sequestrum formation [35]. In our sample, a heterogenous bone pattern, sequestrum formation, persistence of the alveolar socket, and crater-like defect were seen significantly more in the MRONJ+ group. Still, sequestrum appears to be a pathognomonic feature of MRONJ, as it appeared only in MRONJ+ sites. Though, knowing that this pathology manifests itself early with different bone patterns and absence of clinically exposed bone [21], it may be that some of these findings are a subclinical form of osteonecrosis [36]. This observation stresses the value of diagnostic imaging and a closer follow-up.

Among the limitations of this study, we found those inherent to its retrospective nature, namely, lack of data recording in the patient file, surgical variability, different ARD treatment and drug holiday duration, polypharmacy and comorbidity factors, midst other variables that could not be fully controlled. In addition, the prevalence of MRONJ in this sample, 51% of the patients and 29% of the extracted teeth, seems higher than what is reported in the literature (0.5%-4.6% of the patients[37]), but comparable to a prior Belgian study [38], where the risk of MRONJ increased to 20% after tooth extractions. Lastly, the timing of the exposure to the radiograph before and after the extraction was not homogeneous either, giving room for changes in the appearance of the assessment perceptible to the observer.

The clinical relevance of this study relies on the need for diagnostic imaging prior to tooth extraction in oncologic patients under ARD. Especially to identify a sclerotic and heterogeneous preoperative bone pattern, which in this study we consider as high risk for MRONJ, but perhaps they are already an early stage of osteonecrosis without bone exposure. Likewise, follow-up images can indicate the onset of MRONJ with the presence of a heterogeneous bone pattern, persistence of the alveolar socket, a crater-like defect, and bone sequester. Moreover, when the high-risk local factors are recognized, a treatment plan that involves a closer follow-up after tooth extraction(s), the use of prophylactic antibiotics [39,40], antiseptic mouthwash [41], and L-PRF for MRONJ prevention [39,42,43], are recommended. As a word of caution, we suggest careful interpretation of our results, as the purpose of the univariate and

multifactorial statistical analysis was to identify variables (or combinations of) that have a relationship with the development of osteonecrosis, rather than to establish causality. Further prospective studies are necessary to confirm the present findings.

Conclusion

Osteonecrosis may be anticipated upon recognition of the following associations described in the present study: teeth with absent and incomplete endodontic fillings with caries, widened periodontal ligament space and/or periapical lesions, and a sclerotic and heterogeneous preoperative alveolar bone pattern, in patients with longer ARD treatments. Most findings highlight the relevance of local infectious factors in the development of this pathology. Early identification of these features encourages a patient-specific decision making to take preventive measures during the treatment, giving importance to the quality of the conservative treatment and chronic dental infections in sites needing extraction in oncologic patients under ARD treatment.

Conflicts of interest

Authors declare explicitly that there are no conflicts of interest.

Author contributions

1. C.M.R: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing
2. L.L: Investigation, Writing - Original Draft, Writing - Review & Editing
3. H.G.A: Investigation, Writing - Original Draft, Writing - Review & Editing
4. A.L: Writing - Investigation, Writing - Original Draft, Writing - Review & Editing
5. W.C: Statistical analysis
6. T.VdW: Writing - Review & Editing, Supervision
7. R.J: Conceptualization, Writing - Review & Editing, Supervision

References

1. Campisi G, Fedele S, Fusco V, Pizzo G, Di Fede O, Bedogni A. Epidemiology, clinical manifestations, risk reduction and treatment strategies of jaw osteonecrosis in cancer patients exposed to antiresorptive agents. Future Oncology. 2014 Feb;10(2):257–75.

2. D'Oronzo S, Coleman R, Brown J, Silvestris F. Metastatic bone disease: Pathogenesis and therapeutic options. J Bone Oncol. 2019 Apr 1;15:100205.

3. Coleman RE. Clinical Features of Metastatic Bone Disease and Risk of Skeletal Morbidity. Clinical Cancer Research. 2006 Oct 15;12(20):6243s–9s.

4. Gavaldá C, Bagán JV. Concept, diagnosis and classification of bisphosphonate-associated osteonecrosis of the jaws. A review of the literature. Med Oral Patol Oral Cir Bucal. 2016 May 1;21(3):e260-70.

5. Ruggiero SL, Dodson TB, Assael LA, Landesberg R, Marx RE, Mehrotra B. American Association of Oral and Maxillofacial Surgeons Position Paper on Bisphosphonate-Related Osteonecrosis of the Jaws—2009 Update. Journal of Oral and Maxillofacial Surgery. 2009 May;67(5):2–12.

6. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw--2014 update. J Oral Maxillofac Surg. 2014 Oct;72(10):1938–56.

7. Schiodt M, Otto S, Fedele S, Bedogni A, Nicolatou-Galitis O, Guggenberger R, et al. Workshop of European task force on medication-related osteonecrosis of the jaw-Current challenges. Oral Dis. 2019;25(7):1815–21.

8. Otto S, Pautke C, Van den Wyngaert T, Niepel D, Schiødt M. Medication-related osteonecrosis of the jaw: Prevention, diagnosis and management in patients with cancer and bone metastases. Cancer Treat Rev. 2018 Sep 1;69:177–87.

9. Taylor KHH, Middlefell LSS, Mizen KDD. Osteonecrosis of the jaws induced by anti-RANK ligand therapy. Br J Oral Maxillofac Surg. 2010 Apr;48(3):221–3.

10. Nicolatou-Galitis O, Papadopoulou E, Vardas E, Kouri M, Galiti D, Galitis E, et al. Alveolar bone histological necrosis observed prior to extractions in patients, who received bone-targeting agents. Oral Dis. 2020 Jul;26(5):955–66.

11. Gaêta-Araujo H, Ferreira Leite A, de Faria Vasconcelos K, Coropciuc R, Politis C, Jacobs R, et al. Why do some extraction sites develop medication-related osteonecrosis of the jaw and others do not? A within-patient study assessing radiographic predictors. Int J Oral Implantol (Berl). 2021 Mar 16;14(1):87–98.

12. Cardoso CL, Barros CA, Curra C, Fernandes LMP da SR, Franzolin S de OB, Júnior JSF, et al. Radiographic Findings in Patients with Medication-Related Osteonecrosis of the Jaw. Int J Dent. 2017;2017:1–6.

13. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and elaboration. International Journal of Surgery. 2014 Dec 1;12(12):1500–24.

14. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005 May;37(5):360–3.

15. Moreno-Rabié C, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep 6;24(9):2973–89.

16. Ueda N, Aoki K, Shimotsuji H, Nakashima C, Kawakami M, Imai Y, et al. Oral risk factors associated with medication-related osteonecrosis of the jaw in patients with cancer. J Bone Miner Metab. 2021 Jul 8;39(4):623–30.

17. Dimopoulos MA, Kastritis E, Bamia C, Melakopoulos I, Gika D, Roussou M, et al. Reduction of osteonecrosis of the jaw (ONJ) after implementation of preventive measures in patients with multiple myeloma treated with zoledronic acid. Ann Oncol. 2009 Jan;20(1):117–20.

18. Ripamonti CI, Maniezzo M, Campa T, Fagnoni E, Brunelli C, Saibene G, et al. 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. 2009 Jan;20(1):137–45.

19. Klingelhöffer C, Klingelhöffer M, Müller S, Ettl T, Wahlmann U. Can dental panoramic radiographic findings serve as indicators for the development of medication-related osteonecrosis of the jaw? Dentomaxillofac Radiol. 2016 May;45(5):20160065.

20. Guo Y, Wang D, Wang Y, Peng X, Guo C. Imaging features of medicine-related osteonecrosis of the jaws: comparison between panoramic radiography and computed tomography. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016 Aug 1;122(2):e69–76.

21. Olutayo J, Agbaje JO, Jacobs R, Verhaeghe V, Velde F Vande, Vinckier F. Bisphosphonate-Related Osteonecrosis of the Jaw Bone: Radiological Pattern and the Potential Role of CBCT in Early Diagnosis. J Oral Maxillofac Res. 2010 Apr 23;1(2):e3.

22. Querrer R, Ferrare N, Melo N, Stefani CM, Dos Reis PED, Mesquita CRM, et al. Differences between bisphosphonate-related and denosumab-related osteonecrosis of the jaws: a systematic review. Support Care Cancer. 2021 Jun 2;29(6):2811–20.

23. Bedogni A, Fedele S, Bedogni G, Scoletta M, Favia G, Colella G, et al. Staging of osteonecrosis of the jaw requires computed tomography for accurate definition of the extent of bony disease. British Journal of Oral and Maxillofacial Surgery. 2014 Sep;52(7):603–8.

24. Bedogni A, Fusco V, Agrillo A, Campisi G. Learning from experience. Proposal of a refined definition and staging system for bisphosphonate-related osteonecrosis of the jaw (BRONJ). Oral Dis. 2012 Sep 22;18(6):621–3.

25. Hamada H, Matsuo A, Koizumi T, Satomi T, Chikazu D. A simple evaluation method for early detection of bisphosphonate-related osteonecrosis of the mandible using computed tomography. Journal of Cranio-Maxillofacial Surgery. 2014 Sep;42(6):924–9.

26. Torres SR, Chen CSK, Leroux BG, Lee PP, Hollender LG, Lloid M, et al. Mandibular inferior cortical bone thickness on panoramic radiographs in patients using bisphosphonates. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015 May;119(5):584–92.

27. Aghaloo TL, Dry SM, Mallya S, Tetradis S. Stage 0 osteonecrosis of the jaw in a patient on denosumab. J Oral Maxillofac Surg. 2014 Apr;72(4):702–16.

28. Şahin O, Odabaşı O, Demiralp KOÖ, Kurşun-Çakmak EŞ, Aliyev T, Sahin O, et al. Comparison of findings of radiographic and fractal dimension analyses on panoramic radiographs of patients with early-stage and advanced-stage medication-related osteonecrosis of the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019 Jul;128(1):78–86.

29. Hutchinson M, O'Ryan F, Chavez V, Lathon P V., Sanchez G, Hatcher DC, et al. Radiographic findings in bisphosphonate-treated patients with stage 0 disease in the absence of bone exposure. J Oral Maxillofac Surg. 2010 Sep;68(9):2232–40.

30. Ueda N, Nakashima C, Aoki K, Shimotsuji H, Nakaue K, Yoshioka H, et al. Does inflammatory dental disease affect the development of medication-related osteonecrosis of the jaw in patients using high-dose bone-modifying agents? Clin Oral Investig. 2021 May 14;25(5):3087–93.

31. Fedele S, Porter SR, D'Aiuto F, Aljohani S, Vescovi P, Manfredi M, et al. Nonexposed variant of bisphosphonate-associated osteonecrosis of the jaw: a case series. Am J Med. 2010;123(11):1060–4.

32. Arce K, Assael LA, Weissman JL, Markiewicz MR. Imaging findings in bisphosphonate-related osteonecrosis of jaws. J Oral Maxillofac Surg. 2009 May;67(5):75–84.

33. Ruggiero S, Gralow J, Marx RE, Hoff AO, Schubert MM, Huryn JM, et al. Practical guidelines for the prevention, diagnosis, and treatment of osteonecrosis of the jaw in patients with cancer. J Oncol Pract. 2006 Jan;2(1):7–14.

34. de Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, et al. Treatment Outcome in Endodontics: The Toronto Study—Phase 4: Initial Treatment. J Endod. 2008 Mar;34(3):258–63.

35. Koth VS, Figueiredo MA, Salum FG, Cherubini K. Interrelationship of clinical, radiographic and haematological features in patients under bisphosphonate therapy. Dentomaxillofac Radiol. 2017 Apr;46(4):20160260.

36. Matsuo A, Hamada H, Kaise H, Chikazu D, Yamada K, Kohno N. Characteristics of the early stages of intravenous bisphosphonate-related osteonecrosis of the jaw in patients with breast cancer. Acta Odontol Scand. 2014 Nov 12;72(8):656–63.

37. Nicolatou-Galitis O, Schiødt M, Mendes RA, Ripamonti C, Hope S, Drudge-Coates L, et al. Medication-related osteonecrosis of the jaw: definition and best practice for prevention, diagnosis, and treatment. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019 Feb 1;127(2):117–35.

38. Van den Wyngaert T, Delforge M, Doyen C, Duck L, Wouters K, Delabaye I, et al. Prospective observational study of treatment pattern, effectiveness and safety of zoledronic acid therapy beyond 24 months in patients with multiple myeloma or bone metastases from solid tumors. Support Care Cancer. 2013 Dec 18;21(12):3483–90.

39. Şahin O, Tatar B, Ekmekcioğlu C, Aliyev T, Odabaşı O. Prevention of medication related osteonecrosis of the jaw after dentoalveolar surgery: An institution's experience. J Clin Exp Dent. 2020 Aug 1;12(8):e771–6.

40. Spanou A, Nelson K, Ermer MA, Steybe D, Poxleitner P, Voss PJ. Primary wound closure and perioperative antibiotic therapy for prevention of bisphosphonate-related osteonecrosis of the jaw after tooth extraction. Quintessence Int. 2020;51(3):220–8.

41. Pippi R, Giuliani U, Tenore G, Pietrantoni A, Romeo U. What is the Risk of Developing Medication-Related Osteonecrosis in Patients With Extraction Sockets Left to Heal by Secondary Intention? A Retrospective Case Series Study. Journal of Oral and Maxillofacial Surgery. 2021 Oct 28;79(10):2071–7.

42. Miranda M, Gianfreda F, Raffone C, Antonacci D, Pistilli V, Bollero P. The Role of Platelet-Rich Fibrin (PRF) in the Prevention of Medication-Related Osteonecrosis

of the Jaw (MRONJ). Scribante A, editor. Biomed Res Int. 2021 May 15;2021:1–8.

43. Asaka T, Ohga N, Yamazaki Y, Sato J, Satoh C, Kitagawa Y. Platelet-rich fibrin may reduce the risk of delayed recovery in tooth-extracted patients undergoing oral bisphosphonate therapy: a trial study. Clin Oral Investig. 2017 Sep 1;21(7):2165–72.

CHAPTER 2: THREE-DIMENSIONAL ASSESSMENT IN ONCOLOGIC PATIENTS

Three-dimensional clinical assessment for MRONJ risk in oncologic patients following tooth extractions

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Abstract

Objective: To identify clinical and local radiographic predictors for Medication-Related Osteonecrosis of the Jaws (MRONJ) by the assessment of pre-operative cone beam computed tomography (CBCT) images of oncologic patients treated with antiresorptive drugs (ARDs) undergoing tooth extractions.

Methods: This retrospective, longitudinal, case-control study included clinical and imaging data of 97 patients, divided into study and control group. Patients in the study group (n=47; 87 tooth extractions) had received at least one dose of ARD, undergone tooth extraction(s), and had a pre-operative CBCT. An age-, gender-, and tooth extraction-matched control group (n=50; 106 tooth extractions) was selected. Three calibrated, blinded, and independent examiners evaluated each tooth extraction site. Statistical analysis used Chi-Square/Fisher's exact/Mann-Whitney U test to contrast control and study group, ARD type used, and sites with or without MRONJ development. P-value ≤0.05 was considered significant.

Results: From the study group, 15 patients (32%) and 33 sites (38%) developed MRONJ after tooth extraction. When controls were compared to study sites, the latter showed significantly more thickening of the lamina dura, widened periodontal ligament space, osteosclerosis, osteolysis, and sequestrum formation. In the study group, MRONJ risk significantly increased in patients who had multiple tooth extractions, were smokers, and had shorter drug holidays. Periosteal reaction and sequestrum formation may indicate latent MRONJ lesions. Additionally, patients given bisphosphonates showed considerably more osteosclerosis than those given denosumab.

Conclusions: Periosteal reaction and sequestrum formation are suspected to be preclinical MRONJ lesions. Furthermore, ARD induced bony changes and radiographic variations between ARD types were seen.

Keywords: Diphosphonates, Denosumab, Tooth Extraction, Osteonecrosis, Cone-Beam Computed Tomography

Introduction

The skeleton is one of the most common locations for metastases, with breast and prostate cancer accounting for more than 80% of their incidence. Metastatic bone disease can be accompanied by skeletal related events (SRE), which are distressing signs and symptoms comprising pain, impaired mobility, hypercalcemia, fractures, and spinal cord compression.[1] Fortunately, treatments are available to prevent or treat SREs, including antiresorptive drugs (ARDs) such as bisphosphonates and monoclonal antibodies.

Among the available treatments, ARDs interfere with bone turnover by impeding osteoclastic activity through different pathways.[2,3] For instance, nitrogen-containing bisphosphonates bind to calcium ions on the bone surface and are internalized by resorbing osteoclasts, inhibiting the protein farnesyl pyrophosphate (FPP) synthase, which is required for osteoclast function.[4,5] On the other hand, monoclonal antibodies, like denosumab, inhibit the receptor activator of nuclear factor kappa-B (RANK) ligand pathway impairing osteoclast formation.[3] Both strategies result in a reduction of bone resorption and destruction.

Patients receiving ARDs require special attention in the dental practice because of the higher risk of developing medication-related osteonecrosis of the jaws (MRONJ).[6] MRONJ is clinically defined as exposed bone or bone that can be probed through an intraoral or extraoral fistula(e) in the maxillofacial region persisting for more than eight weeks in patients treated with ARDs, with no history of radiation therapy or metastatic disease to the jaws.[7] Interestingly, previous studies have reported changes in the radiographic appearance of the jawbones in these patients even before the manifestation of exposed necrotic bone. While panoramic radiographs may thus provide a good overview and early indicator of patients at risk of MRONJ, they may fall short in identifying even earlier signs due to their low sensitivity to detect minor variations.[8] Instead, cone beam computed tomography (CBCT) has been recommended in these patients [9–12], where thickening of the mandibular cortical and lamina dura [11], osteosclerosis [11,13], and osteolysis can be observed.[13]

Several risk factors for MRONJ have been acknowledged, including high cumulative doses of ARDs [14,15] and tooth extractions.[14] Particularly when tooth extractions take place, local predisposing factors for MRONJ have been recognized using panoramic radiographs, endorsing an increased susceptibility at sites with dental infections and osteosclerotic and osteolytic changes.[16–18] The latter together with cortical bone erosion, sequestrum, and sinus inflammatory signs have been associated to histological evidence of osteonecrosis. Nevertheless, it remains unclear whether any of these CBCT based features are pathognomonic for MRONJ.[12] Therefore, the main objective of this retrospective case-control study was to identify clinical and local radiographic predictors for MRONJ using CBCT images of oncologic patients treated with ARD undergoing tooth extractions. Secondary objectives included comparing radiographic findings between patients treated with ARD versus those without, and between patients treated with bisphosphonates or denosumab. It was hypothesized that CBCT can provide early visualization of preclinical stages of MRONJ.

Material and Methods

Study design and settings

The ethical committee of UZ/KU Leuven was consulted prior to the start of this retrospective case-control study (protocol number: S63934). All procedures were performed according to the ethical standard of the Declaration of Helsinki and the institutional review board. The STROBE guidelines were followed for reporting.[19]

Participant selection

Clinical records of 525 patients treated with antiresorptive drugs and seen at the oral and maxillofacial surgery department at University Hospitals Leuven for CBCT acquisition between 2010 and 2020 were reviewed retrospectively. Patients were included if they (1) received ARD therapy in oncological doses, (2) had tooth extraction(s) within one year after CBCT acquisition, and (3) had documented clinical follow-up of the extraction socket. Patients with prior head and neck radiation, MRONJ

at the extraction site, and poor image quality that would impair the image assessment were excluded.

Additionally, a control group was selected to match the study group regarding age, gender, and extracted tooth. Patients with a CBCT and tooth extractions within a year without a history of antiresorptive medication use were included in this group. Further exclusion criteria were the same as for the study group. Tooth extractions were performed following the description of Moreno-Rabié et al 2023.[16]

Data selection

Medical records (i.e., clinical data and CBCT images) were revised. The following information was retrieved: year of birth, gender, systemic condition, concomitant medication, previous chemotherapy and/or radiotherapy (i.e. other than to the head and neck region), antiresorptive drug, dose, treatment duration, smoking status,[20] alcohol habits, date of CBCT acquisition, extracted teeth, indication for extraction, surgery date, duration of follow-up, and if applicable, the date of diagnosis and stage of MRONJ according to the American Association of Oral and Maxillofacial Surgeons [7], site of MRONJ, drug holiday (i.e., treatment interruption before the tooth extraction), use of leukocyte- and platelet-rich fibrin (L-PRF), prophylactic antibiotics, antiseptic mouthwash, and date when mucosal healing was reached.

Radiographic assessment

CBCT images were acquired with 3D Accuitomo 170 (J. Morita Corp., Kyoto, Japan) or Newtom VGi evo (Cefla Dental Group, Imola, Italy). The field of view (FOV), voxel size (ranging from 80µm to 300µm), and exposure protocol for each exam were determined according to the patient's specific diagnostic or therapeutic indication. All images were assessed using IMPAX software (version 6.5.5, Agfa-Gevaert, Mortsel, Belgium).

Three blinded and independent oral and maxillofacial radiologists evaluated all images and scored the parameters explained below at each tooth extraction site. A calibration session took place before the start of the observations, using a set of 21 CBCTs external to the study to achieve baseline diagnostic consensus. All evaluations were done in a quiet room with low lighting using a high-resolution display (HP

EliteDisplay E243 23.8-inch Monitor; HP Inc.; Palo Alto; USA). The brightness and contrast settings were adapted according to the examiner's judgment. Forty-eight tooth extraction sites were reassessed one month after the evaluation was completed to determine the intra-observer agreement. The outcome was calculated using the mode of the observations, which meant that at least two of the observers had to agree on whether a characteristic was present or absent. Individual sites with no concordance were discussed until agreement was achieved.

Radiological evaluation included an examination of the tooth to be extracted and the surrounding bone (medullary and cortical bone), excluding the crown due to artifacts generated by high-density materials (e.g., fillings and metallic crowns) that prevented its proper visualization. The parameters assessed are shown in Figure 1 and listed hereafter:

1. Alveolar bone loss, considering the absence or presence of horizontal bone loss and angular bone defects as described by Gaeta-Araujo et al. 2021.[18] In multirooted teeth, it was considered the worst outcome.
2. Furcation involvement, classified as not applicable/absent or present.
3. Lamina dura, normal or thickened.
4. Periodontal ligament space, normal or widened, if doubled in width.
5. Endodontic treatment, following the description of Nascimento et al. 2019,[21] was described as absent, present with adequate filling, or present with inadequate filling, if underfilling of more than 2mm coronal to the apex, overfilling, nonhomogeneous filling, non-filled canal, presence of fractured instruments in the canal, or deviation of the natural course of the canal.
6. Periapical lesion, considering presence, size, and involvement of the cortical bone, based on the description of Fontenele et al. 2021.[22] A lesion was deemed present if there was hypodensity in the periapical area wider than 1mm. They were classified as small if their largest diameter was $\leq$3 mm or large if >3 mm. Cortical involvement was divided into four categories: none, thinning, expansion, and destruction.
7. Root remnant, absent or present.

8. Trabecular bone pattern, considering osteosclerosis, osteolysis, periosteal reaction, and sequestrum formation, based on the description of Walton et al. 2019.[23] All characteristics were classified as absent, localized if only in the examined tooth, or extensive if involving further than the immediate neighboring tooth.

Furthermore, measurements of the mandibular cortical width (MCW) were performed once per side per patient to compare control and study groups, and within the latter, MRONJ+ and MRONJ- patients. Figure 2 depicts the measurement methodology adopted based on the description of Castro et al. 2020.[24]

Statistical analysis

The statistical analysis was performed using the software RStudio version 2023.3.1.446 (RStudio, Boston, MA, US), and a p-value $\leq$0.05 was considered significant. Fleiss' Kappa test was used to calculate inter-observer agreement, and Cohen's Kappa test for intra-observer agreement. The results of the Kappa tests were interpreted according to the following scale: $\geq$0.21 - 0.40 was considered fair; moderate when it was $\geq$0.41 - 0.60; substantial when it was $\geq$0.61 - 0.80; almost perfect when it was $\geq$0.81 - 0.99.[25] Furthermore, the Chi-square/Fisher's exact test, for categorical data, and the Mann–Whitney U test, for ordinal variables, were used to test the independence of radiographic characteristics and clinical data documented for each patient/extracted tooth. In these analyses, control and antiresorptive-treated groups were compared. Additionally, comparisons were made by splitting the study group into extraction sites MRONJ+ and MRONJ-. Finally, the radiographic characteristics associated with the use of bisphosphonates and denosumab were investigated. For this purpose, patients who had only been exposed to one type of drug were selected, and the aforementioned tests were used to compare the distribution of radiographic features.

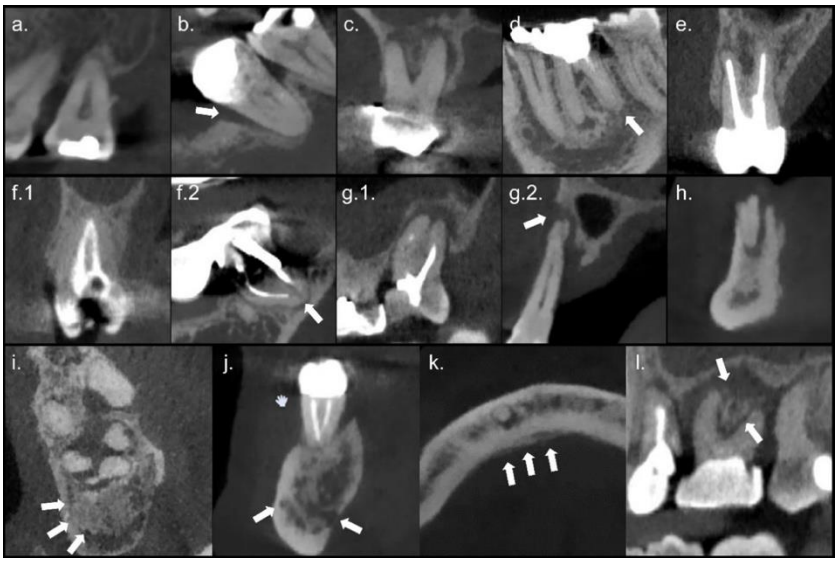

Figure 1. Cutoffs of CBCT reconstructions showing the assessed parameters. These images illustrate severe horizontal bone loss (a), an angular bone defect (b), furcation involvement (c), thickening of the lamina dura (d), widening of the periodontal ligament space (e), an adequate (f.1) and inadequate (f.2) endodontic treatment, a large periapical lesion with cortical expansion (g.1) and with buccal cortical plate destruction (g.2), a root remnant (h), osteosclerosis (i), osteolysis (j), periosteal reaction (k), and sequestrum formation (l).

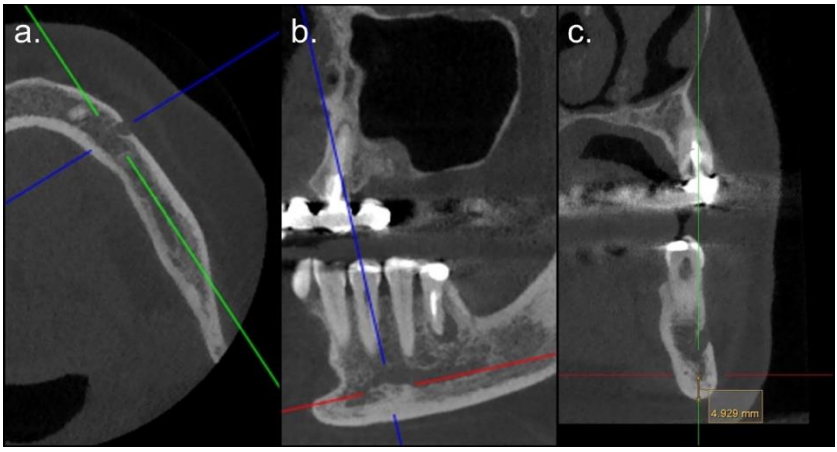

Figure 2. Example of measurement of the mandibular cortical width from the left mandibular side. The measurement was performed after completing three steps. (a) First, the axial reconstruction was selected where the largest dimension of the mental foramen was visible. Then, the orientation lines were rotated so that they could pass through the long axis of the mandible's body and its tangent through the middle of the foramen. (b) Once these lines were in place, the line of the sagittal reconstruction was modified so that it would be parallel to the base of the mandible. (c) Finally, the MCW was measured in the coronal reconstruction using a line that passed through the posterior border of the foramen.

Results

Forty-seven oncologic patients who had 87 teeth extracted met the inclusion criteria. MRONJ was observed in 15 patients (32%) and involved 33 sites (38%). In addition, the control group included 50 patients who had 106 tooth extractions, which showed no significant differences in age (p=0.218), gender (p=0.941), number of teeth to be extracted (p=0.480), tooth type (p=0.643), and arch (p=0.201) with the study group. Other post-extraction complications included one bleeding and three oroantral communications, all successfully treated. Table 1 shows a summary of the patient's data. The time between CBCT acquisition and tooth extraction ranged from 0 to 12 months, with an average time of 2 months for oncologic patients and 1 month for control patients.

Overall, observers had a substantial agreement between their assessments (k=0.69), ranging from a moderate agreement in periodontal ligament space and osteolysis (k=0.41) to almost perfect agreement in endodontic treatment (k=0.95). Furthermore, no significant differences were found among the observer's measurements of mandibular cortical width (right side p=0.87; left side p=0.96). Finally, the intra-observer agreement had an almost perfect concordance (k=0.87).

Patients with malignant disease received at least one dose of zoledronic acid 4mg, denosumab 120mg, or pamidronate 90mg. These patients were diagnosed with breast cancer (n=19, 40.4%), multiple myeloma (n=11, 23.4%), prostate (n=10, 21.3%), renal cell (n=3, 6.4%), lung (n=2, 4.3%), stomach (n=1, 2.1%), and pancreatic cancer (n=1, 2.1%). There were no significant differences in the type of cancer and the onset of osteonecrosis of the jaws (p=0.642) nor in the type of antiresorptive drug used (p=0.779) or the length of treatment (p=0.568) (Table 1). Additionally, complementary cancer therapies at the time of tooth extraction such as the use of anti-angiogenic drugs (n=9, p=1.000) and hormone therapy (n=17, p=0.961) also failed to demonstrate a significant effect on the development of MRONJ.

Patients who developed MRONJ had a significantly shorter drug holiday than those without exposed bone (p=0.021). Furthermore, when bisphosphonates and

denosumab were studied separately, patients on bisphosphonates had a mean drug holiday of 36 months (MRONJ+: 14 months and MRONJ-: 44 months; p=0.094), while those on denosumab had a mean drug holiday of 8 months (MRONJ+: 1.9 months and MRONJ-: 11 months; p=0.041). Moreover, a younger age (MRONJ+ mean 64 years, MRONJ- mean 71 years, p=0.025), multiple tooth extractions simultaneously (MRONJ+ mean 3 teeth, MRONJ- mean 1 tooth, p=0.006), and smoking (p=0.004) significantly increased the risk of developing MRONJ.

The clinical variables studied for each extracted tooth are detailed in Table 2, and the results of the radiographic assessment are displayed in Table 3. None of the clinical factors were found to be significant in the onset of osteonecrosis. It was noted that mucosal lining at the extraction site was achieved on an average of 2.4 weeks in the control group. In contrast, the study group took significantly longer for this sign to develop, averaging 14.2 weeks post-extraction (p<0.001).

Concerning the radiographic signs predisposing to MRONJ, the presence of localized and extensive periosteal reaction was associated with a higher risk of bone exposure compared to its absence (p=0.051). All teeth in sites showing periosteal reaction, presented extensive caries lesions, periapical radiolucencies and/or periodontitis, accompanied by pain, increased response to cold stimuli, tenderness to percussion, periapical fistula, or abscess formation. Interestingly, sequester formation was exclusively seen in the study group, both in sites that later did and did not develop MRONJ. All sites with sequester formation were also accompanied by teeth with caries or periodontal disease, and presenting tenderness to percussion or mobility, respectively.

Finally, among the oncologic patients, 21 received only bisphosphonates, while 24 received denosumab. There were 42 extractions in each group. Results showed no significant differences in the distribution of lamina dura appearance (p=0.646), periodontal ligament space (p=0.602), osteolysis (p=0.401), periosteal reaction (p=0.180), and sequestrum formation (p=0.568) when comparing both types of medication. However, patients who received bisphosphonates (BP) had significantly more localized and extensive osteosclerosis than those who received denosumab (DB)

(localized: 12% in BP vs. 9% in DB, extensive: 74% in BP vs. 45% in DB, p=0.003) (Figure 3).

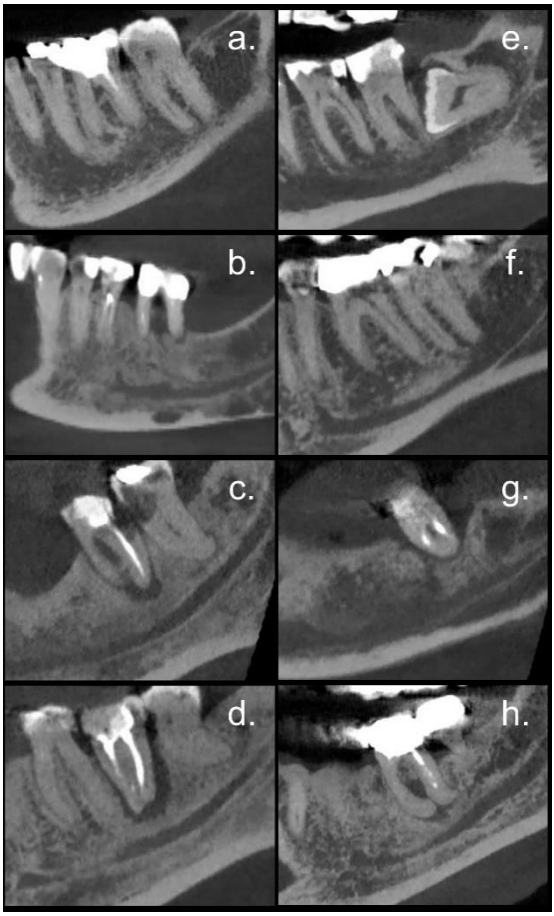

Figure 3. Sagittal reconstruction cuts showing trabecular and cortical bone in the mandibular molar areas of patients treated with bisphosphonates (a-d) and denosumab (e-h). Patients treated with bisphosphonates had significantly more localized (a) or extensive (b, c, d) osteosclerosis, whereas those treated with denosumab showed more frequently normal medullary spaces (e, f) and less often localized (g) or extensive (h) osteosclerosis.

Discussion

Currently, there is no widely agreed recommendation on the best imaging approach for detecting patients at increased risk of clinically overt MRONJ. As a result, osteonecrosis lesions are often only evaluated when clinically exposed bone is present, overlooking early radiographic alterations and potential risk factors.[8,11,26] Studies have revealed that

exposed bone locations may exhibit earlier symptoms of infection or trabecular bone alterations.[16–18] However, all these studies were performed using panoramic radiographic assessment, whose inherent limitations include overlapping of anatomical structures, magnification, and absence of a buccolingual evaluation, which might impair their diagnostic performance. Thus, our primary goal was to identify clinical and local predisposing factors in oncologic patients treated with ARD and undergoing tooth extractions using three-dimensional images.

The use of CBCT for diagnosing MRONJ is advocated due to its better resolution than panoramic radiography, the possibility of assessing the true extent of a lesion, and the visibility of structures without overlap.[11] Through CBCT assessment, it has been possible to observe the presence of osteolysis, cortical bone erosion, sequestrum formation, and osteosclerosis in lesions with bone exposure at all clinical stages as defined by the American Association of Oral and Maxillofacial Surgeons.[7] Adding to these results, the present investigation describes the distinguishing three-dimensional features of antiresorptive drug use even before the presence of clinically exposed necrotic bone. Given that our patients demonstrated significantly more thickening of the lamina dura, widening of the periodontal ligament space, osteosclerosis, osteolysis and sequestrum formation than the control group. All these findings are consistent with prior two-dimensional investigations.[9,17,27,28]

Regarding clinical risk factors associated with osteonecrosis exposure, a higher risk was seen in younger patients, with more than one simultaneous tooth extraction, smokers, and with shorter discontinuation of antiresorptive drugs when the tooth extractions took place. The latter is particularly important in light of the pharmacokinetics of BP and DB, which have half-lives of up to 10 years [29] and 1 month [30], respectively. Besides, BPs, unlike DB, are deposited in bone tissue and only exert their effect on osteoclasts at the time of their uptake.[29] This explains why patients who had stopped taking BP for an average of 14 months and DB for an average of 2 months at the time of tooth extractions developed osteonecrosis.

Table 1. Summary of data collected at a patient level for oncologic and control groups.

Characteristic		Oncologic						Control	
Number of patients, n		47						50	
Development of osteonecrosis, n (%)		MRONJ+ 15	31.9%	MRONJ- 32	68.1%	Total 47	p-value	NA	p-value
Age (years)	Mean (range)*	63.8 (46 - 83)		71.3 (46 - 89)		68.9 (46 - 89)	*0.025*	71.5 (47 – 87)	0.218
Sex, n (%)	Female	10	40%	15	60%	25	0.340	28	0.941
	Male	5	22.7%	17	77.3%	22		22	
Extracted teeth, n	Mean (range)*	2.8 (1 – 8)		1.4 (1 - 4)		1.9 (1 - 8)	*0.006*	2.1 (1 – 6)	0.480
Chemo- and radiotherapy, n (%) [a]	None	1	50%	1	50%	2	0.603	43	*<0.001*
	Chemotherapy	4	44.4%	5	55.6%	9		2	
	Radiotherapy	2	20%	8	80%	10		1	
	Both	8	30.8%	18	69.2%	26		4	
ARD type, n (%)	Bisphosphonate	6	28.6%	15	71.4%	21	0.779	NA	NA
	Denosumab	8	33.3%	16	66.7%	24		NA	
	Both	1	50%	1	50%	2		NA	
Specific ARD used, n (%)	Zoledronic Acid	5	23.8%	16	76.2%	21	0.467	NA	NA
	Denosumab	9	34.6%	17	65.4%	26		NA	
	Alendronate	1	100%	0	0%	1		NA	
	Pamidronate	1	33.3%	2	66.7%	3		NA	
	Ibandronate	1	50%	1	50%	2		NA	
Number of ARD, n (%) [b]*	1	13	31.7%	28	68.3%	41	0.953	NA	NA
	2	2	33.3%	4	66.7%	6		NA	
Time on ARD (months)	Mean (range)*	40.4 (7 - 173)		29.5 (1 - 86)		32.9 (1 - 173)	0.568	NA	NA
Drug holiday (months), n (%)	No	5	38.5%	8	61.5%	13	0.728	NA	NA
	Yes	10	29.4%	24	70.6%	34		NA	
	Mean (range)*	7.8 (0.1 - 29)		27.5 (0.3 - 119)		21.6 (0.1 - 119)	*0.021*	NA	
Corticosteroid use (months), n (%)	No	8	26.7%	22	73.3%	30	0.484	48	*<0.001*
	Yes	7	41.2%	10	58.8%	17		1	
	Mean (range)*	38.5 (10 - 96)		61.3 (3 - 420)		51.9 (3 - 420)	0.115	2.5	0.191
Osteoporosis, n (%)	No	11	30,6%	25	69,4%	36	0.725	46	0.069
	Yes	4	36,4%	7	63,6%	11		4	
Alcohol consumption, n (%) *	No consumption	6	40%	9	60%	15	0.293	16	0.365
	1-2 units week	1	10%	9	90%	10		17	
	3-4 units week	0	0%	0	0%	0		4	
	>5 units week	3	42%	4	57.1%	7		8	
	Ex-abuser	0	0%	2	100%	2		1	
	Unknown	5	38.5%	8	61.5%	13		4	
Tobacco use, n (%) [c]	Never smoker	5	22.7%	17	77.3%	22	*0.004*	36	*0.034*
	Current smoker	5	100%	0	0%	5		7	
	Former smoker	4	25%	12	75%	16		7	
	Unknown	1	25%	3	75%	4		0	
Mandibular Cortical Width (MCW)*	Right	4.11		4.72		4.50	0.071	4.23	0.247
	Left	4.32		4.70		4.56	0.190	4.15	0.071

P-values represent the results of the Chi-Square/Fisher's exact test when comparing MRONJ+ and MRONJ- patients in the study group, as well as the study and control groups. Variables denoted with an asterisk (*) represent ordinal/numerical data analyzed with the Mann–Whitney U test. Significant p-values ($p \leq 0.05$) are *italicized.* [a] No head and neck radiotherapy or antiresorptive drugs were given to control patients with a history of cancer. [b] Referring to the

number of different antiresorptive drugs used sequentially. [c] Following the definition provided by the Center for Disease Control and Prevention (CDC) from the United States, which states as never smoker a person who has never smoked or has smoked less than a 100 cigarettes in their lifetime, as current smoker a person who has smoked at least 100 cigarettes in their lifetime and currently smokes, and as former smoker a person who has smoked at least a 100 cigarettes in their lifetime but who had quit at the time of interview. MRONJ: Medication-related osteonecrosis of the jaws, ARD: antiresorptive drug, NA: Not applicable.

Knowing that antiresorptive drugs have distinct modes of action, it is conceivable that the radiographic characteristics of osteonecrosis related to bisphosphonate- (BRONJ) and denosumab- (DRONJ) are distinct.[31] Pichardo et al. found significant differences in their radiographic appearance, with BRONJ having significantly more sequester formation and cortical bone osteolysis and DRONJ showing less frequent radiographic signs leading to a later diagnosis and treatment.[32] In our results, even before the development of MRONJ, sites exposed to bisphosphonates had significantly more osteosclerosis, while those exposed to denosumab showed no significant features. The latter is relevant because the absence of early radiographic differences in denosumab-treated patients could affect their timely follow-up, as they tend to show late signs.[32]

Periosteal reaction yielded a borderline significance, suggesting that its presence alone may not conclusively predict the development of an exposed variant of necrosis. Nonetheless, sites exhibiting periosteal reaction could potentially harbor latent osteonecrosis lesions. Although this study lacks histopathological evidence, the use of ARD, coupled with the subsequent impairment of bone's reparative response, in combination with dental infections, creates a favorable environment for osteonecrosis.[33] Thus, the possibility of a masked necrotic process cannot be ruled out. Additionally, even though not significant for MRONJ, bone sequestrum was observed only in the study group. Barragan-Adjemian et al. described in CBCTs that bone islands surrounded by an osteolytic halo were a natural response to expel necrotic bone in the direction where there was the least resistance, resulting in clinical MRONJ.[13] We hypothesize that having bone exposure at these sites was a matter of timing, and very

likely, in the MRONJ- sites, surgical removal of sequesters during tooth extraction was curative.[34,35]

Table 2. Description of patient data collected per extracted tooth in the oncologic and study groups.

Characteristic		**Oncologic**						**Control**	
Number of extracted teeth, n		87						106	
Development of osteonecrosis, n (%)		MRONJ+		MRONJ-		Total	p-value	NA	p-value
		33	37.9%	54	62.1%	87			
Extraction indication, n (%)	Caries	20	48.8%	21	51.2%	41	0.338	45	*<0.001*
	Periodontitis	11	34.4%	21	65.6%	32		16	
	Fracture	0	0%	2	100%	2		11	
	Root remnant	2	22.2%	7	77.8%	9		25	
	Pericoronitis	0	0%	1	100%	1		7	
	NA	0	0%	2	100%	2		2	
Type of teeth, n (%) *	Incisors + canines	6	28.6%	15	71.4%	21	0.843	30	0.643
	Premolars	11	57.9%	8	42.1%	19		21	
	Molars	16	34%	31	66%	47		55	
Arch, n (%)	Maxilla	14	29.8%	33	70.2%	47	0.140	68	0.201
	Mandible	19	47.5%	21	52.5%	40		38	
Region, n (%)	Anterior maxilla	3	27.3%	8	72.7%	11	0.221	18	0.459
	Posterior maxilla	11	30.6%	25	69.4%	36		50	
	Anterior mandible	3	30%	7	70%	10		12	
	Posterior mandible	16	53.3%	14	46.7%	30		26	
Antibiotic prophylaxis, n (%)	Yes	30	36.1%	53	63.9%	83	0.151	20	*<0.001*
	No	3	75%	1	25%	4		86	
Antiseptic mouthwash, n (%)	Yes	33	37.9%	54	62.1%	87	1.000	106	1.000
	No	0	0%	0	0%	0		0	
Use of L-PRF, n (%)	Yes	24	33.8%	47	66.2%	71	0.166	4	*<0.001*
	No	9	56.3%	7	43.8%	16		102	
Time until mucosal healing, n (%)	0 - ≤4 weeks	0	0%	26	100%	26	NA	99	*<0.001*
	>4 - ≤8 weeks	0	0%	28	100%	28		7	
	>8 weeks	33	100%	0	0%	33		0	
	Mean (weeks)*	42.8		4.09		14.19	*<0.001*	2.41	*<0.001*
MRONJ worse stage, n (%)	Stage 1	21	63.6%	NA		NA	NA	NA	NA
	Stage 2	10	30.3%	NA		NA		NA	
	Stage 3	2	6.1%	NA		NA		NA	

The p-values described under oncologic and control correspond to those obtained with the Chi-square/Fisher's exact test or Mann–Whitney U test when data were ordinal (*). Comparisons were made between MRONJ+ and MRONJ-sites, and study and control groups. Significant p-values ($p \leq 0.05$) are *italicized*. MRONJ: Medication-related osteonecrosis of the jaws, L-PRF: leukocyte- and platelet-rich fibrin, NA: Not applicable.

Table 3. Results of the preoperative CBCT assessment at each tooth extraction site in the study and control groups.

Observed parameter		Oncologic						Control	
Number of extracted teeth, n		87						106	
Development of osteonecrosis, n (%)		MRONJ+		MRONJ-		Total	p-value	NA	p-value
		33	37.9%	54	62.1%	87			
Horizontal bone loss	Absent/initial	21	35.6%	38	64.4%	59	0.678	81	0.242
	Moderate/severe	12	42.9%	16	57.1%	28		25	
Angular bone defect	Absent	26	37.1%	44	62.9%	70	0.977	83	0.850
	Present	7	41.2%	10	58.8%	17		23	
Furcation involvement	Absent	27	39.7%	41	60.3%	68	0.705	86	0.740
	Present	6	31.6%	13	68.4%	19		20	
Lamina dura	Normal	21	36.2%	37	63.8%	58	0.815	86	*0.033*
	Thickened	12	41.4%	17	58.6%	29		20	
Periodontal ligament space	Normal	8	42.1%	11	57.9%	19	0.875	51	*<0.001*
	Widened	25	36.8%	43	63.2%	68		55	
Endodontic treatment	Absent	20	36.4%	35	63.6%	55	0.905	65	0.955
	Adequate filling	5	38.5%	8	61.5%	13		16	
	Inadequate filling	8	42.1%	11	57.9%	19		25	
Periapical lesion size*	Absent	16	31.4%	35	68.6%	51	0.229	66	0.593
	Small (≤3mm)	7	58.3%	5	41.7%	12		14	
	Large (>3mm)	10	41.7%	14	58.3%	24		26	
Periapical lesion cortical*	Absent	16	31.4%	35	68.6%	51	0.193	66	0.607
	None	5	50%	5	50%	10		12	
	Thinning	4	44.4%	5	55.6%	9		7	
	Expansion	3	75%	1	25%	4		8	
	Destruction	5	38.5%	8	61.5%	13		13	
Root remnant	Absent	32	39.5%	49	60.5%	81	0.401	85	*0.018*
	Present	1	16.7%	5	83.3%	6		21	
Osteoclerosis*	Normal	8	32%	17	68%	25	0.247	51	*0.006*
	Localized Sclerosis	2	22.2%	7	77.8%	9		10	
	Extended Sclerosis	23	43.4%	30	56.6%	53		45	
Osteolysis*	Absent	26	36.6%	45	63.4%	71	0.546	102	*<0.001*
	Localized lysis	4	36.4%	7	63.6%	11		3	
	Extensive lysis	3	60%	2	40%	5		1	
Periosteal reaction*	Absent	29	35.4%	53	64.6%	82	0.051	104	0.155
	Localized reaction	2	100%	0	0%	2		1	
	Extensive reaction	2	66.7%	1	33.3%	3		1	
Sequestrum formation*	Normal	32	38.1%	52	61.9%	84	0.879	106	0.055
	Localized sequester	1	33.3%	2	66.7%	3		0	
	Extensive sequester	0	0%	0	0%	0		0	

P-values represent the results of the Chi-Square/Fisher's exact test when comparing MRONJ+ and MRONJ- patients in the study group, as well as the study and control groups. Variables denoted with an asterisk (*) represent ordinal/numerical data analyzed with the Mann–Whitney U test. Significant p-values (p≤0.05) are *italicized.* NA: Not applicable.

Variations in the surgical technique could affect the occurrence of osteonecrosis even when all patients are exposed to the same risk factor. According to Seidel et al., tooth extractions combined with alveolectomy and the use of platelet-rich

fibrin membranes significantly decreased the incidence of MRONJ. It is believed that surgically removing the alveolar bone, which is anticipated to remodel, would aid healing and reduce the risk of osteonecrosis.[36] Despite surgical variability, most patients included in the current investigation had dental extractions under antibiotic prophylaxis (amoxicillin 875mg/clavulanic acid 125mg or clindamycin 300mg), local anesthetic without vasoconstrictor, L-PRF membranes, and resorbable sutures. All factors that could decrease the incidence of MRONJ. Yet, regardless of preventive measures, the study group took longer to achieve mucosal coverage than the control group. Under a normal setting, the mucosal lining of the exposed post-extraction socket is expected within two weeks,[37–40] but it took a mean of 14 weeks in the ARD-treated patients. Similar evidence supporting BP-use to heal at a slower rate after tooth extractions had been reported with a median of 5 weeks.[40]

The retrospective design of our study has some limitations, such as missing data in patient files, surgical variability, different ARD treatments, polypharmacy, and comorbidity factors, among other variables that may impact the development of osteonecrosis. However, this design allowed us to include a larger number of patients meeting our criteria. Specifically, we studied 47 ARD-treated patients who had 85 tooth extractions, from which 33 sites developed MRONJ. The high incidence of MRONJ can be attributed to two factors. Firstly, our hospital is a specialized referral center for ARD-treated patients, resulting in a higher concentration of MRONJ cases. Secondly, all included patients were exposed to a well-known risk factor for MRONJ, which is tooth extraction. Furthermore, the radiographic assessment was performed on a localized area, meaning that osteonecrosis lesions on the opponent quadrant from the assessed teeth, which may have been the reason for CBCT acquisition in the first place, did not impair the assessment of local risk factors.

Periosteal reaction and sequestrum formation are imaging features which may be indicative of osteonecrosis by CBCT assessment. Further studies with larger samples are required to explore these local radiographic features and their histopathological correlation. Nevertheless, by demonstrating significant variations between the study and control groups, ARD administration was found to cause trabecular bone

alterations. Interestingly, individuals treated with bisphosphonates and denosumab exhibited differential trabecular bone patterns. When it comes to clinical advice based on the present sample for the prevention of osteonecrosis in oncological patients, we urge first and foremost prevention, the abstention of smoking, and periodic dental examinations to avoid multiple extractions. For tooth extractions during ARD treatment, consider the drug pharmacokinetics before discontinuation, as a brief pause will not reduce the risk and the presence of infection may even increase the likelihood of osteonecrosis. Lastly, treatment continuation can outweigh the risk for MRONJ due to the potential for fractures and metastatic progression.[41]

Conclusion

The findings suggest that periosteal reaction on CBCT may indicate an elevated risk or possibly a latent MRONJ in oncologic patients. Similarly, sequestrum formation was exclusively seen in the ARD-treated patients and is also suspected of being a preclinical indicator of MRONJ. Additionally, the use of antiresorptive drugs can lead to bony changes, and the type of antiresorptive drug used may influence the radiographic variations observed.

Conflicts of interest

The authors declare explicitly that there are no conflicts of interest.

Author Contribution

1. C.M.R: Conceptualization, Methodology, Data Curation, Investigation, Formal analysis, Writing - Original Draft, Writing - Review & Editing.
2. R.C.F: Investigation, Writing - Review & Editing.
3. N.O.S: Investigation, Writing - Review & Editing.
4. F.N.R: Investigation, Writing - Review & Editing.
5. T.VdW: Writing - Review & Editing, Supervision.
6. R.J: Conceptualization, Writing - Review & Editing, Supervision.

References

1. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat Rev. 2001 Jun 1;27(3):165–76.

2. Hamadeh IS, Ngwa BA, Gong Y. Drug induced osteonecrosis of the jaw. Cancer Treat Rev. 2015 May 1;41(5):455–64.

3. Baron R, Ferrari S, Russell RGG. Denosumab and bisphosphonates: Different mechanisms of action and effects. Bone. 2011 Apr 1;48(4):677–92.

4. Russell RGG. Bisphosphonates: the first 40 years. Bone. 2011 Jul;49(1):2–19.

5. van Beek E, Pieterman E, Cohen L, Löwik C, Papapoulos S. Farnesyl Pyrophosphate Synthase Is the Molecular Target of Nitrogen-Containing Bisphosphonates. Biochem Biophys Res Commun. 1999 Oct 14;264(1):108–11.

6. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg. 2003 Sep 1;61(9):1115–7.

7. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022 May;80(5):920–43.

8. Wongratwanich P, Shimabukuro K, Konishi M, Nagasaki T, Ohtsuka M, Suei Y, et al. Do various imaging modalities provide potential early detection and diagnosis of medication-related osteonecrosis of the jaw? A review. Dentomaxillofac Radiol. 2021 Sep 1;50(6):20200417.

9. Aghaloo TL, Dry SM, Mallya S, Tetradis S. Stage 0 osteonecrosis of the jaw in a patient on denosumab. J Oral Maxillofac Surg. 2014 Apr;72(4):702–16.

10. Moreno-Rabié C, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep 6;24(9):2973–89.

11. Olutayo J, Agbaje JO, Jacobs R, Verhaeghe V, Velde F Vande, Vinckier F. Bisphosphonate-Related Osteonecrosis of the Jaw Bone: Radiological Pattern and the Potential Role of CBCT in Early Diagnosis. J Oral Maxillofac Res. 2010 Apr 23;1(2):e3.

12. Ristow O, Schnug G, Smielowksi M, Moratin J, Pilz M, Engel M, et al. Diagnostic accuracy comparing OPT and CBCT in the detection of non-vital bone changes before tooth extractions in patients with antiresorptive intake. Oral Dis. 2023 Apr 21;29(3):1039–49.

13. Barragan-Adjemian C, Lausten L, Ang DB, Johnson M, Katz J, Bonewald LF. Bisphosphonate-Related Osteonecrosis of the Jaw: Model and Diagnosis with Cone Beam Computerized Tomography. Cells Tissues Organs. 2009 Dec;189(1–4):284–8.

14. Hoff AO, Toth BB, Altundag K, Johnson MM, Warneke CL, Hu M, et al. Frequency and risk factors associated with osteonecrosis of the jaw in cancer patients treated with intravenous bisphosphonates. J Bone Miner Res. 2008 Jun;23(6):826–36.

15. Taylor KH, Middlefell LS, Mizen KD. Osteonecrosis of the jaws induced by anti-RANK ligand therapy. Br J Oral Maxillofac Surg. 2010 Apr;48(3):221–3.

16. Moreno-Rabié C, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, et al. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8;

17. Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, et al. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280.

18. Gaêta-Araujo H, Ferreira Leite A, de Faria Vasconcelos K, Coropciuc R, Politis C, Jacobs R, et al. Why do some extraction sites develop medication-related osteonecrosis of the jaw and others do not? A within-patient study assessing radiographic predictors. Int J Oral Implantol (Berl). 2021 Mar 16;14(1):87–98.

19. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and elaboration. International Journal of Surgery. 2014 Dec 1;12(12):1500–24.

20. Center for Disease Control and Prevention. NHIS - Adult Tobacco Use - Glossary [Internet]. Adult Tobacco use Information. 2017 [cited 2023 Jul 13]. Available from: https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm

21. Nascimento EHL, Nascimento MCC, Gaêta-Araujo H, Fontenele RC, Freitas DQ. Root canal configuration and its relation with endodontic technical errors in premolar teeth: a CBCT analysis. Int Endod J. 2019 Oct 1;52(10):1410–6.

22. Fontenele RC, Nascimento EHL, Gaêta-Araujo H, Cardelli LO de A, Freitas DQ. Which factors related to apical radiolucency may influence its radiographic detection? A study using CBCT as reference standard. Restor Dent Endod. 2021 Aug;46(3):e43.

23. Walton K, Grogan TR, Eshaghzadeh E, Hadaya D, Elashoff DA, Aghaloo TL, et al. Medication related osteonecrosis of the jaw in osteoporotic vs oncologic patients—quantifying radiographic appearance and relationship to clinical findings. Dentomaxillofacial Radiology. 2019 Jan;48(1):20180128.

24. de Castro JGK, Carvalho BF, de Melo NS, de Souza Figueiredo PT, Moreira-Mesquita CR, de Faria Vasconcelos K, et al. A new cone-beam computed tomography-driven index for osteoporosis prediction. Clin Oral Investig. 2020 Sep 7;24(9):3193–202.

25. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005 May;37(5):360–3.

26. Treister NS, Friedland B, Woo SB. Use of cone-beam computerized tomography for evaluation of bisphosphonate-associated osteonecrosis of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 May;109(5):753–64.

27. Hutchinson M, O'Ryan F, Chavez V, Lathon P V., Sanchez G, Hatcher DC, et al. Radiographic findings in bisphosphonate-treated patients with stage 0 disease in the absence of bone exposure. J Oral Maxillofac Surg. 2010 Sep;68(9):2232–40.

28. Klingelhöffer C, Klingelhöffer M, Müller S, Ettl T, Wahlmann U. Can dental panoramic radiographic findings serve as indicators for the development of medication-related osteonecrosis of the jaw? Dentomaxillofac Radiol. 2016 May;45(5):20160065.

29. Lin JH. Bisphosphonates: A review of their pharmacokinetic properties. Vol. 18, Bone. Bone; 1996. p. 75–85.

30. Gibiansky L, Sutjandra L, Doshi S, Zheng J, Sohn W, Peterson MC, et al. Population Pharmacokinetic Analysis of Denosumab in Patients with Bone Metastases from Solid Tumours. Clin Pharmacokinet. 2012 Apr;51(4):247–60.

31. Querrer R, Ferrare N, Melo N, Stefani CM, Dos Reis PED, Mesquita CRM, et al. Differences between bisphosphonate-related and denosumab-related osteonecrosis of the jaws: a systematic review. Support Care Cancer. 2021 Jun 2;29(6):2811–20.

32. Pichardo SEC, Broek FWT, Fiocco M, Appelman-Dijkstra NM, van Merkesteyn JPR. A comparison of the cone beam computed tomography findings in medication-related osteonecrosis of the jaws related to denosumab versus bisphosphonates: an observational pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020 Apr 1;129(4):411–7.

33. Aghaloo T, Hazboun R, Tetradis S. Pathophysiology of Osteonecrosis of the Jaws. Oral Maxillofac Surg Clin North Am. 2015 Nov;27(4):489–96.

34. Fedele S, Bedogni G, Scoletta M, Favia G, Colella G, Agrillo A, et al. Up to a quarter of patients with osteonecrosis of the jaw associated with antiresorptive agents remain undiagnosed. British Journal of Oral and Maxillofacial Surgery. 2015 Jan 1;53(1):13–7.

35. Bedogni A, Fedele S, Bedogni G, Scoletta M, Favia G, Colella G, et al. Staging of osteonecrosis of the jaw requires computed tomography for accurate definition of the extent of bony disease. British Journal of Oral and Maxillofacial Surgery. 2014 Sep;52(7):603–8.

36. 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. 2015 Oct;42(10):922–32.

37. Amler MH. The time sequence of tissue regeneration in human extraction wounds. Oral Surg Oral Med Oral Pathol. 1969 Mar;27(3):309–18.

38. Araújo MG, Silva CO, Misawa M, Sukekava F. Alveolar socket healing: What can we learn? Periodontol 2000. 2015 Jun 1;68(1):122–34.

39. Saia G, Blandamura S, Bettini G, Tronchet A, Totola A, Bedogni G, et al. Occurrence of bisphosphonate-related osteonecrosis of the jaw after surgical tooth extraction. J Oral Maxillofac Surg. 2010 Apr;68(4):797–804.

40. Migliorati CA, Saunders D, Conlon MS, Ingstad HK, Vaagen P, Palazzolo MJ, et al. Assessing the association between bisphosphonate exposure and delayed mucosal healing after tooth extraction. The Journal of the American Dental Association. 2013 Apr 1;144(4):406–14.

41. Liu C, Xiong YT, Zhu T, Liu W, Tang W, Zeng W. Management of Tooth Extraction in Patients Taking Antiresorptive Drugs: An Evidence Mapping Review and Meta-Analysis. J Clin Med. 2022 Dec 28;12(1):239.

CHAPTER 3: TWO-DIMENSIONAL ASSESSMENT IN OSTEOPOROTIC PATIENTS

Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction

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Abstract

Objective: To identify local radiographic risk factors for Medication-Related Osteonecrosis of the Jaws (MRONJ) in osteoporotic patients treated with antiresorptive drugs (ARD) and undergoing tooth extraction.

Material and Methods: Patients were included in this retrospective, longitudinal, case-control study, if having at least one administration of ARD, underwent tooth extraction(s), and had pre- and post-operative panoramic radiographs. Additionally, a matched control group was selected. Three calibrated, blinded, and independent observers assessed each tooth extraction site. Statistical analysis compared control against study group, and within the latter, sites MRONJ+ and MRONJ-.

Results: In total 120 patients (99 females/21 males) with 354 tooth extractions were included, from which nine patients (7.5%) and eleven tooth extraction sites (3.1%) developed MRONJ. When comparing control with study group, the latter showed significantly more thickened lamina dura, persistence of the alveolar socket, heterogeneous bone patterns, and sequestrum formation. In the study group, MRONJ developed significantly more in males (19%,p=0.049), smokers (25%,p=0.008), in the mandible (82%,p=0.027), when identifying a radiolucent or sclerotic trabecular pattern (p=0.004) or when extracting teeth with furcation involvement (p<0.001), root remnants (p=0.017), or unrestored caries lesions (p=0.005).

Conclusions: Tooth extraction sites showing radiographic signs of chronic dental infection are prone to MRONJ.

Keywords: Diphosphonates, Denosumab, Osteoporosis, Tooth Extraction, Osteonecrosis, Panoramic Radiography

Introduction

Osteoporosis is a highly prevalent disease among older individuals. It affects more than 75 million persons in Europe, Japan, and the United States, and causes more than 2.3 million fractures per year [1]. Pharmacologic treatment is indicated to reduce the risk of osteoporotic fractures and their distressing consequences. Frequently used medications are antiresorptive drugs (ARDs) such as bisphosphonates [1,2] and anti-RANKL monoclonal antibodies (denosumab) [2]. As osteoporosis is a chronic disease, patients are exposed to treatment for a long time. In fact, a Norwegian cross-sectional study of 1,402 women reported an average duration of bisphosphonate treatment of 4.3 years [3]. More specifically, the task force of the American Society for Bone and Mineral Research (ASBMR) considers a duration of treatment of 3–6 years for zoledronate and of 5–10 years for alendronate [4].

Although most ARDs are generally well tolerated, medication-related osteonecrosis of the jaws (MRONJ) has been described as a rare side effect [2,5]. MRONJ is clinically defined as exposure of bone or bone that can be probed through an intraoral or extraoral fistula(e) in the oral cavity persisting for more than eight weeks in patients with current or previous treatment with antiresorptive or antiangiogenic agents and with no history of radiation therapy to the jaws or metastatic disease to the jaws [6]. Although, experts suggest to review this definition as osteonecrosis may be present in the jaw bones even earlier [7–9].

According to the European prescribing information, the risk for MRONJ with denosumab treatment in osteoporosis increases with the duration of therapy from 0.04% at 3 years, 0.06% at 5 years and 0.44% at 10 years [10]. For bisphosphonates, the overall reported incidence is considered rare, with less than 0.001% of the treated patients diagnosed with this complication [11,12]. In case of tooth extraction, evidence is contradictory on whether or not to interrupt antiresorptive therapy to reduce the risk of MRONJ [13,14]. Moreover, the potential benefit of interrupting ARD treatment to reduce MRONJ risk is partly offset by the increased fracture risk after interruption, in particular with denosumab, given its quickly reversible effect on bone mass [15].

In addition, the intake of ARDs is not the only factor playing a role in the etiology of this pathology, but also systemic factors for instance the use of corticosteroids and diabetes mellitus [5], as well as local factors including oral infections and traumatic events such as tooth extractions [16]. Consequently, the development of MRONJ in patients with osteoporosis is likely multifactorial and should be examined separately from patients taking these drugs for oncologic indications, due to their lower dosage and considerably longer treatment periods [17].

Taken together, there is an unmet need for better risk stratification of patients with osteoporosis treated with ARDs presenting in the daily dental practice for an invasive dental procedure and to identify those sites susceptible to MRONJ [5]. The broad availability of panoramic radiographs [18,19] represents a potentially valuable source of information, with a sclerotic bone pattern being reported to be more prevalent at sites prior to developing an exposed osteonecrosis [18,20]. Therefore, the main objective of this retrospective, longitudinal, case-control study is to identify the local radiographic characteristics in panoramic images that act as a risk factor for the onset of MRONJ in patients with osteoporosis treated with antiresorptive drugs and undergoing tooth extraction.

Material and Methods

Study design and ethical considerations

The present retrospective, longitudinal, case-control study, was reported following the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines [21]. This study was approved by the ethical committee of UZ/KU Leuven, reference S63934, who waived the need for informed consent. All research was performed in accordance with ethical standards of the Institutional Review Board and the declaration of Helsinki. The extensive methodology of this study was previously published by Moreno-Rabie et al. [22].

Participant selection

A retrospective search was organized in the Oral and Maxillofacial Surgery department at University Hospitals Leuven, Belgium, covering the period between January 1st 2010 and December 31st 2019. Patients were included if, (1) they had at least one administration of ARD in doses related to osteoporotic fracture prevention, (2) underwent one or more tooth extractions, (3) presented a pre- and post-operative panoramic radiograph, and (4) documented follow-up to refute or confirm the clinical onset of exposed bone. Further exclusion criteria included, (1) prior osteonecrosis in the extraction site, (2) image-quality-related factors, (3) prior radiation therapy to the jaws, and (4) presence of other maxillofacial pathologies in the studied site.

A control group was selected from the same database and was chosen to match the study group in age, gender, and extracted tooth. As a requisite, these patients had no history of ARD, nor radiation to the head and neck, and had a pre- and post-operative panoramic image after a tooth extraction.

Data selection

The following clinical information was retrieved from the patients files, age, gender, date of pre- and post-operative panoramic radiograph, tooth extraction date, extracted teeth, prior chemo and/or radiotherapy, concomitant chronic diseases and medications (i.e. corticosteroids), ARD (prior and current treatment courses, type, dosage, and treatment duration), presence of drug holiday ≥60 days before tooth extraction, development of MRONJ, date of diagnosis, site, and classification according to the American Association of Oral and Maxillofacial Surgeons (AAOMS) [6], use of Leukocyte-Platelet Rich Fibrin (L-PRF), antiseptic mouthwash, prophylactic antibiotics, and tobacco and alcohol use.

Surgical procedure

Dental extractions were performed under local anesthesia, without vasoconstrictor in case of patients in the study group. First, an incision was made and a blunt syndesmotomy was performed. The tooth was luxated with an elevator and extracted using forceps. The extraction sockets and the underlying osteitis areas were then

carefully curetted. Then, they were rinsed with 0.9% physiological water and sutured with 3/0 vicryl resorbable sutures. No flaps were made for first intention closure. In some cases, L-PRF membranes (408g RCF/2700rpm for 12 min; IntraSpinTM, Intra-Lock®, Boca) were placed in the extraction socket prior to suture placement.

Radiographic assessment

Panoramic radiographs were collected in TIFF format and assigned a random number. Images were acquired using VistaPano S or S Ceph (Dürr Dental SE, Bietigheim-Bissingen, Germany) at 73 kVp, 12 mA and 7 seconds exposure. Three observers performed the evaluations independently and blindly using Image J program version 1.53j (Wayne Rasband, https://imagej.nih.gov/ij/) in a semi-darkened room with a high-resolution monitor (MD Barco MDRC-2221; Barco, Kortrijk, Belgium). Prior to radiographic evaluation, observers were calibrated to avoid differences in judgement. In cases of doubt a fourth observer (RJ) was consulted. The result of the evaluation was calculated by means of the observation mode. If not applicable, the cases were discussed individually until an agreement was reached. Approximately one month after concluding the initial observation, 10% of the images were re-evaluated to calculate the reliability of the observers.

The radiological evaluation consisted of the identification of certain local characteristics on each tooth that was to be extracted. The pre-operative parameters were first described by Gaêta-Araujo et al. [19], later adapted and described by Moreno et al. [22], and included the assessment of 11 parameters: horizontal bone loss, angular bone defect, furcation involvement, periodontal ligament space, lamina dura, root remnants, periapical lesion, endodontic treatment and its status, prosthodontic treatment, caries and its extent, and the trabecular bone pattern surrounding the tooth. Subsequently, 5 post-operative parameters were evaluated, including bone pattern at the extraction site, visibility of the alveolus, lamina dura, bone sequestrum formation, and crater-like defect and its extent.

Statistical analysis

RStudio version 4.0.4 (RStudio, Boston, MA US) was used to perform the statistical analysis and a p-value ≤0.05 was considered statistically significant. Before performing the image evaluation, control and study groups were tested for significance regarding their distributions of gender, age, and extracted tooth, using an unpaired t-test, a Wilcoxon rank-sum test, and a Chi-Square/Fisher's exact test. Furthermore, intra- and inter-observer agreement were tested using Cohen's (Fleiss) Kappa test, were the agreement was considered fair when the test result was >0.21 - 0.40, moderate when >0.41 - 0.60, substantial when >0.61 - 0.80, and almost perfect when >0.81 [23].

Univariate analysis

The results of the observations for each tooth and the collected data were tested using the Chi-square/Fisher exact test and the Wilcoxon rank sum test for ordinal variables. The aim was to test the independence between the control group and the group treated with ARD, and within the latter, those sites that did and did not develop MRONJ after tooth extraction. The null hypothesis was that the studied parameters are independent in the control and study groups, and in the MRONJ+ and MRONJ- extraction sites. In addition, the McNemar test was used to compare the pre- and post-operative bone pattern. Lastly, data were analyzed using a generalized linear mixed model to study univariate relations in patients with multiple tooth extractions and MRONJ+ and MRONJ- sites.

Multivariate analysis

To identify the combination of variables that had the best relationship with the development of MRONJ among the study group, a stepwise model was employed using a generalized linear model for binary data by using the logit link and considering the patient as a random factor. The assessed variables were horizontal bone loss, angular bone defect, furcation involvement, periodontal ligament, lamina dura, root remnant, periapical lesion, endodontic treatment, presence of composite or crown, presence of caries, pre-operative bone pattern, type of extracted tooth, and location. The selected

variables were subjected to a pairwise comparison and corrected for simultaneous hypothesis testing according to Tukey.

Results

A total of 120 patients on ARDs, who had a mean age of 69 years (SD±10.4) and underwent 354 tooth extractions, complied with the inclusion criteria and were included in the study group. Additionally, 126 patients who had a mean age of 68 years (SD±10.4) and underwent 364 tooth extractions, were selected as control group. When comparing both groups, there were no significant differences regarding age (W=7466.5, p=0.8676), gender (X^2=2.226, p=0.136), number- (W=7765, p=0.699), type- (W=61597, p=0.276), and sextant of the extracted tooth (W=64579, p=0.956). Descriptive data can be seen in Table 1, for a summary of the patients, and in Table 2, for a summary of the extracted teeth.

When the pre-operatory panoramic radiograph took place, the patients on ARDs had an average treatment with ARDs of 63 months (SD±46, range 1 to 240), used 1.4 antiresorptive medications, and underwent in average three tooth extractions. The ARDs were, from most to least used, alendronate (35%), denosumab (26%), zoledronic acid (17%), risedronate (10%), ibandronate (6%), and pamidronate (5%). The mean time between the pre-operative images and the tooth extraction was 2 months (range 0 – 12) for the study and 1.2 months (range 0 – 8.4) for the control group, while the time between the tooth extractions and the post-operative images was 10.9 months (range 0 – 76.8) and 7.7 months (range 0 – 56.4), respectively.

In the control group, no one developed osteonecrosis. From the total number of patients on ARDs, nine developed MRONJ (7.5%) in 11 extraction sites (3.1%). Significantly more men developed this pathology than women (19% vs. 5.1%, p=0.049). On average, patients developing MRONJ underwent six tooth extractions, while those who healed normally had three (p=0.044). The smoking habits also showed significant differences in the onset of osteonecrosis, as 25% of the patients with active tobacco use presented with the pathology in contrast to 6.9% of those who never smoked

(p=0.008). All affected patients had underlying systemic diseases, except one. These comorbidities included arrhythmia, arterial hypertension, chronic obstructive pulmonary disease, chronic hepatitis, diabetes mellitus, hypothyroidism, rheumatoid arthritis, arthrosis, renal insufficiency, and alcoholism. The diseases were grouped by affected organ or system, as shown in Table 1. There were no significant differences regarding age (p=0.690), presence of underlying systemic disease (p=0.443), type- (p=0.607), number- (p=0.523), and duration of treatment with antiresorptive drugs (p=0.513), prior chemo- and/or radiotherapy (p=0.167), alcohol consumption (p=0.183), and corticosteroid intake (p=1.000) among patients on ARDs that did and did not develop MRONJ.

The prevalence of MRONJ was significantly higher in the mandible (82%) compared to the maxilla (18%, p=0.027), and in molars (82%) rather than incisors, canines and premolars (p=0.004). Upon evaluation of the drug holiday, a 2-month holiday period did not show significant differences in the development of osteonecrosis (p=0.298). However, since most patients took long-acting bisphosphonates, an additional analysis was performed considering a recess period of at least 12 months. This analysis did not show significant differences either (p=0.756). The remaining measured factors, namely the use of L-PRF (p=0.433), prophylactic antibiotics (p=0.182), and antiseptic mouthwash (p=0.182), also showed absence of significance in the onset of MRONJ in patients on ARDs.

The overall interobserver agreement was substantial (K=0.68), ranging from a fair agreement in the evaluation of sequestrum formation (K=0.23) and an almost perfect agreement in the presence of endodontic treatment (K=0.95). Furthermore, the intraobserver agreement was also substantial (K=0.77), showing a moderate agreement in the periodontal ligament space assessment (K=0.48) and an almost perfect agreement in the presence of endodontic treatment (K=0.95).

Pre- and post-operative parameters: control vs. study group.

When looking at the pre-operative characteristics of the extraction sites, a significantly higher prevalence of thickened lamina dura was observed in the study group (12%) in

comparison to the control group (3%, p<0.001). In contrast, the occurrence of bone patterns different from the normal trabecular pattern did not differ between groups (p=0.222) (see Table 3).

In the post-operative assessment, a heterogenous bone pattern was seen rarely but exclusively in patients on ARDs (2%, p=0.020). Additionally, a visible extraction socket (60%, p<0.001) and persistence of the lamina dura (65%, p<0.001), were significantly more observed in the study group. Furthermore, sequester formation was seen only in patients treated with ARDs. Finally, a resorption pattern in the form of a crater-like defect was seen more often in the control than in the study group (9% vs 3%, p=0.001).

The pre- and post-operative bone pattern showed no significant difference in appearance within the control group (p=0.889), but a significant difference was seen in the antiresorptive-treated group (p=0.047). In the latter, 36 sites initially showing normal trabeculae exhibited a sclerotic or heterogeneous post-operative appearance, and three initially sclerotic sites were seen after tooth extraction as heterogeneous.

Pre- and post-operative parameters: MRONJ+ vs. MRONJ-.

Regarding the sites that did and did not develop osteonecrosis within the study group, a summary of the data and their p-values can be found in Table 3. It was observed that 11% of the teeth that had furcation involvement developed osteonecrosis while only 1.1% of those with absence of this characteristic (p<0.001). Moreover, there was a higher chance of the onset of the pathology when having root remnants (p=0.017) and caries lesions (p=0.005) (Figure 1). Furthermore, a radiolucent (67%) and a sclerotic bone pattern (4%), increased the chance of developing osteonecrosis in comparison to a normal bone pattern (2%, p=0.004).

Postoperatively, 2% of the sites with a normal bone pattern were associated with MRONJ, in contrast to 5% of the sclerotic, all of the radiolucent, and none with a heterogenous bone pattern (p<0.001). In addition, 40% of the crater-like defects were seen in osteonecrosis sites (p<0.001) and the presence of sequestrum formation was exclusively seen this group (p<0.001).

MRONJ- and MRONJ+ paired analysis (within-patient).

The results of the analysis which accounted for multiple tooth extractions in one patient and with post-operative sites that healed normally and others that developed osteonecrosis, were similar to those reported previously. A more frequent development of osteonecrosis was seen in male patients (p=0.011), with greater number of tooth extractions (p=0.008), and with hepatic comorbidities (p=0.027). At a systemic level the presence of other comorbidities, such as diabetes (p=0.405) or the use of corticosteroids (p=0.751) did not show significant results. Additionally, at a local level, significant results were also seen in terms of an increased development of osteonecrosis in molars (p<0.001), in the mandible (p<0.001), in teeth with moderate or severe radiographic bone resorption (p<0.001), furcation involvement (p<0.001), root remnants (p<0.001), periapical lesion (p=0.007), caries (p<0.001) and a different bone pattern than normal (p<0.001).

Radiographic characteristics of periodontal and endodontic pathology associated with the development of MRONJ.

The variables selected by the pre-operative logistic regression model were tooth type (p<0.001), location (p<0.001), angular bone defect (p=0.036), furcation involvement (p<0.001), root remnant (p<0.001), endodontic treatment (p=0.005), presence of prosthodontic treatment (p<0.001), caries (p<0.001), and trabecular bone pattern (p<0.001). Nonetheless, after pairwise comparisons and correcting for multiple testing, only some variables maintained their significance, as follows: premolar teeth (OR=4.6, 95% CI 1.2 - 17.9, p=0.022) and molars (OR=7.9, 95% CI 1.3 - 47.6, p=0. 019) over canines and incisors, mandibular teeth (OR=1.6, 95% CI 1.3 - 1.9, p<0.001), with furcation involvement (OR=7.3, 95% CI 4.9 - 11.1, p<0.001), angular bone defects (OR=1.3, 95% CI 0.9 - 1.9, p=0.036), root remnant (OR=1.6, 95% CI 1.2 - 2.1, p<0.001), with absence of restorations (OR=1.2, 95% CI 0.9 - 1.5, p=0. 004) and endodontic treatments (OR=1.1, 95% CI 0.9 - 1.4, p=0.005), and in sites with a radiolucent trabecular pattern (OR=18.76, 95% CI 1.1 - 312.5, p=0.036).

Table 1. Descriptive data from study and control patients.

Characteristics of patients		Study group (on ARDs)			Control
Patients, n		120			126
Development of osteonecrosis, n		**Patients** 120	**MRONJ + (%)** 9 (7.5%)	**MRONJ - (%)** 111 (92.5%)	0
Age at tooth extraction (mean ± SD)		69 ± 10.4	70.3 ± 10.6	68.2 ± 10.1	68.4 ± 10.4
Age (years)	30-45	3	1 (33.3%)	2 (66.7%)	3
	46-60	23	1 (4.3%)	22 (95.7%)	25
	61-75	62	3 (4.8%)	59 (95.2%)	69
	76-92	32	4 (12.5%)	28 (87.5%)	29
Sex, n	Female	99	5 (5.1%)	94 (94.9%)	93
	Male	21	4 (19%)	17 (81%)	33
Underlying chronic disease, n	Cardiac	64	6 (9.4%)	58 (90.6%)	23
	Hypertension	48	5 (10.4%)	43 (89.6%)	49
	Respiratory	20	2 (10%)	18 (90%)	11
	Renal	11	1 (9.1%)	10 (90.9%)	5
	Hepatic	7	2 (28.6%)	5 (71.4%)	4
	Pancreatic	2	0 (0%)	2 (100%)	0
	Endocrine	28	3 (10.7%)	25 (89.3%)	33
	Diabetes	16	2 (12.5%)	14 (87.5%)	18
	Thyroid	13	1 (7.7%)	12 (92.3%)	17
	Rheumatoid	14	2 (14.3%)	12 (85.7%)	19
	Gastrointestinal	4	0 (0%)	4 (100%)	0
	Prior cancer	24	1 (4.2%)	23 (95.8%)	26
Corticosteroid use, n	Yes	27	2 (7.4%)	25 (92.6%)	10
	Mean dose (mg/day)	7.6 mg	3 mg	7.9 mg	18 mg
	Length (mean)	75 months	37 months	78 months	35 months
Antiresorptive drug, n	Bisphosphonate	76	7 (9.2%)	69 (90.8%)	NA
	Denosumab	16	0 (0%)	16 (100%)	NA
	Both	28	2 (7.1%)	26 (92.9%)	NA
Number of ARDs, n	1	84	7 (8.3%)	77 (91.7%)	NA
	2	26	2 (7.7%)	24 (92.3%)	NA
	3	9	0 (0%)	9 (100%)	NA
	4	1	0 (0%)	1 (100%)	NA
Time on ARDs (months), n	≤12	17	1 (5.9%)	16 (94.1%)	NA
	>12 - ≤24	17	2 (11.8%)	15 (88.2%)	NA
	>24 - ≤36	17	1 (5.9%)	16 (94.1%)	NA
	>36 - ≤48	8	1 (12.5%)	7 (87.5%)	NA
	>48 - ≤60	14	0 (0%)	14 (100%)	NA
	>60 - ≤120	32	1 (3.1%)	31 (96.9%)	NA
	>120 - ≤240	15	3 (20%)	12 (80%)	NA
Alcohol consumption, n	No consumption	33	1 (3%)	32 (97%)	32
	1 - 2 Units per day	54	5 (9.3%)	49 (90.7%)	62
	> 2 Units per day	7	1 (14.3%)	6 (85.7%)	11
	Prior abuse	5	1 (20%)	4 (80%)	4
	Unknown	21	1 (4.8%)	20 (95.2%)	17
Tobacco use, n	Previous use	33	0 (0%)	33 (100%)	31
	Active use	16	4 (25%)	12 (75%)	7
	Never smoked	58	4 (6.9%)	54 (93.1%)	80
	Unknown	13	1 (7.7%)	12 (92.3%)	8

NA: not applicable.

Table 2. Descriptive data of the extracted teeth in the study and control groups.

Characteristics of extracted teeth		Study group (on ARDs)			Control
Extracted teeth, n		354			364
Development of osteonecrosis, n		**Teeth** 354	**MRONJ + (%)** 11 (3.1%)	**MRONJ - (%)** 343 (96.9%)	NA
Type of teeth, n (%)	Incisors and canines	123	0 (0%)	123 (100%)	137
	Premolars	80	2 (2.5%)	78 (97.5%)	87
	Molars	151	9 (6%)	142 (94%)	140
Region, n (%)	Anterior maxilla	79	0 (0%)	79 (100%)	70
	Posterior maxilla	113	2 (1.8%)	111 (98.2%)	129
	Anterior mandible	44	0 (0%)	44 (100%)	67
	Posterior mandible	118	9 (7.6%)	109 (92.4%)	98
Hemiarch, n (%)	Maxilla	192	2 (1%)	190 (99%)	199
	Mandible	162	9 (5.6%)	153 (94.4%)	165
Underlying dental disease*, n (%)	Nonapparent	36	0 (0%)	36 (100%)	36
	Periodontal pathology	40	0 (0%)	40 (100%)	43
	Endodontic pathology	90	2 (2.2%)	88 (97.8%)	92
	Combined lesion	188	9 (4.8%)	179 (95.2%)	193
Drug holiday >2 months, n (%)	No	84	4 (4.8%)	80 (95.2%)	NA
	Yes	270	7 (2.6%)	263 (97.3%)	NA
Antibiotic prophylaxis, n (%)	No	48	3 (6.3%)	45 (93.8%)	344
	Yes	301	8 (2.7%)	293 (97.3%)	20
Antiseptic mouthwash, n (%)	No	48	3 (6.3%)	45 (93.8%)	14
	Yes	301	8 (2.7%)	293 (97.3%)	350
Use of L-PRF, n (%)	No	168	7 (4.2%)	161 (95.8%)	354
	Yes	186	4 (2.2%)	182 (97.8%)	10
MRONJ worse stage, n (%)	Stage 1	5	5 (45.5%)	NA	NA
	Stage 2	6	6 (55.5%)	NA	NA
	Stage 3	0	0 (0%)	NA	NA

Within the study group, the sites were classified as MRONJ+ and MRONJ-. NA: not applicable. (*): According to the radiographic characteristics, the teeth were classified into: periodontal pathology, those with horizontal bone loss, angular bone defect or furcation involvement; endodontic pathology, those with pulpal caries, widening of the periodontal ligament space, or periapical lesion; and with combined endodontic-periodontal lesions, when they presented characteristics of both groups.

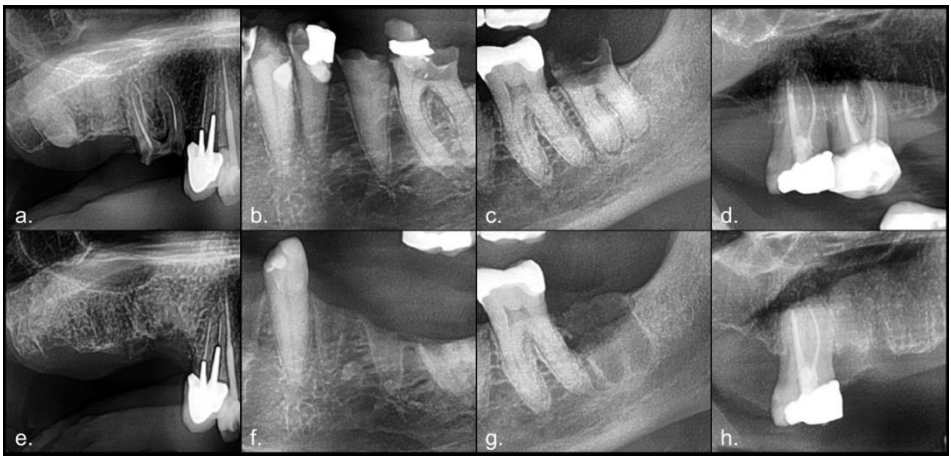

Figure 1. Cropped panoramic images indicating pre- (a-d) and post-operative (e-h) extraction sites. All these sites developed osteonecrosis after tooth extraction and in the pre-operative radiographs it is possible to see the risk factors, furcation involvement (a, b, c, d), root remnants (a, b, c), and pulpal caries (a, b, c).

Table 3. Distribution of the extraction sites in the study (MRONJ+ and MRONJ-) and control group according to the pre- and post-operative radiographic characteristics.

Observed radiographic parameters		Study group (on ARDs)				Control group	
		Sites n = 354	**MRONJ + (%)** n = 11	**MRONJ - (%)** n = 343	**p-value**	n = 364	**p-value**
Pre-operative assessment							
Horizontal bone loss	Absent/initial	136	3 (2.2%)	133 (97.8%)	0.541	138	0.950
	Moderate/severe	218	8 (3.7%)	210 (96.3%)		226	
Angular bone defect	Absent	328	10 (3%)	318 (97%)	0.573	338	1.000
	Present	26	1 (3.8%)	25 (96.2%)		26	
Furcation involvement	Absent	281	3 (1.1%)	278 (98.9%)	*<0.001*	285	0.792
	Present	73	8 (11%)	65 (89%)		79	
Periodontal ligament space	Normal	156	4 (2.6%)	152 (97.4%)	0.761	169	0.575
	Widened	198	7 (3.5%)	191 (96.5%)		195	
Lamina dura	Normal	310	10 (3.2%)	300 (96.8%)	1.000	352	*<0.001*
	Thickened	44	1 (2.3%)	43 (97.7%)		12	
Root remnant	Absent	299	6 (2%)	293 (98%)	*0.017*	297	0.355
	Present	55	5 (9.1%)	50 (90.9%)		67	
Periapical lesion	Absent	233	5 (2.1%)	228 (97.9%)	0.196	249	0.510
	Present	121	6 (5%)	115 (95%)		115	
Endodontic treatment	Absent	253	9 (3.6%)	244 (96.4%)	0.882	218	*0.005*
	Adequate filling	63	1 (1.6%)	62 (98.4%)		89	
	Inadequate filling	38	1 (2.6%)	37 (97.4%)		57	
Prosthodontic treatment	Absent	138	6 (4.3%)	132 (95.7%)	0.350	169	0.052
	Present	216	5 (2.3%)	211 (97.7%)		195	
Caries*	Absent	219	2 (0.9%)	217 (99.1%)	*0.005*	175	*<0.001*
	Dentinal	17	2 (11.8%)	15 (88.2%)		34	
	Pulpal	118	7 (5.9%)	111 (94.1%)		155	
Bone pattern	Normal	298	7 (2.3%)	291 (97.7%)	*0.004*	294	0.222
	Sclerotic	52	2 (3.8%)	50 (96.2%)		69	
	Radiolucent	3	2 (66.7%)	1 (33.3%)		1	
	Heterogenous	1	0 (0%)	1 (100%)		0	
Post-operative assessment							
Bone pattern	Normal	279	6 (2.2%)	273 (97.8%)	*0.001*	288	*0.020*
	Sclerotic	65	3 (4.6%)	62 (95.4%)		73	
	Radiolucent	2	2 (100%)	0 (0%)		3	
	Heterogenous	8	0 (0%)	8 (100%)		0	
Alveolar socket	Absent	201	3 (1.5%)	198 (98.5%)	0.121	260	*<0.001*
	Visible	153	8 (5.2%)	145 (94.8%)		104	
Lamina dura	Absent	214	4 (1.9%)	210 (98.1%)	0.074	289	*<0.001*
	Visible	140	7 (5%)	133 (95%)		75	
Sequestrum formation	Absent	352	9 (2.6%)	343 (97.4%)	*<0.001*	364	0.243
	Visible	2	2 (100%)	0 (0%)		0	
Crater-like defect	Absent	344	7 (2%)	337 (98%)	*<0.001*	332	*0.001*
	Visible	10	4 (40%)	6 (60%)		32	

Statistically significant values are shown in italics. These results were obtained using the chi-square/Fisher's exact test, except (*), which used the Wilcoxon rank sum test.

Discussion

The aim of this study was to identify local radiographic predictors for MRONJ in patients with osteoporosis treated with ARDs, considering pre- and post-operative parameters. For such purpose, a case-control design was presented. Pre-operative radiographic evaluation demonstrated that extraction of teeth with furcation involvement, root remnants, and untreated caries lesions led more frequently to an exposed form of osteonecrosis. Postoperatively, sites with osteonecrosis presented with persistence of alveolar socket, thickening of the lamina dura, a heterogeneous trabecular bone pattern, and bone sequestrum formation.

As life expectancy lengthens, certain diseases such as osteoporosis become more prevalent, and with it the use of antiresorptive drugs as therapy. Although today the incidence of MRONJ in these patients is considered rather low, 21 to 22.9 per 100,000 person-years [24,25], many efforts have been made to identify the elements that make a patient susceptible to this pathology. Considering the local factors, it is crucial to identify them prior to the dental extractions to minimize the risk of MRONJ development.

Dental extractions have been widely reported as a triggering factor for the development of osteonecrosis of the jaws [2,5,24,25]. However, the risk in osteoporotic patients is low. A recent 12-month observational cohort study described only one patient (0.6%) presenting with MRONJ after tooth extractions in a cohort of 45 patients (43 females and 2 males) with 159 surgeries performed [26]. MRONJ was also developed by only a minority of the studied patients in the present study, although the frequency was higher and may be due to our inclusion criteria and the fact that our hospital is a referral center for ARD-treated patients. A systematic review that collected data on 680 osteoporotic patients who had MRONJ, reported a mean age of 69.7 ± 5.2 years, that the majority were women (93.5%), took alendronate (72.6%), and had a mean treatment duration of 51.9 months (ranging from 2 to 93 months) [5]. In our results the mean age of the MRONJ+ group was also 70 years, and most were in treatment with alendronate or zoledronic acid. Yet, the mean duration of treatment was somewhat longer, 81 months, and osteonecrosis was seen more frequently in men than in women.

At a systemic level, the duration of antiresorptive therapy has been mentioned in multiple publications as a major risk factor [5,18]. In fact, a Korean nationwide cohort study found no association between the route of administration and the development of MRONJ, but a positive association with cumulative dose among bisphosphonate users [24]. In the present study, although there were no significant differences between drug type and duration of the treatment, a longer treatment (mean 81 months) was observed among patients who developed osteonecrosis than for those who healed normally (mean 61 months). Yet, studies have shown that treatment discontinuation is a subject of debate without evidence to confirm a reduced risk of osteonecrosis [13,27]. Moreover, interruption of ARDs would only increase the occurrence of fractures in the period of cessation, especially in patients treated denosumab because of its quick reversible effect [13].

Systemic diseases such as hypertension [5,24], diabetes mellitus [5,24,28], rheumatoid arthritis [24,25,28], corticosteroid use [5,25,28], and tobacco consumption [28] have also been mentioned as risk factors. In our sample, smoking proved to be a risk factor and all but one patient who developed osteonecrosis presented with an underlying systemic disease and two had long-term corticosteroid use. Nonetheless, no statistical differences were found when comparing those with systemic pathologies with those without.

At a local level, osteonecrosis occurs most frequently in the mandible (70.6%)[5] and infections seem to be the most important risk factor [5,24]. Concretely, studies point to periodontal disease as a risk factor [24,25], which is consistent with our results, as we found a higher incidence of the pathology in the mandible, in molars, and in teeth with furcation involvement. The terminal circulation can explain the predilection for affecting the molars of the mandible. When angiogenesis is inhibited due to ARDs [6], the molar regions would be the most affected, in contrast to the anterior region which receives circulation from the contralateral side. In addition, molars are more than twice the volume of incisors, canines, and premolars, thereby requiring a considerably greater surface area to heal after extraction [29].

The importance of timely control of local infection cannot be overemphasized, considering that the longer the wait with infection, the higher the risk. Supporting this statement, a Japanese study described that patients who waited more than two months to undergo tooth extraction had significantly more delayed healing than patients who underwent tooth extraction within one month after consultation, regardless of whether the ARD was still administered [30]. Yet, as in the case of these study patients, prevention is not always timely and tooth extractions are inevitable [31]. To some extent, all teeth had some degree of infection or susceptibility to infection, but an important point to emphasize is that there are patients who had several teeth extracted at the same time but only one developed osteonecrosis. This raises the question of local associated factors and whether there are differences in the type of infection or radiological appearance of the extraction site [19].

It is well-known that the use of antiresorptive medication induces changes in bone [20], with some of these changes considered as a sign of medication use, while others indicate a risk factor for osteonecrosis. In particular, thickening of the lamina dura and of the mandibular cortex have been reported significantly more often in patients treated with these drugs than in the control group, but no differences were found when comparing patients who did and did not develop osteonecrosis [18,32,33]. This was also observed in our sample. On the other hand, the presence of focal and diffuse sclerosis was seen in both the ARD treatment group and in MRONJ+ sites. Based on these findings, Kubo et al. considered sclerosis as a local risk factor for MRONJ and lamina dura thickening as a local feature in patients treated with ARDs [18].

Postoperatively, these drugs also cause changes in the healing process following tooth extraction. These changes have been reported by other studies [20] and in the present sample we saw a significantly higher persistence of the extraction socket in patients treated with ARD than in the control group. Other postoperative findings in the ARD treatment group are a greater persistence of lamina dura, a sclerotic and heterogeneous trabecular bone pattern, and bone sequestrum formation. These features persist in sites that develop osteonecrosis. Koth et al. described a correlation between visible bone sclerosis on panoramic radiographs and the persistence of the

alveolar cavity and osteolysis at the same site. Furthermore, osteolysis was correlated with the formation of a sequestration [34]. The latter appear to be a pathognomonic sign of osteonecrosis, as they were only observed at MRONJ+ sites.

This study has several limitations. First, those related to the retrospective nature that inherits limited generalizability and risk of recall bias. Secondly, given the small prevalence of osteonecrosis, this sample includes few patients and extraction sites that led to the onset of the pathology. Third, our sample included patients with corticoid induced osteoporosis and their comorbidities may also be predisposing factors [28]. Finally, we focused on the development of exposed necrotic bone in the oral cavity, disregarding that histological bone necrosis may be present already on those sites showing changes in their radiodensity but with intact mucosa [7]. However, despite the limitations, the clinical relevance of this study lies in supporting prior findings which demonstrate that patients treated with ARDs exhibit distinctive bony characteristics visible on panoramic radiographs and in highlighting the importance of dental infection control as a risk factor for the development of osteonecrosis.

Conclusion

To conclude, among patients with osteoporosis treated with antiresorptive medications and undergoing tooth extraction, radiographic signs of chronic dental infection seem to predict MRONJ onset since a greater development of the pathology was seen in sites with furcation involvement, root remnants or that had untreated dentinal or pulpal caries lesions. It was also possible to confirm that ARDs induced bony changes that are visible in the diagnostic images commonly used at the dental practice. Postoperatively, these bony changes induced by ARDs are persistence of the alveolar socket and of the lamina dura, a heterogeneous bone pattern, and sequestration formation. In addition, sequestrum formation appears to be a pathognomonic feature of MRONJ, as it only appeared in MRONJ+ sites. The identification of the pre-operative radiographic predictors of MRONJ is crucial for developing a preventive strategy.

Furthermore, the early identification of the post-operative MRONJ findings may result in an early initiation of treatment with improved patient's outcomes.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Author contribution

1. C.M.R: Conceptualization, Methodology, Data Curation, Investigation, Formal analysis, Writing - Original Draft, Writing - Review & Editing
2. H.G.A: Conceptualization, Investigation, Writing - Review & Editing
3. A.L: Investigation, Writing - Review & Editing
4. W.C: Formal analysis
5. E.G: Writing - Review & Editing
6. T.VdW: Writing - Review & Editing, Supervision
7. R.J: Conceptualization, Writing - Review & Editing, Supervision

References

1. WHO Scientific Group of Osteoporosis. Prevention and management of osteoporosis: report of a WHO scientific group. Geneva, Switzerland: World Health Organization; 2003. p. 192 p. (WHO technical report series, no. 921).

2. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw--2014 update. J Oral Maxillofac Surg. 2014 Oct;72(10):1938–56.

3. Hoff M, Skovlund E, Meyer HE, Langhammer A, Søgaard AJ, Syversen U, et al. Does treatment with bisphosphonates protect against fractures in real life? The HUNT study, Norway. Osteoporosis International. 2021 Jul 1;32(7):1395–404.

4. Adler RA, El-Hajj Fuleihan G, Bauer DC, Camacho PM, Clarke BL, Clines GA, et al. Managing Osteoporosis in Patients on Long-Term Bisphosphonate Treatment: Report of a Task Force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2016 Jan 1;31(1):16–35.

5. Aljohani S, Fliefel R, Ihbe J, Kühnisch J, Ehrenfeld M, Otto S. What is the effect of anti-resorptive drugs (ARDs) on the development of medication-related osteonecrosis of the jaw (MRONJ) in osteoporosis patients: A systematic review. Journal of Cranio-Maxillofacial Surgery. 2017 Sep 1;45(9):1493–502.

6. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022 May;80(5):920–43.

7. Nicolatou-Galitis O, Papadopoulou E, Vardas E, Kouri M, Galiti D, Galitis E, et al. Alveolar bone histological necrosis observed prior to extractions in patients, who received bone-targeting agents. Oral Dis. 2020 Jul;26(5):955–66.

8. Hamada H, Matsuo A, Koizumi T, Satomi T, Chikazu D. A simple evaluation method for early detection of bisphosphonate-related osteonecrosis of the mandible using computed tomography. Journal of Cranio-Maxillofacial Surgery. 2014 Sep;42(6):924–9.

9. Campisi G, Bedogni A, Fusco V. Raccomandazioni clinico-terapeutiche sull'osteonecrosi delle ossa mascellari (ONJ) farmaco-relata e sua prevenzione. Versione 2. Palermo University Press; 2020. 1–234 p.

10. European Medicines Agency. Prolia (denosumab). Product information. 05/05/2022 Prolia - EMEA/H/C/001120 - II/0093. [Internet]. https://www.ema.europa.eu/en/documents/product-information/prolia-epar-product-information_en.pdf. 2022 [cited 2022 Oct 12]. p. 1–44. Available from: https://www.ema.europa.eu/en/documents/product-information/prolia-epar-product-information_en.pdf

11. European Medicines Agency. Fosavance (alendronic acid/colecalciferol). Product information. 27/10/2021 Fosavance - EMEA/H/C/000619 - N/0052. [Internet]. https://www.ema.europa.eu/en/documents/product-information/fosavance-epar-product-information_en.pdf. 2021 [cited 2022 Dec 5]. p. 1–36. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/fosavance

12. European Medicines Agency. Aclasta (zoledronic acid). Product information. 09/11/2022 Aclasta - EMEA/H/C/000595 - T/0079. [Internet]. https://www.ema.europa.eu/en/documents/product-information/aclasta-epar-product-information_en.pdf. 2022 [cited 2022 Dec 5]. p. 1–41. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/aclasta

13. Taguchi A, Shiraki M, Tsukiyama M, Miyazaki T, Soen S, Ohta H, et al. Impact of Osteonecrosis of the Jaw on Osteoporosis Treatment in Japan: Results of a Questionnaire-Based Survey by the Adequate Treatment of Osteoporosis (A-TOP) Research Group. Calcif Tissue Int. 2015 Jul 26;97(6):542–50.

14. Nicolatou-Galitis O, Schiødt M, Mendes RA, Ripamonti C, Hope S, Drudge-Coates L, et al. Medication-related osteonecrosis of the jaw: definition and best practice for prevention, diagnosis, and treatment. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019 Feb 1;127(2):117–35.

15. Cosman F, Huang S, McDermott M, Cummings SR. Multiple Vertebral Fractures After Denosumab Discontinuation: FREEDOM and FREEDOM Extension Trials Additional Post Hoc Analyses. J Bone Miner Res. 2022 Nov 12;37(11):2112–20.

16. Otto S, Tröltzsch M, Jambrovic V, Panya S, Probst F, Ristow O, et al. Tooth extraction in patients receiving oral or intravenous bisphosphonate administration: A trigger for BRONJ development? J Craniomaxillofac Surg. 2015 Jul 1;43(6):847–54.

17. Adler RA. Update on Rare Adverse Events from Osteoporosis Therapy and Bisphosphonate Drug Holidays. Endocrinol Metab Clin North Am. 2021 Jun;50(2):193–203.

18. Kubo R, Ariji Y, Taniguchi T, Nozawa M, Katsumata A, Ariji E. Panoramic radiographic features that predict the development of bisphosphonate-related

osteonecrosis of the jaw. Oral Radiol. 2018 May 12;34(2):151–60.

19. Gaêta-Araujo H, Ferreira Leite A, de Faria Vasconcelos K, Coropciuc R, Politis C, Jacobs R, et al. Why do some extraction sites develop medication-related osteonecrosis of the jaw and others do not? A within-patient study assessing radiographic predictors. Int J Oral Implantol (Berl). 2021 Mar 16;14(1):87–98.

20. Moreno-Rabié C, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep 6;24(9):2973–89.

21. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and elaboration. International Journal of Surgery. 2014 Dec 1;12(12):1500–24.

22. Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, et al. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Dec 4;12(1):11280.

23. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005 May;37(5):360–3.

24. Kim SH, Lee YK, Kim TY, Ha YC, Jang S, Kim HY. Incidence of and risk for osteonecrosis of the jaw in Korean osteoporosis patients treated with bisphosphonates: A nationwide cohort-study. Bone. 2021 Feb 1;143.

25. Ishimaru M, Ono S, Morita K, Matsui H, Hagiwara Y, Yasunaga H. Prevalence, Incidence Rate, and Risk Factors of Medication-Related Osteonecrosis of the Jaw in Patients With Osteoporosis and Cancer: A Nationwide Population-Based Study in Japan. J Oral Maxillofac Surg. 2022 Apr;80(4):714–27.

26. Cuozzo A, Iorio-Siciliano V, Vaia E, Mauriello L, Blasi A, Ramaglia L. Incidence and risk factors associated to Medication-Related Osteo Necrosis of the Jaw (MRONJ) in patients with osteoporosis after tooth extractions. A 12-months observational cohort study. J Stomatol Oral Maxillofac Surg. 2022 Nov;123(6):616–21.

27. Ottesen C, Schiodt M, Gotfredsen K. Efficacy of a high-dose antiresorptive drug holiday to reduce the risk of medication-related osteonecrosis of the jaw (MRONJ): A systematic review. Heliyon. 2020 Apr 1;6(4):e03795.

28. Everts-Graber J, Lehmann D, Burkard J, Schaller B, Gahl B, Häuselmann H, et al. Risk of Osteonecrosis of the Jaw Under Denosumab Compared to Bisphosphonates in Patients With Osteoporosis. Journal of Bone and Mineral Research. 2022 Feb;37(2):340–8.

29. Couso-Queiruga E, Ahmad U, Elgendy H, Barwacz C, González-Martín O, Avila-Ortiz G. Characterization of Extraction Sockets by Indirect Digital Root Analysis. Int J Periodontics Restorative Dent. 2021 Jan;41(1):141–8.

30. Kamimura M, Taguchi A, Komatsu M, Koiwai H, Ashizawa R, Ichinose A, et al. Long waiting time before tooth extraction may increase delayed wound healing in elderly {Japanese}. Osteoporos Int. 2019 Mar 5;30(3):621–8.

31. Campisi G, Mauceri R, Bertoldo F, Bettini G, Biasotto M, Colella G, et al. Medication-Related Osteonecrosis of Jaws (MRONJ) Prevention and Diagnosis: Italian Consensus Update 2020. Int J Environ Res Public Health. 2020 Aug 18;17(16):5998.

32. Torres SR, Chen CSK, Leroux BG, Lee PP, Hollender LG, Lloid M, et al. Mandibular inferior cortical bone thickness on panoramic radiographs in patients using bisphosphonates. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015 May;119(5):584–92.

33. Barngkgei I, Halboub E, Almashraqi A. Effect of bisphosphonate treatment on the jawbone: an exploratory study using periapical and panoramic radiographic evaluation. Oral Radiol. 2019 May 7;35(2):159–70.

34. Koth VS, Figueiredo MA, Salum FG, Cherubini K. Interrelationship of clinical, radiographic and haematological features in patients under bisphosphonate therapy. Dentomaxillofac Radiol. 2017 Apr;46(4):20160260.

CHAPTER 4: THREE-DIMENSIONAL ASSESSMENT IN OSTEOPOROTIC PATIENTS

Key Insights into Antiresorptive Drug Use and Osteonecrosis in Osteoporotic Patients Undergoing Tooth Extractions: a clinical and CBCT assessment

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Abstract

Purpose: To describe the effects of antiresorptive drugs (ARD) in the jawbones and risk factors for Medication-Related Osteonecrosis of the Jaws (MRONJ) in osteoporotic patients undergoing tooth extractions using clinical data and cone beam computed tomography (CBCT).

Methods: This retrospective cohort study collected clinical and CBCT data from 176 patients. The study group (n=78; 224 extractions) received ARD treatment, underwent tooth extraction, and had a pre-operative CBCT. Additionally, age-, sex-, and tooth-matched controls were selected (n=98; 227 extractions). Radiographic examinations were performed independently by three calibrated examiners. Statistical analysis employed Chi-Square/Fisher's exact/Mann-Whitney U/t tests to contrast study and control, MRONJ+ and MRONJ-, and bisphosphonate and denosumab patients/sites. Significance was set at $p \leq 0.05$.

Results: From the study group, 4 patients (5%) and 5 sites (2%) developed MRONJ after tooth extraction. Controls did not develop MRONJ. ARD-treated patients exhibited significantly more thickening of the lamina dura ($p<0.001$) and a longer average mucosal healing time (4.4 weeks) than controls (2.6 weeks, $p<0.001$). In the study group, MRONJ risk significantly increased with corticosteroid intake ($p=0.021$) and in multi-rooted teeth ($p=0.041$). No significant differences between bisphosphonates and denosumab use were seen in the tomographic features ($p>0.05$). Lastly, the presence of bone sequestrum was exclusively seen in osteoporotic patients, who showed post-operative exposed bone or histological evidence of osteonecrosis.

Conclusion: Osteoporotic patients under ARD may exhibit thickening of the lamina dura and prolonged post-operative healing. Among these patients, multi-rooted teeth are at higher risk for MRONJ than single-rooted teeth. Sequester formation is an indicator of osteonecrosis.

Keywords: Diphosphonates, Denosumab, Tooth Extraction, Osteonecrosis, Osteoporosis, Cone-Beam Computed Tomography

Introduction

Osteoporosis is a systemic skeletal disorder characterized by decreased bone mass and microarchitectural deterioration, which is a growing concern in an aging population [1]. Antiresorptive drugs (ARDs), including bisphosphonates and denosumab, play a crucial role in managing osteoporosis by inhibiting bone resorption and reducing fracture risk [1,2]. However, their prolonged use has been associated with a rare but severe complication known as medication-related osteonecrosis of the jaw (MRONJ) [2,3].

MRONJ is an oral pathology characterized by exposed bone or bone that can be probed through an intraoral or extraoral fistula(e) in the oral cavity persisting for more than eight weeks in patients with current or previous treatment with ARD or antiangiogenic agents and with no history of radiation therapy to the jaws or metastatic disease to the jaws [4]. Although MRONJ incidence is relatively low, affecting between 0.001% and 0.4% of the ARD-treated osteoporotic patients [5–7], studies have reported an increased risk after tooth extractions, ranging from 2.3% to 3.4% [8–10].

As osteonecrosis can negatively impact an individual's quality of life, it is important not only to timely diagnose MRONJ but also to identify the predisposing factors or latent lesions when tooth extractions are needed and anticipate the best perioperative approach [11]. In this regard, the duration of ARD treatment [10], the use of corticosteroids, and diabetes mellitus [3] have been linked to MRONJ. In addition, specifically when dental extractions are involved, local risk factors for this pathology such as osteosclerosis or osteolysis, teeth with furcation involvement or untreated dentinal caries, multi-rooted teeth, and mandibular extraction sites have been identified [12].

Several studies have employed diagnostic imaging to investigate the effects of ARDs on the jaw and their potential contribution to osteonecrosis development [13]. While some investigations have been carried out with panoramic radiographs [12,14], the use of cone beam computed tomography (CBCT) images in osteoporotic patients for this diagnostic task has been rarely used. CBCT studies have predominantly focused on the mandibular cortical area [15,16], leaving a gap in the comprehensive evaluation of

broad-spectrum changes in this population. Hence, the objective of this retrospective cohort study is to describe the effects of ARDs in the jawbones and identify risk factors for the development of osteonecrosis in osteoporotic patients undergoing tooth extractions, relying on clinical data and CBCT assessment. This study aimed to answer two main research questions: 1) Are there clinical and radiographic differences between patients with and without treatment with ARDs? and, 2) What are the clinical and local radiographic risk factors for the development of MRONJ? We hypothesize that low doses of ARDs induce detectable changes in the maxillary bones that are evidenced by clinical data and three-dimensional imaging. Furthermore, local risk factors for MRONJ can be identified using CBCTs.

Material and Methods

Study Design and Settings

Prior to the initiation of this retrospective cohort study, the ethical committee of UZ/KU Leuven was consulted (protocol number: S63934). The study followed the ethical standards outlined in the Declaration of Helsinki and the institutional review board. Reporting of the study adhered to the STROBE guidelines [17].

Participant Selection

The medical records of 525 patients who received ARD treatment and underwent CBCT imaging at the oral and maxillofacial surgery department of University Hospitals Leuven between 2010 and 2020 were revised. The inclusion criteria comprised (1) patients with osteoporosis on active or prior treatment with ARDs, (2) having tooth extraction(s) within one year after CBCT imaging, and (3) documented clinical follow-up. Exclusion criteria included (1) prior head and neck radiation, (2) bone exposure or prior MRONJ at the extraction site, and (3) poor image quality.

Subsequently, patients from the same imaging department who had a CBCT and tooth extractions but no history of antiresorptive treatment were selected as controls. This control group was age-, sex- and type-of-tooth-matched to the study group and complied with the same exclusion criteria. Tooth extractions followed the

methodology outlined by Moreno-Rabié et al. 2023 [12]. Particularly, study group patients on active ARD therapy did not have a drug holiday at the time of tooth extractions. In addition, the study group was prescribed amoxicillin 875mg/clavulanic acid 125mg or clindamycin 300mg 3 times daily to start two days prior to surgery for a duration of one week and a 0.12% chlorhexidine mouthwash to start the day after the extraction for two weeks.

Clinical Data Selection

The patients' electronic medical records were reviewed, including clinical data and diagnostic images. Collected information included age, sex, systemic condition (i.e., comorbidities), concomitant medication, antiresorptive drug scheme (i.e., type, dose, duration, and time since the last ARD administration), smoking status [18], alcohol habits, date of CBCT acquisition, extracted teeth (i.e., surgery date, indication for extraction, and clinical follow-up), use of leukocyte- and platelet-rich fibrin (L-PRF), prophylactic antibiotics, antiseptic mouthwash, and, if developed, date of MRONJ diagnosis, stage [4], and date of mucosal healing (i.e., "epithelial continuity obtained by granulation of the extraction socket with no fistula connected to the underlying bone" [19,20]).

CBCT assessment

The imaging assessment protocol has been previously described by Moreno-Rabie et al 2023 [21]. In a nutshell, CBCT images were obtained using 3D Accuitomo 170 (J. Morita Corp., Kyoto, Japan) or Newtom VGi evo (Cefla Dental Group, Imola, Italy). Image assessment was carried out using IMPAX software (version 6.5.5, Agfa-Gevaert, Mortsel, Belgium).

Three independent oral and maxillofacial radiologists, blinded to the study variables, evaluated the CBCT images. Before the imaging assessment, a calibration session was conducted to establish diagnostic consensus using 21 CBCTs external to this study sample. Parameters assessed at each tooth extraction site included alveolar bone loss, furcation involvement, lamina dura, periodontal ligament space, endodontic treatment, periapical lesion, root remnant, and trabecular bone pattern. Additionally, measurements of the mandibular cortical width (MCW) were performed bilaterally at

the mental foramen level. To assess intra-observer agreement, 49 extraction sites were re-assessed one month after the initial assessment completion.

Statistical analysis

Statistical analyses were conducted using RStudio software (version 2023.3.1.446, RStudio, Boston, MA, U.S.). The collected data were grouped by patient and extraction site and separated into control and study groups, as outlined in Table 1 and 2. Imaging assessment was only performed at an extraction site level (Table 3). Comparisons were made between control and antiresorptive-treated patients/sites, as well as between MRONJ+ and MRONJ- patients/sites within the study group. To determine the independence of clinical data and radiographic features, the Chi-square/Fisher's exact test was used for categorical data, the Mann–Whitney U test for ordinal variables, and the t-test for continuous data.

Furthermore, Pearson correlation test was used in the study group to analyze the association of ARD duration with MCW and time until mucosal healing. The association between specific antiresorptive drugs (i.e., bisphosphonates and denosumab) and radiographic features was also investigated within the study group. For this, patients who had only been exposed to one type of drug were selected, and the distribution of radiographic characteristics was compared using the aforementioned tests.

Inter-observer agreement was calculated using Fleiss' Kappa test, while Cohen's Kappa test was used for assessing intra-observer agreement [22]. The significance level was set at $p \leq 0.05$.

Results

Characteristics of the selected patients

A total of 78 osteoporotic patients who underwent 224 tooth extractions were included in the present study. In addition, 98 patients with 227 tooth extractions were selected for the control group. Both groups showed no significant differences in the patient's

age (p=0.260), sex (p=0.827), type of extracted tooth (p=0.997), nor in the presence of systemic diseases (p=0.583). Four patients with osteoporosis (5%; 5 sites (2%)) and cero controls developed MRONJ. Other peri- and post-operative complications included seven oroantral communications, three inflammations, three post-operative bleedings, and one abscess. All complications were successfully treated.

Clinical Data Assessment

The clinical characteristics investigated as risk factors for MRONJ are described in Table 1 at the patient level, and in Table 2 at the tooth level. These tables also provide comparative data with the control group. At the patient level, a significantly higher risk of developing MRONJ was seen in patients with respiratory diseases, comprising sarcoidosis and chronic obstructive pulmonary disease (p=0.014). Of which two patients were treated with a budesonide inhaler. Corticosteroid treatment was also identified as a risk factor for MRONJ (p=0.021), although the treatment duration did not show significant differences (p=0.225). No other variable proved to be a predisposing factor for MRONJ. However, it is important to note that all patients with osteonecrosis had been treated with injectable antiresorptive drugs (i.e., subcutaneous or intravenous). Three of them had been on these drugs for at least four years, and two had received their last administration less than six months before tooth extraction.

When examining the extraction sites, it was observed that molars developed significantly more osteonecrosis than premolars and single-rooted teeth (p=0.041). All sites that developed osteonecrosis exhibited spontaneous and percussion pain at the time of extraction. Among these, two showed radiographic signs of moderate periodontitis, and three had dentinal caries. Finally, when analyzing the time taken to observe mucosal healing, a longer healing time was observed in the study group, with a mean of 4.4 weeks (ranging from 1 to 86 weeks), while the control group took 2.6 weeks (ranging from 1 to 7 weeks) (p<0.001). Specifically, the bisphosphonate-exposed sites took 4.1 weeks (ranging from 1 to 11 weeks) for mucosal closure, while denosumab-exposed sites took an average of 4.4 weeks (ranging from 1 to 86 weeks). No significant correlation was found between the period on ARDs and the time until mucosal healing (r= -0.022, p=0.849).

CBCT Assessment

All tooth extractions occurred within 12 months of CBCT acquisition, with an average of 3 months for osteoporotic patients and 2 months for the control group.

The inter-observer agreement was substantial (K=0.695), and there was no significant difference between the observer's MCW measurements (p=0.921). In addition, the intra-observer agreement ranged from substantial to almost perfect ($K_{OBSERVER1}$=0.829, $K_{OBSERVER2}$=0.979, $K_{OBSERVER3}$=0.790). There was also no significant difference in the reproducibility of MCW measurements ($p_{OBSERSER1}$=0.923, $p_{OBSERVER2}$=0.960, $p_{OBSERVER3}$=0.538).

The radiographic findings at each extraction site are shown in Table 3. When comparing the control and study groups, it was observed that the latter exhibited significantly more thickening of the lamina dura (p<0.001). In contrast, the control group had significantly more periapical lesions (p=0.009) and osteosclerosis at the extraction sites (p<0.001). It should be noted that 55% of the control teeth with periapical radiopacities had periapical lesions, and 73% of the remaining teeth had thickened periodontal ligaments.

The presence of radiographic bone sequestrum was exclusively observed in the ARD-treated group, and it exhibited a significantly higher prevalence in extraction sites that subsequently developed post-operative osteonecrosis (Figure 1) (p<0.001). Notably, only one of these sites had a histopathological study using a sample taken during tooth extraction. This specific site was the sole one with bone sequestrum and did not manifest post-operative exposed bone (Figure 2). The examination confirmed the presence of necrotic bone and a radicular cyst. At this site, complete mucosal healing was observed five weeks after surgery.

On average, the mandibular cortical width was 4 mm in the study group (MRONJ+ 4.6 mm, MRONJ- 3.9 mm) and 4.2 mm in the control group. No significant differences were observed between the control and study group (p=0.129), MRONJ+ and MRONJ- (p=0.639), MRONJ+ and control (p=0.774), nor between MRONJ- and

control patients (p=0.099). Lastly, a non-significant correlation (p=0.827, r= -0.029) was found between the duration of ARDs and MCW.

Finally, in order to identify three-dimensional features associated with each type of antiresorptive medication, 137 sites exposed exclusively to bisphosphonates and 52 to denosumab were selected. None of the three-dimensional characteristics observed showed an association with drug type (p>0.05).

Table 1. Data at a patient level for osteoporosis and control subjects.

Characteristic		**Osteoporosis**						**Control**	
Number of patients, n		**78**						**98**	
Development of osteonecrosis, n (%)		**MRONJ+**		**MRONJ-**		**Total**	**p-value**	**NA**	**p-value**
		4	**5%**	**74**	**95%**	**78**			
Age (years)	Mean (range)*	70.3 (51 - 82)		67.4 (16 - 92)		67.6 (16 - 92)	0.496	66.2 (45 – 86)	0.260
Sex, n (%)	Female	4	6%	60	94%	64	1.000	78	0.827
	Male	0	0%	14	100%	14		20	
Extracted teeth, n	Mean (range)*	2.0 (1 – 3)		2.9 (1 - 16)		2.9 (1 - 16)	0.828	2.3 (1 – 13)	0.182
Systemic disease, n (%)	Yes	4	8%	47	92%	51	0.292	59	0.583
	No	0	0%	27	100%	27		39	
Underlying disease, n (%)	Cardiac	1	3%	31	97%	32	0.640	38	0.882
	Hypertension	1	5%	20	95%	21	1.000	33	0.424
	Respiratory	3	23%	10	77%	13	*0.014*	3	*0.004*
	Renal	0	0%	4	100%	4	1.000	2	0.408
	Hepatic	1	33%	2	67%	3	0.148	5	1.000
	Endocrine	2	13%	13	87%	15	0.165	20	0.997
	Diabetes	0	0%	7	100%	7	1.000	6	0.668
	Thyroid	2	22%	7	78%	9	0.063	17	0.387
	Rheumatoid	0	0%	7	100%	7	1.000	10	0.986
	Gastrointestinal	0	0%	1	100%	1	1.000	1	1.000
	Neurological	0	0%	1	100%	1	1.000	1	1.000
	Oncologic	1	5%	19	95%	20	1.000	21	0.633
ARD type, n (%)	Bisphosphonate	1	2%	45	98%	46		NA	
	Denosumab	1	5%	19	95%	20	0.112	NA	NA
	Both	2	17%	10	83%	12		NA	
Specific ARD used, n (%)	Zoledronic Acid	1	6%	15	94%	16		NA	
	Denosumab	3	9%	29	91%	32		NA	
	Alendronate	1	3%	32	97%	33		NA	
	Pamidronate	0	0%	4	100%	4	0.889	NA	NA
	Ibandronate	0	0%	9	100%	9		NA	
	Risedronate	0	0%	8	100%	8		NA	
	Etidronate	0	0%	1	100%	1		NA	
Number of sequential ARD, n (%) *	1	3	5%	56	95%	59		NA	
	2	1	8%	12	92%	13	0.964	NA	NA
	3	0	0%	6	100%	6		NA	
Time on ARD (months)	Mean (range)*	55.8 (6 - 124)		68.9 (3 - 266)		68.2 (3 - 266)	0.865	NA	NA

Characteristic		Osteoporosis						Control	
Number of patients, n		**78**						**98**	
Development of osteonecrosis, n (%)		**MRONJ+**		**MRONJ-**		**Total**	**p-value**	**NA**	**p-value**
		4	**5%**	**74**	**95%**	**78**			
Time since last ARD (months), n (%)	Not stopped	0	0%	17	100%	17	0.571	NA	NA
	Mean (range)*	7.4 (2 - 19)		20.8 (1 - 97)		19.9 (1 - 97)	0.205	NA	
	Mean B.P. (range)*	19		30.4 (1 - 97)		30 (1 - 97)	1.000	NA	NA
	Mean D.B. (range)*	3.6 (2 - 4)		10.9 (1 - 47)		10.1 (1 - 47)	0.106	NA	
Corticosteroid use (months), n (%)	Yes	3	20%	12	80%	15	*0.021*	7	*0.029*
	No	1	2%	62	98%	63		91	
	Mean (range)*	44.1 (17 - 63)		88.5 (18 - 161)		79 (17 - 161)	0.225	46.8 (1 – 154)	0.127
Alcohol consumption, n (%) *	No consumption	1	4%	25	96%	26		26	
	1-2 units week	2	9%	21	91%	23		39	
	3-4 units week	0	0%	3	100%	3	0.829	4	0.684
	>5 units week	0	0%	11	100%	11		18	
	Ex-abuser	0	0%	2	100%	2		2	
	Unknown	1	8%	12	92%	13		9	
Tobacco use, n (%)	Never smoker	2	5%	39	95%	41		68	
	Current smoker	1	8%	11	92%	12	0.415	10	0.092
	Former smoker	0	0%	22	100%	22		18	
	Unknown	1	33%	2	67%	3		2	

The p-values correspond to the outcomes of the Chi-Square/Fisher's exact test for comparing MRONJ+ and MRONJ- sites in the study group, and the latter with the control group. Variables marked with an asterisk (*) indicate ordinal/numerical data analyzed using the Mann-Whitney U/t test. Significant p-values ($p \leq 0.05$) are indicated *in italics*. MRONJ: Medication-related osteonecrosis of the jaws, ARD: antiresorptive drugs, B.P.: bisphosphonates, D.B.: denosumab, NA: Not applicable.

Discussion

The incidence of MRONJ in osteoporotic patients remains relatively low, ranging from 0.001% to 0.4% [5–7,23]. Tooth extractions elevate MRONJ risk to 3.4% [8–10,23,24]. However, avoiding extractions solely due to MRONJ concerns is unwarranted, as infection may be the primary cause of osteonecrosis [19]. Regarding identifying risk factors for MRONJ, the limited cases in osteoporotic patients [25] and the restricted use of diagnostic images pose a challenge. Thus, we aimed to explore the effects of ARDs on the jawbones and the development of MRONJ using clinical data and CBCT in osteoporotic patients undergoing tooth extractions. Our findings identified that patients under low doses of ARDs present thicker lamina dura, have a higher risk for MRONJ if under corticosteroid

treatment or having extractions of multi-rooted teeth, found sequestrum formations as indicators for MRONJ, and noted extended post-operative healing times.

Table 2. Summary of the data at a tooth level in the osteoporotic and control groups.

Characteristic		Osteoporosis						Control	
Number of extracted teeth, n		224						227	
Development of osteonecrosis, n (%)		MRONJ+		MRONJ-		Total	p-value	NA	p-value
		5	2%	219	98%	224			
Extraction indication, n (%)	Caries	1	1%	67	99%	68	0.282	77	0.333
	Cyst	0	0%	2	100%	2		1	
	Difficult Higiene	0	0%	0	0%	0		3	
	Fracture	1	5%	21	95%	22		14	
	Internal Resorption	0	0%	0	0%	0		1	
	Pericoronitis	0	0%	0	0%	0		2	
	Periodontitis	1	1%	86	99%	87		81	
	Root remnant	2	7%	28	93%	30		33	
	Trauma	0	0%	2	100%	2		1	
	NA	0	0%	13	100%	13		14	
Type of teeth, n (%) *	Incisors + canines	0	0%	84	100%	84	*0.041*	77	0.178
	Premolars	1	2%	53	98%	54		46	
	Molars	4	5%	82	95%	86		104	
Arch, n (%)	Maxilla	2	1%	143	99%	145	0.348	136	0.338
	Mandible	3	4%	76	96%	79		91	
Region, n (%)	Anterior maxilla	0	0%	64	100%	64	0.280	51	0.454
	Posterior maxilla	2	2%	79	98%	81		85	
	Anterior mandible	0	0%	20	100%	20		26	
	Posterior mandible	3	5%	56	95%	59		65	
Antibiotic prophylaxis, n (%)	Yes	5	2%	209	98%	214	1.000	25	*<0.001*
	No	0	0%	10	100%	10		202	
Antiseptic mouthwash, n (%)	Yes	5	2%	212	98%	217	1.000	227	*0.007*
	No	0	0%	7	100%	7		0	
Use of L-PRF, n (%)	Yes	3	2%	148	98%	151	0.662	3	*<0.001*
	No	2	3%	71	97%	73		224	
Time until mucosal healing, n (%)	0 - ≤4 weeks	0	0%	111	100%	111	NA	205	0.059
	>4 - ≤8 weeks	0	0%	108	100%	108		22	
	>8 weeks	5	100%	0	0%	5		0	
	Mean (weeks)*	26.61		3.88		4.40	*<0.001*	2.59	*<0.001*
MRONJ worse stage, n (%)	Stage 1	3	60%	NA		NA	NA	NA	NA
	Stage 2	2	40%	NA		NA		NA	
	Stage 3	0	0%	NA		NA		NA	

The p-values described under osteoporosis and control correspond to those obtained with the Chi-square/ Fisher's exact test or Mann–Whitney U test (*) when data were ordinal. Comparisons were made between MRONJ+ and MRONJ-sites in the study group, and between study and control groups. Significant p-values (p≤0.05) are *italicized*. MRONJ: Medication-related osteonecrosis of the jaws, L-PRF: leukocyte- and platelet-rich fibrin, NA: Not applicable.

Concurrent clinical risk factors for MRONJ during tooth extractions have been recognized, including osteoporotic patients older than 65 years [10,11], females [26], prolonged ARD therapy [10,11,23], rheumatoid arthritis [23], corticosteroid use [9], and mandibular extraction sites [10,11]. While Jeong et al. identified the mandible as a predisposing arch, no differences were found in dental arch location [10]. Although, the present results did not find a mandibular predilection, a preference for multi-rooted sites over single-rooted teeth was observed. This propensity can be attributed to alveolar socket size, which in the absence of severe bone resorption as seen in our MRONJ+ teeth, the wound area can be twice as large in in molars than canines or incisors [27].

Diverse tooth extraction protocols are reported, which may impact MRONJ development. Like ours, some employ prophylactic antibiotics [19,26] and discourage drug holidays during ongoing ARD treatment [19,20]. Lesclous et al. agreed that discontinuing ARD is not recommended due to increased fracture risk in the cessation period, in particular with denosumab [20]. Our results support that drug continuation does not increase the risk for MRONJ. Besides, while some studies omit first intention closure [19,26], others compare L-PRF and mucoperiosteal flap use [28]. Poxleitner et al. found no significant healing differences between L-PRF and mucoperiosteal flap use, suggesting L-PRF as a minimally invasive, efficient, and cost-effective alternative, countering drawbacks of mucoperiosteal flaps like invasiveness and reduced vestibular depth impacting dental rehabilitation [28]. Complementarily, a meta-analysis of 2098 subjects found no significant differences in the use of L-PRF and alveolectomy for MRONJ protection [25]. Likewise, we could not prove a significant protective effect of L-PRF use for MRONJ.

Few radiographic studies exist in osteoporotic patients investigating the local effects of ARDs on jawbones and their relationship with MRONJ. In this sense, one of the most researched structures is the mandibular cortical width [14–16]. Three-dimensional examinations have revealed a notably thicker MCW in ARD-treated patients compared to controls [15,16], averaging 4.3mm and 3.4mm, respectively [15]. Although, these differences are not evident in panoramic radiographs [14]. Our findings

similarly showed no significant contrasts between ARD-treated patients and the control group, nor a correlation between the duration of ARD treatment and the thickness of the MCW. This lack of association might arise from a treatment duration of less than one year with ARD in about 20% of the study patients, and from the fact that 25% of the study group and 20% of the control group lacked MCW measurements, as this structure was not visible on their CBCT scans.

One of the novel aspects of the present investigation is the comprehensive assessment of bony changes seen on CBCT images, which has been studied in oncologic patients treated with high-doses of ARDs [21,29] but to a lesser extent in patients treated with low-doses. In osteoporotic patients under ARDs, no significant differences in the trabecular bone pattern have been demonstrated in two- [12] or three-dimensional examinations [16] when compared to a control group. Nevertheless, these patients demonstrated significantly more thickening of the lamina dura in panoramic radiographs [12]. All findings are corroborated by our current results. In contrast, imaging outcomes related to oncologic ARD doses revealed not only a higher incidence of thickening of the lamina dura but also of osteosclerotic and osteolytic regions [30], which have been identified as local risk factors for MRONJ [30–32]. Lesser changes in the radiodensity of the bone trabeculae is consistent with a lower incidence of MRONJ in low-dose ARD treatment.

Among the examined radiographic features, only the presence of bone sequesters demonstrated an association with MRONJ development. Notably, all instances of radiographic sequestrum formation in our sample corresponded to sites with osteonecrosis. Two cases exhibited post-operative exposed bone, exceeding eight weeks, while the remaining case displayed histological osteonecrosis. In the latter case, tooth extraction and sequestrum removal within a single surgical procedure was curative, as post-operative bone exposure was absent. Shudo et al. advocated for biopsy during tooth extraction in suspected latent MRONJ cases [19]. Tooth extraction is not the trigger for MRONJ but rather the unveiling factor in these cases. Thus, combining perioperative biopsy and radiographic assessment could promptly identify a latent pathology.

While some studies observed no exposed bone postoperatively, they noted a longer healing period in osteoporotic patients on bisphosphonates [19,20]. Shudo et al. linked longer bisphosphonate treatment to delayed mucosal healing, particularly beyond 5 years [19]. Similarly, Lesclous et al. found ARD-treated patients experienced delayed healing, contrasting controls healing within 4 weeks [20]. Our results showed controls achieved mucosal healing in an average of 2.6 weeks, whereas ARD-treated patients, whether under bisphosphonates or denosumab, needed 4.4 weeks. Yet, no correlation was found between ARD treatment duration and healing time. Consistently, other studies also found no significant impact of ARD type or treatment duration [28], corticosteroids [19,20], diabetes [19,20], smoking [20], number or type of tooth extracted [28], or systemic diseases [28] on socket healing.

This retrospective design inherently holds limitations compared to prospective studies. While efforts were made to match controls, ideally, they would have been drug-naïve osteoporosis or osteopenia patients, which was unattainable. Furthermore, the study's limited sample size precluded comprehensive exploration of the effects of diverse bisphosphonate types. Additionally, different surgeons with varied experience levels performed the tooth extractions, despite surgeries taking place in the same center under similar protocols and materials. Lastly, due to the lack of histopathological reports, the incidence of MRONJ may be higher in this sample owing to dental infections rather than tooth extractions per se.

Conclusion

In conclusion, osteoporotic patients under low-dose ARDs manifested a distinct thickening of the lamina dura, corticosteroid intake and multi-rooted teeth were identified as a risk factor for MRONJ, and sequestrum formation observed on CBCT can be considered a strong indicator of osteonecrosis. In addition, a prolonged post-operative healing period is expected in patients taking antiresorptive drugs, even when there is no development of exposed bone. These results contribute to understanding

the effects of ARDs and osteonecrosis in osteoporotic patients undergoing tooth extractions.

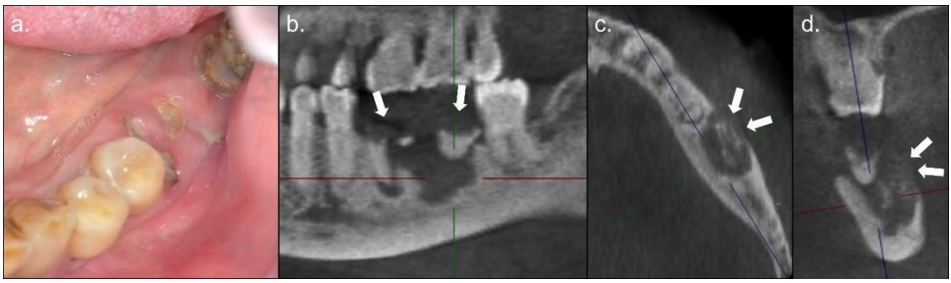

Figure 1. Clinical (a) and CBCT reconstructions (b, c, d) of an 83-year-old female with osteoporosis treated with zoledronic acid. Clinically, there was spontaneous pain and suppuration from the root remnants of the mandibular left first and second molars. No evidence of exposed bone was observed. In the sagittal CBCT slice (b), the root remnants are pointed out by white arrows. While in the axial (c) and coronal (d) views, white arrows depict sequestrum formation. Tooth extractions were carried out under local anesthesia. Seven weeks postoperatively, bone exposure and loose sequesters were clinically seen. Therefore, sequestrectomy was performed. The duration from tooth extractions to mucosal healing was 11 weeks. No histopathological analysis was conducted.

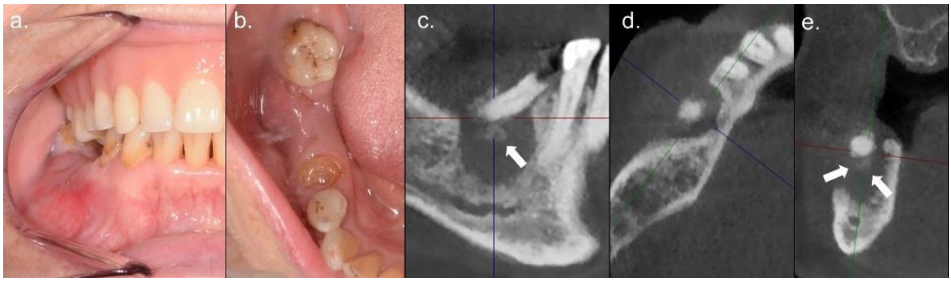

Figure 2. Clinical (a, b) and CBCT reconstructions (c, d, e) of a 90-year-old female with osteoporosis treated with denosumab. A root remnant of the mandibular right second premolar with an accompanying vestibular abscess and absence of bone exposure were clinically observed. In the CBCT, a radiolucent lesion surrounding the tooth and a bony island (white arrows) can be seen. Tooth extraction and debridement of the alveolar socket were performed under local anesthesia. A sample of the bone and lesion were taken for histopathological analysis where it was confirmed the diagnosis of osteonecrosis and radicular cyst. Complete mucosal healing and absence of inflammation were seen five weeks postoperatively.

Table 3. Pre-operative CBCT characterization of the tooth extraction sites in the study (MRONJ+ and MRONJ-) and control groups.

Observed parameter		Osteoporosis						Control	
Number of extracted teeth, n		224						227	
Development of osteonecrosis, n (%)		MRONJ+		MRONJ-		Total	p-value	NA	p-value
		5	2%	219	98%	224			
Horizontal bone loss	Absent/initial	3	2%	137	98%	140	1.000	150	0.487
	Moderate/severe	2	2%	82	98%	84		77	
Angular bone defect	Absent	4	2%	171	98%	175	1.000	187	0.309
	Present	1	2%	48	98%	49		40	
Furcation involvement	Absent	5	3%	189	97%	194	1.000	180	0.053
	Present	0	0%	30	100%	30		47	
Lamina dura	Normal	3	2%	124	98%	127	1.000	183	*<0.001*
	Thickened	2	2%	95	98%	97		44	
Periodontal ligament space	Normal	2	3%	71	97%	73	0.662	92	0.098
	Widened	3	2%	148	98%	151		135	
Endodontic treatment	Absent	4	3%	138	97%	142	0.533	143	0.979
	Adequate filling	1	3%	32	97%	33		35	
	Inadequate filling	0	0%	49	100%	49		49	
Periapical lesion size*	Absent	3	2%	155	98%	158	0.852	138	*0.009*
	Small (≤3mm)	2	7%	27	93%	29		22	
	Large (>3mm)	0	0%	37	100%	37		67	
Periapical lesion cortical*	Absent	3	2%	156	98%	159	0.917	138	*0.009*
	None	2	13%	14	87%	16		14	
	Thinning	0	0%	18	100%	18		22	
	Expansion	0	0%	7	100%	7		9	
	Destruction	0	0%	24	100%	24		44	
Root remnant	Absent	3	1%	201	99%	204	0.064	196	0.151
	Present	2	10%	18	90%	20		31	
Osteoclerosis*	Normal	2	1%	144	99%	146	0.285	105	*<0.001*
	Localized Sclerosis	1	6%	17	94%	18		16	
	Extended Sclerosis	2	3%	58	97%	60		106	
Osteolysis*	Absent	4	2%	205	98%	209	0.253	213	0.775
	Localized lysis	1	11%	8	89%	9		13	
	Extensive lysis	0	0%	6	100%	6		1	
Periosteal reaction*	Absent	5	2%	217	98%	222	0.847	226	0.559
	Localized reaction	0	0%	1	100%	1		0	
	Extensive reaction	0	0%	1	100%	1		1	
Sequestrum formation*	Normal	3	1%	218	99%	221	*<0.001*	227	0.081
	Localized sequester	0	0%	1	100%	1		0	
	Extensive sequester	2	100%	0	0%	2		0	

P-values obtained as results from the Chi-Square/Fisher's exact test when comparing MRONJ+ and MRONJ- patients in the study group, as well as the study and control groups. Variables denoted with an asterisk (*) represent ordinal/numerical data analyzed with the Mann–Whitney U test. Significant p-values ($p \leq 0.05$) are *italicized.* NA: Not applicable

Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

1. C.M.R: Conceptualization, Methodology, Data Curation, Investigation, Formal analysis, Writing - Original Draft, Writing - Review & Editing.
2. R.C.F: Investigation, Writing - Review & Editing
3. N.O.S: Investigation, Writing - Review & Editing
4. F.N.R: Investigation, Writing - Review & Editing
5. T.VdW: Writing - Review & Editing, Supervision
6. R.J: Conceptualization, Writing - Review & Editing, Supervision

References

1. WHO Scientific Group of Osteoporosis. Prevention and management of osteoporosis: report of a WHO scientific group. Geneva, Switzerland: World Health Organization; 2003. p. 192 p. (WHO technical report series, no. 921).

2. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw--2014 update. J Oral Maxillofac Surg. 2014 Oct;72(10):1938–56.

3. Aljohani S, Fliefel R, Ihbe J, Kühnisch J, Ehrenfeld M, Otto S. What is the effect of anti-resorptive drugs (ARDs) on the development of medication-related osteonecrosis of the jaw (MRONJ) in osteoporosis patients: A systematic review. Journal of Cranio-Maxillofacial Surgery. 2017 Sep 1;45(9):1493–502.

4. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022 May;80(5):920–43.

5. European Medicines Agency. Aclasta (zoledronic acid). Product information. 09/11/2022 Aclasta - EMEA/H/C/000595 - T/0079. [Internet]. https://www.ema.europa.eu/en/documents/product-information/aclasta-epar-product-information_en.pdf. 2022 [cited 2022 Dec 5]. p. 1–41. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/aclasta

6. European Medicines Agency. Fosavance (alendronic acid/colecalciferol). Product information. 27/10/2021 Fosavance - EMEA/H/C/000619 - N/0052. [Internet]. https://www.ema.europa.eu/en/documents/product-information/fosavance-epar-product-information_en.pdf. 2021 [cited 2022 Dec 5]. p. 1–36. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/fosavance

7. European Medicines Agency. Prolia (denosumab). Product information. 05/05/2022 Prolia - EMEA/H/C/001120 - II/0093. [Internet]. https://www.ema.europa.eu/en/documents/product-information/prolia-epar-product-information_en.pdf. 2022 [cited 2022 Oct 12]. p. 1–44. Available from: https://www.ema.europa.eu/en/documents/product-information/prolia-epar-product-information_en.pdf

8. Hansen PJ, Knitschke M, Draenert FG, Irle S, Neff A. Incidence of bisphosphonate-related osteonecrosis of the jaws (BRONJ) in patients taking bisphosphonates for osteoporosis treatment-a grossly underestimated risk? Clin Oral Investig. 2013 Nov 1;17(8):1829–37.

9. Taylor T, Bryant C, Popat S. A study of 225 patients on bisphosphonates presenting to the bisphosphonate clinic at King's College Hospital. Br Dent J. 2013 Apr 12;214(7):E18.

10. Jeong HG, Hwang JJ, Lee JH, Kim YH, Na JY, Han SS. Risk factors of osteonecrosis of the jaw after tooth extraction in osteoporotic patients on oral bisphosphonates. Imaging Sci Dent. 2017 Mar;47(1):45.

11. Kwack DW, Park SM. Prediction of medication-related osteonecrosis of the jaw (MRONJ) using automated machine learning in patients with osteoporosis associated with dental extraction and implantation: a retrospective study. J Korean Assoc Oral Maxillofac Surg. 2023 Jun 1;49(3):135–41.

12. Moreno-Rabié C, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, et al. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8;

13. Moreno-Rabié C, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep 6;24(9):2973–89.

14. Barngkgei I, Halboub E, Almashraqi A. Effect of bisphosphonate treatment on the jawbone: an exploratory study using periapical and panoramic radiographic evaluation. Oral Radiol. 2019 May 7;35(2):159–70.

15. Diniz-Freitas M, Fernández-Montenegro P, Fernández-Feijoo J, Limeres-Posse J, González-Mosquera A, Vázquez-García E, et al. Mandibular cortical indices on cone-beam computed tomography images in osteoporotic women on treatment with oral bisphosphonates. Gerodontology. 2016 Jun;33(2):155–60.

16. Barngkgei I, Khattab R. Detecting the effect of bisphosphonates during osteoporosis treatment on jawbones using multidetector computed tomography: The OSTEOSYR project. J Investig Clin Dent. 2018 Aug 25;9(3):e12332.

17. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg. 2014 Dec 1;12(12):1500–24.

18. Center for Disease Control and Prevention. NHIS - Adult Tobacco Use - Glossary [Internet]. Adult Tobacco

use Information. 2017 [cited 2023 Jul 13]. Available from: https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm

19. Shudo A, Kishimoto H, Takaoka K, Noguchi K. Long-term oral bisphosphonates delay healing after tooth extraction: a single institutional prospective study. Osteoporosis International. 2018 Oct 1;29(10):2315–21.

20. Lesclous P, Cloitre A, Catros S, Devoize L, Louvet B, Châtel C, et al. Alendronate or Zoledronic acid do not impair wound healing after tooth extraction in postmenopausal women with osteoporosis. Bone. 2020 Aug 1;137.

21. Moreno Rabie C, Cavalcante Fontenele R, Oliveira Santos N, Nogueira Reis F, Van den Wyngaert T, Jacobs R. Three-dimensional clinical assessment for MRONJ risk in oncologic patients following tooth extractions. Dentomaxillofacial Radiology. 2023 Oct 24;

22. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005 May;37(5):360–3.

23. Chiu WY, Yang WS, Chien JY, Lee JJ, Tsai KS. The influence of alendronate and tooth extraction on the incidence of osteonecrosis of the jaw among osteoporotic subjects. Trackman PC, editor. PLoS One. 2018 Apr 25;13(4):e0196419.

24. Seki K, Kaneko T, Kamimoto A, Wada M, Takeuchi Y, Furuchi M, et al. Medication-related osteonecrosis of the jaw after tooth extraction in patients receiving pharmaceutical treatment for osteoporosis: A retrospective cohort study. J Dent Sci. 2022 Oct 1;17(4):1619–25.

25. 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. Vol. 42, Journal of Clinical Periodontology. J Clin Periodontol; 2015. p. 922–32.

26. Cuozzo A, Iorio-Siciliano V, Vaia E, Mauriello L, Blasi A, Ramaglia L. Incidence and risk factors associated to Medication-Related Osteo Necrosis of the Jaw (MRONJ) in patients with osteoporosis after tooth extractions. A 12-months observational cohort study. J Stomatol Oral Maxillofac Surg. 2022 Nov 1;123(6):616–21.

27. Couso-Queiruga E, Ahmad U, Elgendy H, Barwacz C, González-Martín O, Avila-Ortiz G. Characterization of Extraction Sockets by Indirect Digital Root Analysis. Int J Periodontics Restorative Dent. 2021 Jan;41(1):141–8.

28. Poxleitner P, Steybe D, Kroneberg P, Ermer MA, Yalcin-Ülker GM, Schmelzeisen R, et al. Tooth extractions in patients under antiresorptive therapy for osteoporosis: Primary closure of the extraction socket with a mucoperiosteal flap versus application of platelet-rich fibrin for the prevention of antiresorptive agent-related osteonecrosis of. Journal of Cranio-Maxillofacial Surgery. 2020 Apr 1;48(4):444–51.

29. Yanaguizawa WH, Barros FB, Kobayashi-Velasco S, Salineiro FC, Alves FA, Cavalcanti MG. Computed tomography bone density variations in oncological patients undergoing antiresorptive medication. Med Oral Patol Oral Cir Bucal. 2022 Nov 1;27(6):e489–96.

30. Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, et al. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280.

31. Bedogni A, Fedele S, Bedogni G, Scoletta M, Favia G, Colella G, et al. Staging of osteonecrosis of the jaw requires computed tomography for accurate definition of the extent of bony disease. British Journal of Oral and Maxillofacial Surgery. 2014 Sep;52(7):603–8.

32. Hamada H, Matsuo A, Koizumi T, Satomi T, Chikazu D. A simple evaluation method for early detection of bisphosphonate-related osteonecrosis of the mandible using computed tomography. J Craniomaxillofac Surg. 2014 Sep;42(6):924–9.

PART 2: THERAPEUTIC PROGNOSTIC RISK FACTORS FOR MRONJ

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CHAPTER 5:

Moreno Rabie C, García-Larraín S, Contreras Diez de Medina D, Cabello-Salazar I, Cavalcante Fontenele R, Van den Wyngaert T, Jacobs R. How does the clinical and tomographic appearance of MRONJ influence its treatment prognosis? Dentomaxillofac Radiol. 2023 Oct 23:20230304. doi: 10.1259/dmfr.20230304.

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CHAPTER 5: CLINICAL AND TOMOGRAPHIC PROGNOSTIC RISK FACTORS FOR MRONJ

How does the clinical and tomographic appearance of MRONJ influence its treatment prognosis?

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Abstract

Objective: To identify clinical and tomographic prognostic factors for conservative and surgical treatment of medication-related osteonecrosis of the jaws (MRONJ).

Methods: A retrospective search identified patients treated with antiresorptive drugs (ARDs), diagnosed with stage 1, 2 or 3 MRONJ, and having CBCT scans previous to conservative or surgical treatment. Following data collection, imaging assessment of the following parameters on each MRONJ site was performed: involvement of teeth and/or implants, presence of osteosclerosis, osteolysis, sequestrum formation, periosteal reaction, and pathological fractures. For statistical analysis, patients and lesions were divided into conservative and surgical treatment. Comparisons were made between successful and unsuccessful outcomes. Significance was set at $p \leq 0.05$.

Results: One-hundred fifteen ARD-treated patients who developed 143 osteonecrosis lesions were selected. Forty patients and 58 lesions received conservative treatment, of which 14 patients (35%) and 25 lesions (43%) healed. Additionally, 75 patients and 85 lesions underwent surgery, with 48 patients (64%) and 55 lesions (65%) that healed. Clinical and tomographic risk factors for conservative treatment were MRONJ staging, tooth involvement, extensive osteosclerosis, and deep sequestrum formation ($p<0.05$). Complementarily, poor prognostic indicators for surgical therapy were a short bisphosphonate (BP) holiday, MRONJ staging, absence of sequestrum formation, and presence of periosteal reaction ($p<0.05$).

Conclusions: Lesions at stage 3 MRONJ, with tooth involvement, or sequestrum formation showed poor outcomes when conservative treatment is chosen. Alternatively, surgical treatment is most effective when BPs are discontinued, in stage 1 lesions, in the presence of sequestrum formation, and absence of periosteal reaction.

Keywords: osteonecrosis, cone-beam computed tomography, diagnostic imaging, prognosis, therapy

Introduction

Medication-Related Osteonecrosis of the Jaw (MRONJ) can be defined as exposed bone or fistula that probes to bone in the maxillofacial region persisting for more than eight weeks in patients treated with antiresorptive drugs (ARDs).[1,2] These medications effectively and safely treat skeletal-related events (SREs) and prevent bone fractures among patients with bone metastases and osteoporosis, respectively.[2–4] However, whilst diagnostic and treatment methodologies for MRONJ remain debatable, no gold standard has been agreed upon.[5]

Several efforts have been made to find the best treatment option for MRONJ. As a general thought, the treatment aims to control infection, minimize pain, and avoid necrosis progression.[1,2,6] Various possible treatment schemes with approaches ranging from conservative to surgical management are indicated based on MRONJ-staging, age, primary disease, comorbidities, and type of ARD. Conservative treatment includes the use of antibiotics and antiseptic mouthwashes but using a variety of compounds and doses.[7] While the surgical technique mainly consists of removing necrotic and infected bone, softening of the sharp edges, and wound closure with a free-of-tension mucoperiosteal flap. However, some protocols also include the use of laser therapy or local application of autologous platelet concentrates (APC).[7,8] Therapeutic success is usually considered when reaching mucosal healing in the necrotic site. Yet, success rates have shown different results, being 28.8% in conservative treatment [7] and ranging from 27.6% [9] to 91.6% [10] in surgical removal.

In this context, clinical aspects, including the dosage of ARD, C-reactive protein (CRP), and alkaline phosphate, have been identified as treatment prognostic factors.[5,11] Nevertheless, few authors have also considered the three-dimensional radiographic appearance of the lesion in this assessment.[5,12–15] Yet, tomographic images of clinically exposed necrotic bone are variable and may show osteolysis, cortical bone erosion, sequestrum formation, osteosclerosis as well as periosteal reaction.[2,16,17] In this sense, Shin et al. described that osteonecrosis lesions larger than one third of the jaw had a worse surgical prognosis than smaller lesions.[5] Likewise, periosteal reaction was also

found to be a poor prognostic outcome indicator.[12,13] However, a better understanding of the factors that predict the postoperative outcome of MRONJ is still necessary.[5,18]

Given the need for a comprehensive assessment using Cone-Beam Computed Tomography (CBCT) to aid treatment prognosis, the present study aims to identify clinical and tomographic prognostic factors for conservative and surgical treatment of MRONJ. A secondary objective is to investigate the imaging features associated with lesion relapse.

Material and Methods

Study design and settings

Ethical approval was granted by the research ethics committee of University Hospitals Leuven (reference number: S66635). Informed consent was waived given the retrospective longitudinal cohort design. All procedures and data collection were conducted in accordance with the ICH-GCP principles and declaration of Helsinki. The database of the department of oral and maxillofacial surgery at University Hospitals Leuven was reviewed to identify eligible patients between January 1st 2010, and May 31st 2022.

Participant selection

Patients were included if: (1) older than 18 years, (2) treated with at least one administration of ARDs, (3) diagnosed with stage 1, 2, or 3 MRONJ according to the American Association of Oral and Maxillofacial Surgeons (AAOMS) [1,2], and (4) had a CBCT of the MRONJ lesion prior to conservative or surgical treatments. Exclusion criteria included: (1) prior radiotherapy targeted to the jawbones, (2) metastasis in the jaws, (3) stage 0 MRONJ, (4) absence of documented follow-up (at least two clinical follow-up consultations less than one year apart), (5) insufficient image quality to perform their assessment, (6) CBCTs acquired after a surgical procedure, (7) relapse of a preceding MRONJ lesion, and (8) former reconstructive surgery.

Treatment protocol

All patients were initially given conservative care. Surgical treatment was advised in presence of pain, persistent infection after antibiotic initiation, presence of a mobile sequester, or progression of the lesion's extension, if the patient's health status allowed it.

Conservative treatment

Conservative treatment involved the prescription of antiseptic mouthwashes, such as 0.12% chlorhexidine or 0.5% sodium hypochlorite during the first two weeks of treatment. Subsequently, 0.05% chlorhexidine was used for maintenance throughout the follow-up period. Additionally, amoxicillin 875mg/clavulanic acid 125mg or clindamycin 300mg three times per day were prescribed in the first two weeks. Afterwards, the medication was switched to amoxicillin 500mg or doxycycline 100mg per day for treatment maintenance until the infection subsided or mucosal healing was achieved. Control visits were initially scheduled every two weeks, later transitioning to monthly or three-monthly appointments, or sooner if the patient experienced worsening symptoms.

Surgical treatment

Patients who underwent surgical therapy initially received conservative treatment. Once the outpatient surgery was scheduled, they were prescribed amoxicillin 875mg/clavulanic acid 125mg or clindamycin 300mg three times per day two days before surgery. This medication regimen was continued for two weeks before transitioning to amoxicillin 500mg or doxycycline 100mg per day until the infection subsided or mucosal healing was achieved.

The surgeries occurred under local anaesthesia without vasoconstrictor and, in some cases, intravenous sedation (midazolam 0.03mg/kg and fentanyl 2ug/kg) was administered. The procedure consisted of wound debridement, sequestrectomy, and occasionally marginal osteotomy of the bone, depending on the extent of the lesion and patients' symptoms. If teeth or implants were located immediately adjacent to or

within the osteonecrosis lesion, they were also removed. For closure, either leukocyte- and platelet-rich fibrin (L-PRF) membranes (408g/2700rpm for 12 min; IntraSpinTM, Intra-Lock®, Boca) or a mucoperiosteal free-of-tension flap were placed. The surgical sites were rinsed with 0.9% physiological saline solution and sutured with 3/0 vicryl resorbable sutures. Control visits were scheduled in the same manner as the conservative group.

The primary endpoint was the presence of mucosal healing during the clinical follow-up. Lesion relapse was noted as a secondary endpoint. Treatment outcome was assessed in the last documented consultation, and it was considered successful when mucosal healing and absence of symptoms, including swelling, pain, and pus discharge, was achieved. Treatment failure meant a persistent lesion, one that became clinically worse (i.e., stage-up), or an increase in the lesion's size.

Data collection

Together with the CBCTs, the following clinical information was collected: age, gender, systemic condition, comorbidities, tobacco and alcohol use, corticosteroid intake, previous chemotherapy and/or radiotherapy, antiresorptive drug (including dosage and treatment duration), date of MRONJ diagnosis, staging at diagnosis according to the AAOMS,[2] site of development, oral factors (e.g. use of dentures, oral trauma, tooth extraction, etc.), date of CBCT and staging at acquisition, drug holiday (i.e., discontinuation of medication at treatment initiation), treatment scheme, surgery date, use of L-PRF, antibiotics, and antiseptic mouthwash, date of mucosal healing, relapse information, and date and staging at follow-up consultations.

Radiographic assessment

Diagnostic images were acquired at the Dentomaxillofacial Radiology Centre at the Imaging and Pathology Department in St. Raphael Hospital, using 3D Accuitomo 170 (J. Morita Corp., Saitama, Japan) or Newtom VGi evo (Cefla s.c., Imola, Italy). The selection of the field of view (FOV), voxel size (ranging from 80µm to 300µm), and exposure protocol was determined according to the patient's specific diagnostic or therapeutic

indication. Images were assessed using Xero Viewer software (Agfa-Gevaert, Mortsel, Belgium).

A blinded and independent assessment of the CBCT scans was performed by two dentomaxillofacial radiologists and one general dentist. Prior to the commencement of the observations, a calibration session was held using a set of 16 CBCTs involving 22 lesions external to this study to reach baseline diagnostic consensus. All observations were conducted in a quiet room with dim light using a high-resolution display (HP EliteDisplay E243 23.8-inch Monitor; HP inc; Palo Alto; USA). Brightness and contrast setup were left at the discretion of the examiner. In cases where consensus was not achieved, individual discussions were held to reach an agreement. One month after completion of the evaluation, 22 CBCTs involving 26 lesions were randomly selected and reassessed to calculate the intra-observer agreement.

The imaging assessment was performed at each MRONJ site. When multiple examinations were available, the CBCT closest to the date of treatment initiation or surgery was selected, depending on whether the patient received conservative or surgical treatment, respectively. The evaluation included the following assessments:

1. Involvement of teeth and/or implants in the lesion, as well as imaging signs of periodontal disease/peri-implantitis. These signs included furcation involvement, horizontal bone loss greater than 1/3 of the root/implant length, angular bone defects, and periapical/peri-implant lesions.[19] Tooth/implant compromise was considered when immediately adjacent to or embedded in osteolysis, bone sequestrum, or an osteosclerotic area.
2. Osteosclerosis, osteolysis, and sequestrum formation. Considering osteosclerosis as hyperdense areas in the body of the maxilla or mandible; as osteolysis hypodense areas in the cortical and/or trabecular bone; and as sequestrum formation a bony island surrounded by an osteolytic halo. These characteristics were assessed based on depth [14] and extension.[5] The lesions were classified as superficial if they were localized to the alveolar process. In contrast, they were considered deep if they extended further than the

mandibular canal, maxillary sinus, or nasal cavity. The extension of the lesions was categorized as localized if they were contained in 1/3 of the jaw or generalized if they extended beyond 1/3 of the jaw.

3. Periosteal reaction in the mandible. Considering periosteal reaction as a uniform outer layer of bone formation along the mandibular surface. When present, it was considered localized if it included only the buccal or lingual side of the mandible without involving the lower edge or extensive, if present in both buccal and lingual sides beyond the inferior mandibular border.[20]
4. Pathological fractures.[14]

Statistical analysis

The collected data were analysed using RStudio Software version 2023.3.1.446 (RStudio, Boston, MA US). The significance level was set at 5% ($p \leq 0.05$). Cohen's (Fleiss) Kappa test was used to calculate intra- and inter-observer agreement. Considering a fair agreement when the test result was ≥ 0.21 - 0.40, moderate when ≥ 0.41 - 0.60, substantial when ≥ 0.61 - 0.80, and almost perfect when ≥ 0.81 - 0.99.[21]

Further statistical analysis was conducted to identify clinical and imaging variables that could serve as treatment prognosis predictors. Patients and lesions were initially grouped based on the treatment received, either conservative or surgical. Subsequently, comparisons were made between treatment success and failure. The Chi-square/Fisher's exact test was used to test the independence of categorical variables, while a Mann Whitney U test was used to assess ordinal variables. The same statistical tests were used to assess the independence of the radiographic features and lesion relapse among both treatment groups.

To examine the relationship between predictor variables and the outcome on each treatment group, a Generalized Linear Mixed Model (GLMM) was used. The fixed effects included age, gender, underlying diagnosis, chemotherapy and/or radiotherapy, duration of ARD-therapy, MRONJ staging, presence of teeth and implants, osteosclerosis, osteolysis, sequestrum formation, periosteal reaction, fracture, duration of drug holiday at the start of the treatment, arcade, use of L-PRF, and

antibiotics. Patients were included as a random effect to account for multiple lesions per person. A logit link function was used to model the healing probability. LASSO regression was applied to select significant variables, which were then used in a simplified GLMM.

Results

A total of 115 ARD-treated patients who developed 143 osteonecrosis lesions were included in the present study. They were on average 70 years old (ranging from 43 to 88 years) at the time of diagnosis. Overall, 96 patients (83%) received ARDs in a higher dose for oncologic purposes, while the remaining 19 (17.5%) took lower doses for osteoporosis prevention. Malignancy diagnoses included breast cancer (33.9%, n=39), prostate cancer (22.6%, n=26), multiple myeloma (14.8%, n=17), lung cancer (5.2%, n=6), renal cell cancer (5.2%, n=6), and other types of cancer (1.8%, n=2). Most oncologic patients were treated with both chemotherapy and radiotherapy (53.9%, n=62), whereas others received only chemotherapy (16.5%, n=19), radiotherapy (13%, n=15), or other treatments (16.5%, n=19).

Forty patients received conservative therapy and 75 underwent surgery. In the conservative group, 14 patients (35%) healed and required an average of 8.4 months (ranging from 1 to 43 months) from MRONJ diagnosis to mucosal healing, while 26 patients (65%) showed persistence of the lesion and were followed up for an average of 11.5 months (ranging from 1 to 50 months). In the surgical group, 48 patients (64%) healed, which took 14 months (ranging from 1 to 63 months) until achieving mucosal healing. The remaining 27 patients (36%) did not heal during the mean follow-up period of 17 months (ranging from 1 to 61 months). A significantly higher healing rate was observed in patients that received surgical rather than conservative treatment ($p<0.05$).

Different clinical variables were assessed to explain the treatment outcome. The reason for ARD use, whether it was for osteoporosis or malignancy, did not show a

statistically significant difference when comparing treatment outcomes in both the conservative and surgical group ($p>0.05$). Similarly, age, gender, type-, number-, and duration of ARD, arcade of MRONJ lesion, alcohol consumption, and tobacco use also did not have a significant effect in both treatment groups concerning treatment success ($p>0.05$). The investigation of the drug holiday at treatment initiation revealed no significant differences in the conservative group, both overall and when analyzing bisphosphonates (BP) and denosumab separately ($p>0.05$). For the surgical group, no significance was seen in the overall examination nor when isolating denosumab use ($p>0.05$). Yet, a lengthier BP withdrawal had a significant ($p<0.05$) effect on the healing outcome, with a mean interruption in healed patients of 21 months and of 2.3 months at patients who had persisting lesions. A summary of these data at a patient level can be found in Table 1.

Clinical data regarding lesions in the conservative and surgical group can be found in Table 2 and the results of the CBCT assessment in Table 3. An illustrative example of the observed features can be seen in Figure 1. Overall, intra-observer agreement ranged from substantial to almost perfect ($K_{OBSERVER\ 1}=0.828$, $K_{OBSERVER\ 2}=0.669$, $K_{OBSERVER\ 3}=0.899$) and inter-observer agreement was substantial ($K_{OVERALL}=0.725$, ranging from 0.691 to 0.744).

Conservative treatment

From a total of 143 lesions, 58 (41%) received conservative treatment, and 25 (43%) of them achieved healing. Among the 58 lesions, 10 (17%) experienced recurrence, and out of those, only 3 remained unhealed. MRONJ lesions that received conservative treatment were monitored for a mean of 16 months (ranging from 1 to 59 months). Healing occurred on average 10 months (ranging from 1 to 36 months) from the start of treatment until mucosal healing was first observed. CBCT scans were acquired on average 2.6 months after diagnosis (ranging from 0 to 16 months). When evaluating the clinical risk factors, only the staging of the lesion showed significant results. Lesions in stage 1 and 2 showed a significant healing (circa 50%) compared to absence of healing seen in stage 3 lesions ($p<0.05$).

Tomographic characteristics that indicated resistance to treatment included lesions with tooth involvement as 86% of these lesions did not heal, hyperdense trabecular pattern extending to more than 1/3 of the mandible or maxilla with 81% of these lesions persisting, and presence of sequesters involving the maxillary sinus or mandibular canal as 89% of these lesions remained unhealed during follow-up ($p<0.05$) (Figure 2). It is worth noting that from the 14 teeth involved in lesions, 9 had periapical radiolucency or radiographic signs of periodontal disease. Moreover, none of the studied imaging features showed an association with lesion relapse ($p>0.05$).

The variables selected for the GLMM were age, teeth involvement, osteosclerosis extension, and sequester depth. Results indicate that older age, tooth involvement, an extensive osteosclerosis, and deep sequesters significantly decrease the chance of achieving mucosal healing after conservative treatment ($p<0.05$).

Surgical treatment

A total of 85 lesions (59%) received surgical treatment resulting in 55 lesions (65%) that healed. Among these 85 lesions, 9 (11%) had relapse after surgery, with 2 of them remaining unhealed. The surgeries were performed on average 7 months (ranging from 0 to 64 months) after the initiation of conservative treatment, and 61 (72%) of lesions underwent surgical procedure within 6 months of conservative treatment initiation. The mean follow-up duration for this treatment group was of 25 months (ranging from 1 to 106 months) and mucosal healing was first achieved in average 16 months (ranging from 1 to 83 months) after diagnosis and 7 months (ranging from 0.4 to 40 months) after surgery. CBCT examinations took place in an average of 2.3 months (ranging from 0 to 10 months) prior to surgery. In terms of lesion staging, a significant association was found, as lesions in stage 1 achieved the highest healing rate after surgery (86%), with rates diminishing in stage 2 (59%), and 3 (44%) ($p=0.028$).

Tomographic characteristics associated with persistence of the lesion after treatment were the absence of sequestrum formation and the presence of periosteal reaction, while lesions with sequestrum formation presented the most success ($p<0.05$)

(Figure 3). Additionally, no imaging feature under study revealed an association with lesion relapse ($p>0.05$).

The selected variables for the GLMM were gender, duration of ARD treatment, MRONJ staging at the start of treatment, presence of implants, osteolysis extension, sequestrum depth, periosteal reaction, drug holiday, arcade, and use of L-PRF. In the tailored model, only a worse MRONJ staging showed a significant association with a poorer treatment outcome ($p<0.05$).

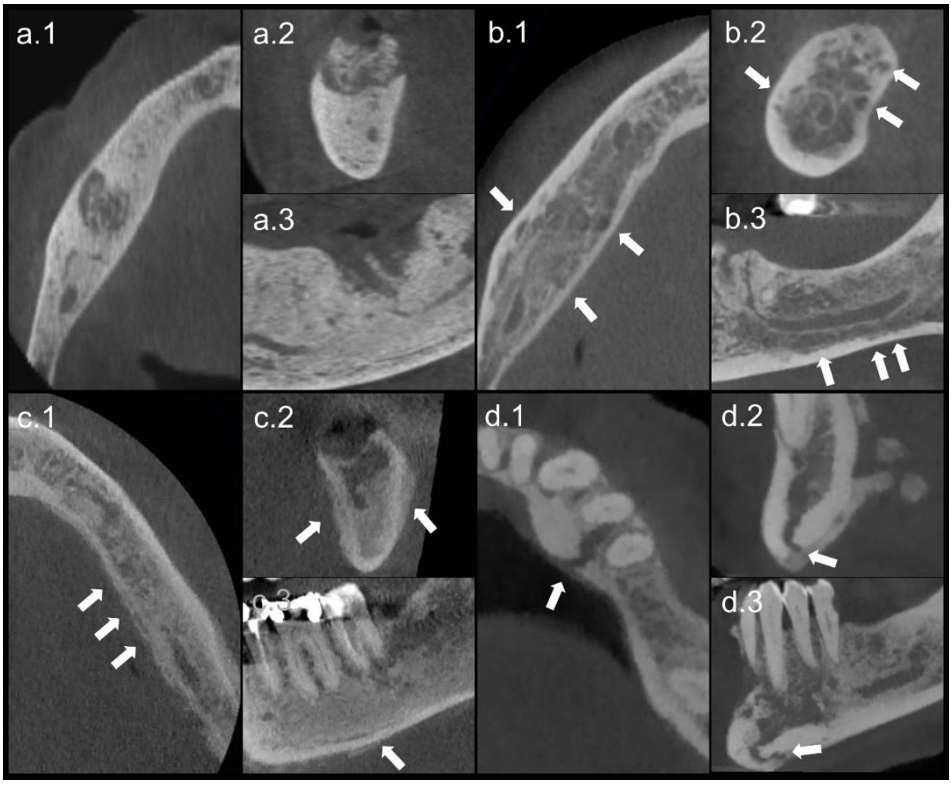

Figure 1. Cropped CBCT reconstructions exampling the imaging features associated with MRONJ in (.1) axial, (.2) coronal, and (.3) sagittal slides of CBCTs. (a) Deep and extensive osteosclerosis with a superficial and localized bone sequestrum. (b) Deep and extensive osteolytic lesions pointed out with white arrows. (c) Extensive periosteal reaction. (d) Mandibular fracture.

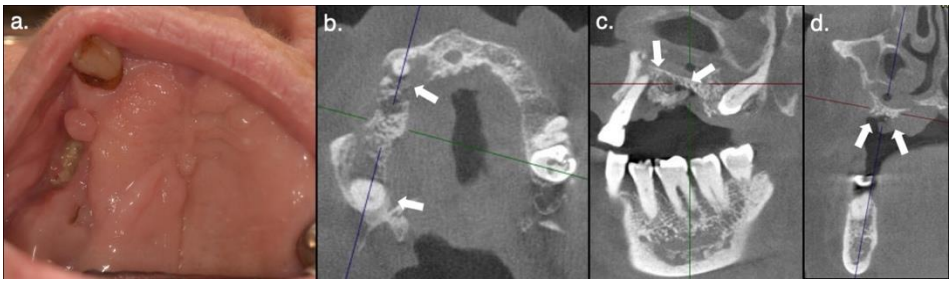

Figure 2. Conservative treatment of osteonecrosis. An 84-year-old female with breast cancer and bone metastases, under denosumab treatment, presented with a year-old osteonecrosis lesion (a). Clinically, there was increased mobility in the upper right canine, absence of pain, oro-nasal or antral communication, inflammation, or suppuration. Conservative treatment was initiated and a CBCT image was taken. Axial (b), sagittal (c), and coronal (d) CBCT slices revealed tooth involvement (b, white arrows), localized osteosclerosis, and bone sequestrum involving the lower nasal wall (c, d, white arrows). At six-month follow-up, loosening of bone fragments was reported, but the lesion showed minor changes. Subsequent visits were not attended due to the patient's deteriorating health.

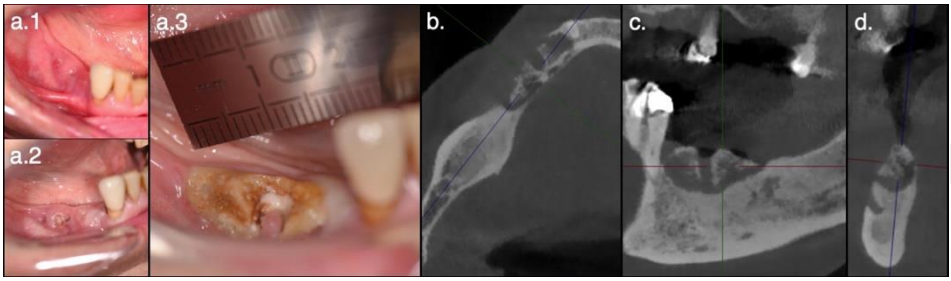

Figure 3. Surgical management of osteonecrosis. A 70-year-old female with breast cancer and bone metastases, treated with denosumab, presented with MRONJ following tooth extractions of the lower right premolars. Initially, there was a small fistula probing to bone accompanied by pain and suppuration (a.1). Despite antibiotic and antiseptic mouthwash administration, the lesion progressed (a.2, one month and, a.3, three months after diagnosis). Axial (b.), sagittal (c.), and coronal (d.) CBCT slices show involvement of the lower right canine, deep and extensive osteosclerosis, and superficial and localized sequestrum formation. Sequestrectomy and L-PRF application under sedation were performed one month after CBCT acquisition (clinical photograph a.3.). Mucosal healing and symptom resolution was observed three months later. No relapse was documented.

Table 1. Descriptive data of patients with MRONJ receiving conservative and surgical treatment.

Characteristic		Conservative Treatment					Surgical Treatment				
		Healed		Persistent		p-value	Healed		Persistent		p-value
Number of patients, n (%)		14	35%	26	65%		48	64%	27	36%	
Age at diagnosis (years)	Mean (range)*	64.8 (46 - 80)		68.4 (45 - 85)		0.347	71.9 (43 - 85)		70.5 (50 - 88)		0.649
Sex, n (%)	Female	9	41%	13	59%	0.594	31	69%	14	31%	0.404
	Male	5	28%	13	72%		17	57%	13	43%	
Staging at diagnosis, n (%)	Stage 1	7	33%	14	67%	0.164	16	76%	5	24%	0.168
	Stage 2	7	50%	7	50%		30	63%	18	38%	
	Stage 3	0	0%	5	100%		2	33%	4	67%	
Arch, n (%)	Maxilla	5	33%	10	67%	1.000	23	70%	10	30%	0.439
	Mandible	8	36%	14	64%		21	60%	14	40%	
	Both	1	33%	2	67%		4	67%	2	33%	
Time on ARD (months)	Mean (range)*	50.3 (3 - 173)		41.8 (10 - 119)		0.470	44.7 (1 - 240)		32.1 (5 - 153)		0.148
Type of ARD, n (%)	Bisphosphonate	4	29%	10	71%	0.698	18	67%	9	33%	0.843
	Denosumab	7	44%	9	56%		23	61%	15	39%	
	Both	3	30%	7	70%		7	70%	3	30%	
Specific ARD, n (%)	Zoledronic Acid	5	25%	15	75%	0.287	17	63%	10	37%	0.292
	Denosumab	10	38%	16	62%		30	63%	18	38%	
	Alendronate	1	25%	3	75%		7	100%	0	0%	
	Pamidronate	1	100%	0	0%		2	67%	1	33%	
	Ibandronate	0	0%	0	0%		1	50%	1	50%	
	Risedronate	1	100%	0	0%		0	0%	0	0%	
Number of ARDs, n (%) *	1	10	36%	18	64%	1.000	39	62%	24	38%	0.394
	2	3	27%	8	73%		9	75%	3	25%	
	3	1	100%	0	0%		0	0%	0	0%	
Drug holiday (months), n (%)	Yes	13	35%	24	65%	1.000	42	62%	26	38%	0.410
	No	1	33%	2	67%		6	86%	1	14%	
	Mean (range)*	5.5 (0 - 26)		7.2 (0 - 88)		0.854	10.3 (0 - 129)		3.4 (0 - 35)		0.084
Corticosteroid use (months), n (%)	No	10	45%	12	55%	0.230	36	72%	14	28%	0.074
	Yes	4	22%	14	78%		12	48%	13	52%	
	Mean (range)*	26.9 (3 - 57)		28 (1 - 127)		0.956	44.4 (3 - 150)		23.4 (1 - 78)		0.152
Alcohol consumption, n (%)	No consumption	5	38%	8	62%	0.710	13	62%	8	38%	0.831
	1-2 units weekly	5	45%	6	55%		19	73%	7	27%	
	3-4 units weekly	0	0%	1	100%		1	100%	0	0%	
	≥5 units weekly	1	17%	5	83%		7	70%	3	30%	
	Ex-abuser	0	0%	0	0%		2	50%	2	50%	
	Unknown	3	33%	6	67%		6	46%	7	54%	
Tobacco use, n (%)	Never smoked	4	31%	9	69%	0.815	23	66%	12	34%	0.947
	Active user	4	44%	5	56%		9	64%	5	36%	
	Previous user	4	31%	9	69%		11	61%	7	39%	
	Unknown	2	40%	3	60%		5	63%	3	38%	

P-values obtained using Chi-Square/Fisher's exact test for categorical data or (*) Mann Whitney U test for ordinal data, to compare at a patient level the treatment outcomes in the surgical and conservative groups. A significant p-value was considered when $p \leq 0.05$. ARD: antiresorptive drugs.

Table 2. Descriptive data of MRONJ lesions receiving conservative and surgical treatment.

Characteristic		Conservative Treatment					Surgical Treatment				
		Healed		Persistence		p-value	Healed		Persistence		p-value
Number of sites, n		25	43%	33	57%		55	65%	30	35%	
Staging at treatment start, n (%) *	Stage 1	10	42%	14	58%	*0.011*	19	86%	3	14%	*0.028*
	Stage 2	15	58%	11	42%		32	59%	22	41%	
	Stage 3	0	0%	8	100%		4	44%	5	56%	
Arch, n (%)	Maxilla	10	40%	15	60%	0.883	28	74%	10	26%	0.184
	Mandible	15	45%	18	55%		27	57%	20	43%	
Reason for MRONJ	Implant	0	0%	0	0%	0.226	2	67%	1	33%	0.881
	Infected tooth	0	0%	0	0%		1	50%	1	50%	
	Periodontitis	0	0%	0	0%		2	40%	3	60%	
	Prosthesis	4	33%	8	67%		8	73%	3	27%	
	Spontaneous	5	29%	12	71%		10	63%	6	38%	
	Tooth extraction	14	54%	12	46%		29	64%	16	36%	
	NS	2	67%	1	33%		3	100%	0	0%	
Antibiotics, n (%)	Yes	24	42%	33	58%	0.431	54	64%	30	36%	1.000
	No	1	100%	0	0%		1	100%	0	0%	
Use of L-PRF, n (%)	Yes	0	0%	0	0%	NA	36	61%	23	39%	0.409
	No	25	43%	33	57%		19	73%	7	27%	

The p-values described under conservative and surgical treatment correspond to those obtained with the Chi-square/Fisher's exact test or Mann–Whitney U test when data were ordinal (*). Comparisons were made between healed and persistent sites in both treatment groups. Significant p-values ($p \leq 0.05$) are *italicized*. MRONJ: Medication-related osteonecrosis of the jaws, NS: Not specified, L-PRF: leukocyte- and platelet-rich fibrin, NA: Not applicable.

Discussion

The present study aimed to identify clinical and three-dimensional imaging findings that serve as therapeutic prognosis risk factors for MRONJ. Our sample included 58 lesions treated non-operatively and 85 lesions treated surgically. Risk factors found for conservative treatment were MRONJ staging, presence of teeth in the lesion, extensive osteosclerosis, and deep sequestrum formation. Complementarily, poor prognostic indicators for surgical therapy were MRONJ staging, length of BP holiday, absence of sequestrum formation, and presence of periosteal reaction.

The choice of whether to recommend conservative or surgical treatment is still a matter of debate. [13] The AAOMS suggests that both approaches can be considered for all clinical stages judging on the disease progression and patient's comorbidities.[2] Similar recommendations are given by the Japanese position paper.[22] Even though,

systematic reviews have indicated that surgical treatment offer superior results to those of conservative therapy.[18,23,24] For this reason, Kawaoka et al. recommend surgical therapy as first choice in all MRONJ stages.[13] In the current investigation, the success rates for non-operative and operative treatment were found to be 43% and 65%, respectively. These rates are comparable to those reported in prior publications ranging from 25% to 46% in conservative treatment [7,11,18,25,26] and from 28% to 92% in surgical therapy.[5,9–11,23,27]

Conservative treatment for MRONJ is proposed to provide symptom relief rather than to reach complete mucosal healing because necrotic bone is unlikely to heal spontaneously. Yet, this approach implies a long-term management, which can potentially lead to progression of the pathology.[11] Although, a Canadian study described that patients' quality of life improved with conservative treatment even if it did not resolve the pathology due to symptom relief.[28] Our results show that despite having asymptomatic patients with unchanged lesions, mucosal healing is improbable if teeth and sequester formation are involved. Moreover, those teeth are often affected by endodontic and/or periodontal disease, which in turn are risk factors for the onset of MRONJ.[29,30] Therefore, tooth extraction is advisable in these patients.

Surgical therapy for MRONJ has been associated with successful outcomes and significantly less recurrence than non-surgical therapy.[27] Variables that could compromise the prognosis are diabetes [13], extensive osteolysis [5,27], absence of sequester [12], severe osteosclerosis [12,13], presence of periosteal reaction [12,13], absence of drug holiday [11,12], and a history of high-dose antiresorptive therapy with either bisphosphonates or denosumab.[11–13] All these findings are consistent with our results. Additionally, a systematic review described higher healing rates in stages 1 and 2 (72% and 79%, respectively) than in stage 3 (27%) with less invasive surgical approaches [23], which is also supported by our findings.

Once the surgical approach is chosen to treat MRONJ, the extent of the resection and use of healing aids like L-PRF or hyperbaric oxygen are at discretion of the surgeon. It has been reported that a better prognosis is associated with extensive

surgical removal in contrast to a minimally invasive procedure.[5,11,18,23] In our hospital, a less invasive surgical approach is often opted, which may explain the moderate success rates. Besides, when sequesters are absent, determining the appropriate resection size becomes challenging. In such instances, radiographic identification of osteosclerosis can serve as a helpful guide.[15] Particularly, when observing periosteal reaction, Kawaoka et al. suggested to remove its complete extent as healing was reached in 83% and 61% of the cases with complete and partial resection, respectively.[13] In parallel, Kojima et al. investigated the factors related to periosteal reaction and found that mandibular osteonecrosis, severe osteosclerosis, and a diagnosis of malignancy were significantly related to this feature.[12]

The absence of a clinical-radiographic evaluation in the AAOMS categorization of osteonecrosis has drawn criticism.[31] Given that imaging features such as osteolysis, cortical bone erosion, sequestrum formation, and osteosclerosis can occur at any clinical stage [2], and this was also seen in our sample, which challenges the clinical staging-based treatment decision. For instance, a lesion that clinically displays no evidence of infection or inflammation may show imaging signs of these, such as periosteal reaction, osteolysis, or a radiopaque maxillary sinus. In addition, MRONJ lesions and their imaging findings may change over time influencing the treatment's prognosis, which makes imaging diagnostic tools vital to consider.[15]

Limitations of this study include those related to its retrospective nature, such as heterogeneity in the data due to surgical variability, differences in the drug scheme, and comorbidities of the included patients. Furthermore, the data belongs to only one treating center, thus providing a restricted sample. Nevertheless, despite these limitations, this study provides evidence that favors operative treatment when encountering lesions in stage 3, with teeth, or sequesters involved. Likewise, surgical treatment showed outstanding results in stage 1, but significantly reduced its effectiveness in stages 2 and 3. Finally, if the latter is chosen and the patient’s health status allows it, a drug holiday, especially under BP use, seems beneficial to the outcome. Further investigations should be carried out to confirm the present findings and assess suitable treatment alternatives for MRONJ lesions showing resistance to

surgical treatment, which presented absence of sequestrum or presence of periosteal reaction on CBCT.

Conclusion

To conclude, this study reports a comprehensive assessment of risk factors for conservative and surgical management of MRONJ. Conservative treatment yielded poor outcomes for lesions at stage 3 MRONJ, with tooth involvement, or sequestrum formation. Conversely, surgical treatment demonstrated its highest effectiveness for stage 1 lesions, particularly when bisphosphonates were discontinued, and in cases with sequestrum formation, and absence of periosteal reaction.

Conflicts of interest

Authors declare explicitly that there are no conflicts of interest.

Author Contributions

1. C.M.R: Conceptualization, Methodology, Data Curation, Investigation, Formal analysis, Writing - Original Draft, Writing - Review & Editing
2. S.G.L: Investigation
3. D.C: Investigation
4. I.C.S: Data Curation
5. R.C.F: Writing - Review & Editing
6. T.VdW: Methodology, Writing - Review & Editing, Supervision
7. R.J: Writing - Review & Editing, Supervision

Table 3. Three-dimensional imaging assessment of MRONJ lesions that received conservative and surgical treatment.

Characteristic		Conservative Treatment					Surgical Treatment				
		Healed		Persistence		p-value	Healed		Persistence		p-value
Number of sites, n		25	43%	33	57%		55	65%	30	35%	
Teeth	Absent	23	52%	21	48%	*0.029*	41	63%	24	37%	0.765
	Present	2	14%	12	86%		14	70%	6	30%	
Implants	Absent	25	44%	32	56%	1.000	51	64%	29	36%	0.652
	Present	0	0%	1	100%		4	80%	1	20%	
Osteosclerosis depth*	Absent	10	59%	7	41%	0.147	10	67%	5	33%	0.779
	Superficial osteosclerosis	2	40%	3	60%		2	40%	3	60%	
	Deep osteosclerosis	13	36%	23	64%		43	66%	22	34%	
Osteosclerosis extension*	Absent	10	59%	7	41%	*0.010*	10	67%	5	33%	0.906
	Localized osteosclerosis	11	55%	9	45%		22	65%	12	35%	
	Extended osteosclerosis	4	19%	17	81%		23	64%	13	36%	
Osteolysis depth*	Absent	12	44%	15	56%	0.959	18	55%	15	45%	0.249
	Superficial osteolysis	5	38%	8	62%		14	74%	5	26%	
	Deep osteolysis	8	44%	10	56%		23	70%	10	30%	
Osteolysis extension*	Absent	12	44%	15	56%	0.663	18	55%	15	45%	0.210
	Localized osteolysis	12	46%	14	54%		32	73%	12	27%	
	Extended osteolysis	1	20%	4	80%		5	63%	3	38%	
Sequester depth*	Absent	13	57%	10	43%	*0.036*	13	50%	13	50%	*0.020*
	Superficial Sequester	11	42%	15	58%		26	65%	14	35%	
	Deep Sequester	1	11%	8	89%		16	84%	3	16%	
Sequester extension*	Absent	13	57%	10	43%	0.090	13	50%	13	50%	0.063
	Localized Sequester	12	36%	21	64%		39	70%	17	30%	
	Extended Sequester	0	0%	2	100%		3	100%	0	0%	
Periosteal reaction*	Absent	22	46%	26	54%	0.392	52	70%	22	30%	*0.008*
	Localized Reaction	2	40%	3	60%		0	0%	2	100%	
	Extended Reaction	1	20%	4	80%		3	33%	6	67%	
Fracture	Absent	25	45%	31	55%	0.501	55	65%	30	35%	NA
	Present	0	0%	2	100%		0	0%	0	0%	

P-values obtained using Chi-Square/Fisher's exact test for categorical data or (*) Mann Whitney U test for ordinal data when comparing healed and persistent sites in the conservative and surgical group. Significant values are marked in *italic* (p≤0.05). NA: Not applicable.

References

1. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw--2014 update. J Oral Maxillofac Surg. 2014 Oct;72(10):1938–56.

2. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022 May;80(5):920–43.

3. Campisi G, Fedele S, Fusco V, Pizzo G, Di Fede O, Bedogni A. Epidemiology, clinical manifestations, risk reduction and treatment strategies of jaw osteonecrosis in cancer patients exposed to antiresorptive agents. Future Oncology. 2014 Feb;10(2):257–75.

4. Gavaldá C, Bagán JV. Concept, diagnosis and classification of bisphosphonate-associated osteonecrosis of the jaws. A review of the literature. Med Oral Patol Oral Cir Bucal. 2016 May 1;21(3):e260-70.

5. Shin WJ, Kim CH. Prognostic factors for outcome of surgical treatment in medication-related osteonecrosis of the jaw. J Korean Assoc Oral Maxillofac Surg. 2018 Aug 31;44(4):174.

6. Del Fabbro M, Gallesio G, Mozzati M. Autologous platelet concentrates for bisphosphonate-related osteonecrosis of the jaw treatment and prevention. A systematic review of the literature. Eur J Cancer. 2015 Jan;51(1):62–74.

7. Lobekk OK, Dijkstra W, Pedersen TØ. Surgical vs conservative treatment of medication-related osteonecrosis of the jaw: A complex systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2021 Dec 1;132(6):671–9.

8. Ahrenbog G, Gottsauner M, Meier JK, Ettl T, Reichert TE, Klingelhöffer C. Surgical treatment of advanced medication-related osteonecrosis of the jaws: Comparison of soft tissue closure techniques and evaluation of side effects. Journal of Cranio-Maxillofacial Surgery. 2020 Sep 1;48(9):896–901.

9. Klingelhöffer C, Zeman F, Meier J, Reichert TE, Ettl T. Evaluation of surgical outcome and influencing risk factors in patients with medication-related osteonecrosis of the jaws. Journal of Cranio-Maxillofacial Surgery. 2016 Oct 1;44(10):1694–9.

10. Carlson ER, Basile JD. The Role of Surgical Resection in the Management of Bisphosphonate-Related Osteonecrosis of the Jaws. Journal of Oral and Maxillofacial Surgery. 2009 May 1;67(5):85–95.

11. Hayashida S, Soutome S, Yanamoto S, Fujita S, Hasegawa T, Komori T, et al. Evaluation of the Treatment Strategies for Medication-Related Osteonecrosis of the Jaws (MRONJ) and the Factors Affecting Treatment Outcome: A Multicenter Retrospective Study with Propensity Score Matching Analysis. Journal of Bone and Mineral Research. 2017 Oct 1;32(10):2022–9.

12. Kojima Y, Kawaoka Y, Sawada S, Hayashida S, Okuyama K, Yutori H, et al. Clinical significance of periosteal reaction as a predictive factor for treatment outcome of medication-related osteonecrosis of the jaw. J Bone Miner Metab. 2019 Sep 4;37(5):913–9.

13. Kawaoka Y, Kojima Y, Sawada S, Funahara M, Hayashida S, Yutori H, et al. Periosteal reaction as a risk factor for poor outcomes after surgical treatment for medication-related osteonecrosis of the jaw: A retrospective analysis of 205 surgeries. J Oral Maxillofac Surg Med Pathol. 2020 Jan 1;32(1):8–13.

14. Shimizu T, Kim M, Dam TT, Kurihara J, Ogawa M, Makiguchi T, et al. Predictive factors for refractory stage I and II anti-resorptive agent-related osteonecrosis of the jaw. Oral Radiol. 2022 Apr 3;38(2):240–51.

15. Yasui T, Nagamine H, Tanaka K, Kimura M, Karube T, Kawana H, et al. Treatment outcomes and time to healing of medication-related osteonecrosis of the jaw based on image findings. Dentomaxillofacial Radiology. 2023 May 16;20220352.

16. Wilde F, Heufelder M, Lorenz K, Liese S, Liese J, Helmrich J, et al. Prevalence of cone beam computed tomography imaging findings according to the clinical stage of bisphosphonate-related osteonecrosis of the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Dec;114(6):804–11.

17. Treister NS, Friedland B, Woo SB. Use of cone-beam computerized tomography for evaluation of bisphosphonate-associated osteonecrosis of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 May;109(5):753–64.

18. Fliefel R, Tröltzsch M, Kühnisch J, Ehrenfeld M, Otto S. Treatment strategies and outcomes of bisphosphonate-related osteonecrosis of the jaw (BRONJ) with characterization of patients: a systematic review. Int J Oral Maxillofac Surg. 2015 May;44(5):568–85.

19. Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, et al. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280.

20. Soutome S, Otsuru M, Hayashida S, Yanamoto S, Sasaki M, Takagi Y, et al. Periosteal reaction of medication-related osteonecrosis of the jaw (MRONJ): clinical significance and changes during conservative therapy. Supportive Care in Cancer. 2021 Nov 21;29(11):6361–8.

21. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005 May;37(5):360–3.

22. Yoneda T, Hagino H, Sugimoto T, Ohta H, Takahashi S, Soen S, et al. Antiresorptive agent-related osteonecrosis of the jaw: Position Paper 2017 of the Japanese Allied Committee on Osteonecrosis of the Jaw. J Bone Miner Metab. 2017 Jan 1;35(1):6–19.

23. Rupel K, Ottaviani G, Gobbo M, Contardo L, Tirelli G, Vescovi P, et al. A systematic review of therapeutical approaches in bisphosphonates-related osteonecrosis of the jaw (BRONJ). Oral Oncol. 2014 Nov;50(11):1049–57.

24. Khan AA, Morrison A, Hanley DA, Felsenberg D, McCauley LK, O'Ryan F, et al. Diagnosis and Management of Osteonecrosis of the Jaw: A Systematic Review and International Consensus. Journal of Bone and Mineral Research. 2015 Jan 1;30(1):3–23.

25. Freiberger JJ, Padilla-Burgos R, McGraw T, Suliman HB, Kraft KH, Stolp BW, et al. What Is the Role of Hyperbaric Oxygen in the Management of Bisphosphonate-Related Osteonecrosis of the Jaw: A Randomized Controlled Trial of Hyperbaric Oxygen as an Adjunct to Surgery and Antibiotics. Journal of Oral and Maxillofacial Surgery. 2012 Jul;70(7):1573–83.

26. Kaibuchi N, Hoshi K, Yamazaki A, Miyamoto-Sangu N, Akagi Y, Okamoto T. The progress of medication-related osteonecrosis of the jaw with conservative initial treatment: A 12-year retrospective study of 129 patients. Bone Rep. 2021 Jun 1;14:101072.

27. Mucke T, Koschinski J, Deppe H, Wagenpfeil S, Pautke C, Mitchell DA, et al. Outcome of treatment and parameters influencing recurrence in patients with bisphosphonate-related osteonecrosis of the jaws. J Cancer Res Clin Oncol. 2011 May;137(5):907–13.

28. El-Rabbany M, Blanas N, Sutherland S, Lam DK, Shah PS, Azarpazhooh A. Surgical Therapy in Patients With Medication-Related Osteonecrosis of the Jaw Is Associated With Disease Resolution and Improved Quality of Life: A Prospective Cohort Study. J Oral Maxillofac Surg. 2022 Jun;80(6):1084–93.

29. Ueda N, Aoki K, Shimotsuji H, Nakashima C, Kawakami M, Imai Y, et al. Oral risk factors associated with medication-related osteonecrosis of the jaw in patients with cancer. J Bone Miner Metab. 2021 Jul 8;39(4):623–30.

30. Moreno-Rabié C, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, et al. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8;

31. Fusco V, Mauceri R, Campisi G, Bedogni A. RE: American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws—2022 Update: Quantitative Risk Assessment and Controversial Issues. Vol. 80, Journal of Oral and Maxillofacial Surgery. J Oral Maxillofac Surg; 2022. p. 1871–3.

PART 3: ARTIFICIAL INTELLIGENCE AS AN AID FOR MRONJ DIAGNOSIS

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CHAPTER 6:

Baseri Saadi S, Moreno-Rabié C, van den Wyngaert T, Jacobs R. Convolutional neural network for automated classification of osteonecrosis and related mandibular trabecular patterns. Bone Rep. 2022 Oct 29;17:101632. doi: 10.1016/j.bonr.2022.101632. (Shared first authorship)

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CHAPTER 6: AUTOMATED CLASSIFICATION OF MANDIBULAR TRABECULAR PATTERNS

Convolutional neural network for automated classification of osteonecrosis and related mandibular trabecular patterns

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Abstract

Objective: The present study aimed to develop and validate a tool for the automated classification of normal, affected, and osteonecrosis mandibular trabecular bone patterns in panoramic radiographs using convolutional neural networks (CNNs).

Methods: A dataset of 402 panoramic images from 376 patients was selected, comprising 112 control radiographs from healthy patients and 290 images from patients treated with antiresorptive drugs (ARD). The latter was subdivided in 70 radiographs showing thickening of the lamina dura, 128 with abnormal bone patterns, and 92 images of clinically diagnosed osteonecrosis of the jaw (ONJ). Four pre-trained CNNs were fined-tuned and customized to detect and classify the different bone patterns. The best performing network was selected to develop the classification tool. The output was arranged as a colour-coded risk index showing the category and their odds. Classification performance of the networks was assessed through evaluation metrics, receiver operating characteristic curves (ROC), and a confusion matrix. Furthermore, Gradient-weighted Class Activation Mapping (Grad-CAM) was employed to visualise class-discriminative regions.

Results: All networks correctly detected and classified the mandibular bone patterns with optimal performance metrics. InceptionResNetV2 showed the best results with an accuracy of 96%, precision, recall and F1-score of 93%, and a specificity of 98%. Overall, most misclassifications occurred between normal and abnormal trabecular bone patterns.

Conclusion: CNNs offer reliable potentials for automatic classification of abnormalities in the mandibular trabecular bone pattern in panoramic radiographs of antiresorptive treated patients.

Clinical significance: A novel method that supports clinical decision making by identifying sites at high risk for ONJ.

Keywords*: osteonecrosis, panoramic radiography, diagnostic imaging, artificial intelligence, convolutional neural network*

Introduction

The radiographic appearance of the mandibular trabecular bone pattern is a recurring topic of interest in dental research due to its direct impact on the prognosis of bone tissue-related treatments, such as dental implant placement [1]. When beginning with the bone pattern assessment, panoramic radiographs are a useful and widely available diagnostic tool [2,3]. They allow the identification of bony changes, which are caused by different reasons, including systemic diseases like osteoporosis or diabetes [3], condensing osteitis [4], and the use of antiresorptive drugs (ARDs), namely bisphosphonates and denosumab [5].

ARDs are effective medications used to manage oncological conditions secondary to bone metastases and osteoporosis-related fractures [6]. Their mechanism of action alters the bone resorption-apposition cycle by impeding osteoclast activity through different pathways [7], thus favouring bone apposition. Consequently, the use of these drugs has been associated with radiographic findings on panoramic radiographs [5,6] and the development of a side effect known as Medication-Related Osteonecrosis of the Jaws (MRONJ) [6,8]. The latter can be clinically identified as exposed bone in the oral cavity present for more than eight weeks in patients treated with ARD [6,9].

Patients receiving ARD treatment and without bone exposure may show in their panoramic images, osteosclerosis, thickening of the lamina dura and of the mandibular cortical, osteolytic areas, persistence of the extraction socket, and widening of the periodontal ligament space [5]. On the other hand, MRONJ lesions show sclerosis, lytic changes, periosteal reaction, and sequestrum formation [10]. These radiographic findings are important to identify, specially before the onset of osteonecrosis since some may act as predisposing factors for its occurrence. In fact, heterogeneous [11] and sclerotic trabecular bone patterns [12,13] have been identified as risk factors for MRONJ.

It is in the identification of these radiographic findings that clinicians could benefit from an objective and automated approach. The role of deep learning,

specifically with convolutional neural networks (CNNs), has gained great importance in the classification, detection, and segmentation of objects of interest in medical imaging [14], showing promising results in dental applications both with two- and three-dimensional images. For instance, CNNs have been applied to automatically detect and segment teeth [15–17] and cystic lesions [18] in panoramic radiographs. Moreover, examples of applications of CNNs in Cone-Beam Computed Tomography (CBCT) include, mandibular canal segmentation [4] and tooth segmentation and classification [19].

Based on the prior evidence, the main aim of this study is to develop and validate a tool for the automated classification of normal, affected, and osteonecrosis mandibular trabecular bone patterns in panoramic images using CNNs.

Material and Methods

Study design and settings

The ethical committee of UZ/KU Leuven approved the elaboration of this retrospective cohort study (reference number: MP018766) and waived the need for informed consent. In addition, the World Medical Association Declaration of Helsinki and the standards of the Institutional Review Board were obeyed. To perform this study, panoramic radiographs were collected from patients treated in the department of oral and maxillofacial surgery at the University Hospitals of Leuven in Belgium.

Dataset

Panoramic radiographs were obtained from patients older than 18 years, treated with at least one administration of ARD, who fit into one of the following three groups, (1) showing thickening of the lamina dura (TLD), (2) abnormal bone pattern (ABP) such as bone sclerosis or persistence of the extraction socket, or (3) presenting with medication-related osteonecrosis of the jaw (MRONJ). Clinically, the first two patient groups had at the time of radiographic acquisition absence of bone exposure in the oral cavity, while the third group had a diagnosed MRONJ lesion with consequent clinical bone exposure [9]. In addition, a control group of healthy patients without ARD

treatment, absence of maxillofacial pathologies, and with a normal bone pattern, was selected. These images were acquired with two different panoramic radiographic machines (Vista Pano S Ceph, Durr Dental, Bissingen-Bietigheim, Germany and Promax 2D, Planmeca, Helsinki, Finland), had a dimension of 2880 x 1504 pixels, and were anonymized at the time of export. Their indication was diagnosis and/or treatment planning for reasons other than the participation to this study.

A dataset of 402 panoramic images from 376 patients were selected. The mean age of the patients was 61 years (SD±18.6, range 25 – 94) in the control group, 64 years (SD±13.8, range 18 – 94) in the TLD, 69 years (SD±9.6, range 38 – 91) in the ABP, and 70 years (SD±10.3, range 48 – 91) in the MRONJ group. From the total images, 112 belonged to healthy control patients and 290 to patients treated with ARDs. The latter group was subdivided in 70 radiographs showing thickening of the lamina dura, 128 with abnormal bone pattern, and 92 images of clinically diagnosed osteonecrosis of the jaw.

Once all images were collected, mandibular regions of interest (ROIs) of 512 x 512 pixels were cropped using GIMP software (version 2.10.22, GIMP Development Team, CA, USA), resulting in 236 croppings from the control group, 126 with thickened lamina dura, 251 with abnormal bone pattern, and 131 cut-offs with osteonecrosis of the jaw. The image selection, cropping, and labelling was performed by a general dentist (CMR) and revised by a dentomaxillofacial radiologist (RJ) with 30 years of experience, serving as ground truth. The complete dataset was randomly divided into three sets using the python split-folders library (version 0.5.1, licensed from MIT, MA, USA). The same proportion of images from each group was assigned to each set, resulting in 536 images in the training set (70% of the images of each group), 74 images in the validation set (10%), to test the performance of the models during the training phase, and 134 images in the test set (20%), used to evaluate the performance of the models by comparing the results with the ground truth data.

Due to the limited dataset, data augmentation was performed using an open source Python library, Albumentations augmentation library [20]. Transformations from

pixel- to spatial-level were implemented to prevent overfitting and provide optimal results. Augmentation methods led to a dataset of 10000 images. The networks were trained and validated with 7000 (70% of dataset) and 1000 (10% of dataset) images, respectively. The remaining 2000 images (20% of dataset) were used as test set with 500 images obtained for each of the four classes. Figure 1 shows the workflow until reaching the final dataset.

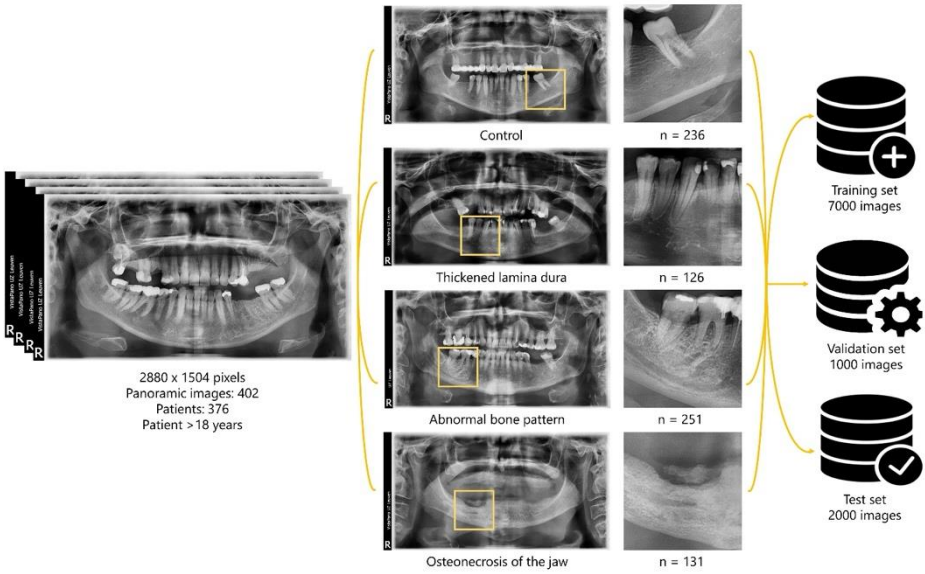

Figure 1. Workflow from image collection to the final number of images used for training, validation and testing of the algorithms. Once collected, the images were classified into different groups, areas of interest of 512x512 pixels were selected and augmentation techniques were performed until the final number of 10000 images was reached.

AI framework

Three popular very deep convolutional neural networks and one mobile design CNN architecture for resource constrained environments were selected to perform the classification task in the present study. The selected networks and their layer numbers were as follows, ResNet152V2 (n=152) [21], InceptionResNetV2 (n=164) [22], Densenet201 (n=201) [23], and MobileNetV2 (n=53) [24].

The models were implemented through Keras library and its applications [25] using transfer learning methods, pre-designed models and pre-trained weights. The selected networks were designed based on a variation of deep residual learning principle [21] and pretrained with ImageNet database [26].Once the models were extracted from Keras library, they were customized by replacing the classification head with a 512-unit dense layer, the 'Sigmoid' activation function using 'He_uniform' as the kernel initializer, a 25% dropout layer, and a fully connected layer with 'Softmax' activation function and four outputs. The last modification aimed to homologate the number of classes in the dataset. All models' layers were set to be untrainable with the exception of the last customised layer. The datasets were read, pre-processed and resized to a resolution of 256 x 256 pixels using OpenCV [27], Matplotlib [28] and Numpy libraries [29]. Then, they were normalized to a fixed range (0,1) for training, validation, and test phases. Training of the models was performed with categorical cross-entropy as the loss function, Adam's algorithm as the optimizer with an initial learning rate of 0.001, a batch size of 32, and a weight decay parameter of 2e-4. The models achieved convergence at different epochs by controlling the validation loss using early stopping with the patience rate of 30.

The best performing model was selected to develop a desktop classification tool. The tool was developed using PyQt5 library (version 5.15.7, Riverbank Computing Limited, Dorchester, UK), a set of cross-platform libraries in C++ that provides high-level application programming interfaces (APIs). The output was presented as a colour-coded clinical risk index to provide clinicians with a straightforward scale to determine the referral and treatment needs of individual patients undergoing treatment with ARD. The color-coded classification indicated in green a normal trabecular bone pattern. Yellow showed thickening of the lamina dura as an indicator of bone changes induced by antiresorptive drugs with an initial advice for a cautionary surgical approach. Orange referred to an abnormal bone pattern attributed to the use of antiresorptive drugs and indicates potential negative bone remodelling with a warning to limit surgical trauma to that area. Finally, red would imply recognition of osteonecrosis of the mandible. The

colour classification was accompanied by a relative probability for the region of interest.

Modelling was performed using Keras deep learning framework (version 2.10.0)[25], Tensorflow (version 2.10.0) and tensorflow-gpu (version 2.10.0) [30] and implemented on an Intel(R) Xeon(R) W-2104 CPU@3.20GHz 3.19 GHz with 32.0 GB Ram, and a graphic card of NVIDIA Quadro P4000 GPU (NVIDIA Corporation, U.S.A) with a memory of 8 GB GDDR5.

Evaluation metrics

The following multiclass classification metrics [31] were used to evaluate the performance of the CNN models on the test dataset:

- Accuracy: percentage of correctly classified images considering the whole sample.

  $Accuracy = \frac{TP+TN}{TP+TN+FP+FN}$

- Precision: percentage of correctly classified positives from all assigned positives.

  $Precision = \frac{TP}{TP+FP}$

- Recall (Sensitivity): percentage of correctly classified positives from the ground truth.

  $Recall = \frac{TP}{TP+FN}$

- F1-score: weighted average between precision and recall in percentage.

  $F1\text{-}score = 2 \cdot (\frac{Precesion \cdot Recall}{precision+Recall})$

- Specificity: percentage of correctly classified negatives from the ground truth.

  $Specificity = \frac{TN}{TN+FP}$

Where true positive (TP) indicates the correctly classified images among the different categories, true negative (TN), the number of images where the model correctly classified as not belonging to a group, false positive (FP), the number of the images where the network misclassified as belonging to a group, and false negative

(FN), express the classifications where the model incorrectly classified an image as not belonging to a group, but it did belong. In other words, these values assess the ability of the system to classify the images properly.

The models were further assessed using tf-keras-vis library (version 0.8.2, MIT Licence, MA, USA) to implement the explainable artificial intelligence (XAI) elements, were a Gradient-weighted Class Activation Mapping (Grad-CAM) [32] was obtained to provide a visual localization of class-discriminative regions. In addition, a Receiver Operating Characteristic (ROC) curve and confusion matrix were acquired with Scikit-learn library [33] from each model to evaluate their classification performance.

Statistical analysis

RStudio version 4.0.4 (RStudio, Boston, MA US) was used to perform the statistical analysis. The metric values were tested for normality using Shapiro–Wilk test and visual inspection with a Q-Q Plot. Then, the Kruskal Wallis test was implemented to test the statistical significance of the accuracy, precision, F1-score, recall (sensitivity), and specificity between the models. A p-value ≤0.05 was considered statistically significant.

Results

The results of the evaluation metrics computed for each network on the test set are presented in Table 1. Overall, the best results were achieved by InceptionResNetV2 and MobileNetV2 with an accuracy of 96%, while Densenet201 showed the lowest performance with 88% of accuracy. Although, when performing the Kruskal Wallis test, the statistical computations demonstrated that there was no significant difference between the accuracy (p=0.152), precision (p=0.150), recall (p=0.119), F1-score (p=0.164), and specificity (p=0.117) of the models. Given the slightly better classification metrics, InceptionResNetV2 was chosen as the CNN with which the classification model would be developed. An example of the interface can be seen in Figure 6.

The training history of the models is presented in Figure 2. The models' performance improved as the number of epochs increased. A slight overfitting was

observed in the accuracy and loss values of the training and validation sets in each model. Moreover, convergence was achieved at different epochs, being in ResNet152V2 at 29, in InceptionResNetV2 at 60, in MobileNetV2 at 46, and in DenseNet201 at 31 epochs.

Figure 3 shows the ROC curves of each network for different classifications. The accuracy of classification, based on the area under the curves, was the highest in MobileNetV2 (mean 0.95), followed with a minor difference by InceptionResNetV2 (mean 0.947), ResNet152V2 (mean 0.937), and lastly, DenseNet201 (mean 0.84). Yet, InceptionResNetV2 had the best performance for the classification of ABP, while MobileNetV2 achieved the best performance when classifying MRONJ, TLD, and control groups. Additionally, the classification performance of the models was plotted through a confusion matrix (Figure 4), comparing the class predictions against the ground truth.

The heat maps of the two best performing networks, InceptionResNetV2 and MobileNetV2, are presented in Figure 5 using a class activation map. Both networks assigned the highest activation regions to the distinguishing features of each trabecular bone pattern, displayed with warm colours in the figure. InceptionResNetV2 was more successful in combining the detection of globally and locally distributed features to discriminate the classes, while MobileNetV2 demonstrated a better performance in the detection of complex localized features.

Discussion

Convolutional neural networks have become a popular technique in the field of dentistry for detecting and classifying various pathologies and objects on radiographs [34–37]. Hence, we proposed in this study the use of CNNs for the automated classification of trabecular bone patterns in patients treated with antiresorptive drugs and who developed MRONJ. To the best of our knowledge, this is the first attempt to use an artificial intelligence model for this purpose.

Table 1. Classification metrics used to evaluate the performance of the CNN models on the test dataset.

Parameters	Accuracy (%)	Model	Precision (%)	F1-Score (%)	Recall (%) (Sensitivity)	Specificity (%)
55.1M	0.96 (0.93, 0.99)	InceptionResNetV2	0.93 (0.84, 1)	0.93 (0.87, 0.99)	0.93 (0.83,1)	0.98 (0.94, 1)
59.3M	0.95 (0.92, 0.99)	ResNet152V2	0.91 (0.84, 0.98)	0.91 (0.82, 0.99)	0.91 (0.79, 1)	0.97 (0.94, 1)
18.8M	0.88 (0.78, 0.98)	Densenet201	0.77 (0.55, 0.98)	0.76 (0.57, 0.94)	0.76 (0.55, 0.96)	0.92 (0.83, 1)
2.9M	0.96 (0.93, 1)	MobileNetV2	0.92 (0.85, 0.99)	0.92 (0.84, 0.99)	0.92 (0.77, 1)	0.98 (0.97, 0.99)

These values were obtained by comparing the results of the classification with the ground truth (mean, 95% CI).

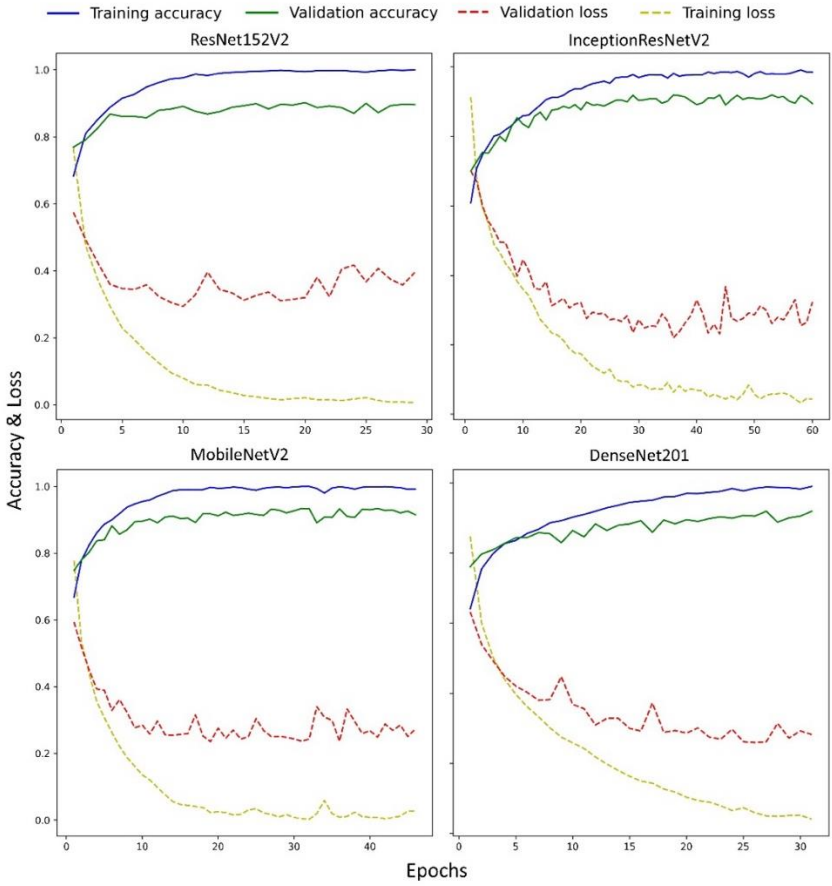

Figure 2. Training history of the networks depicting the loss and accuracy values of the models at different epochs during training and validation phases.

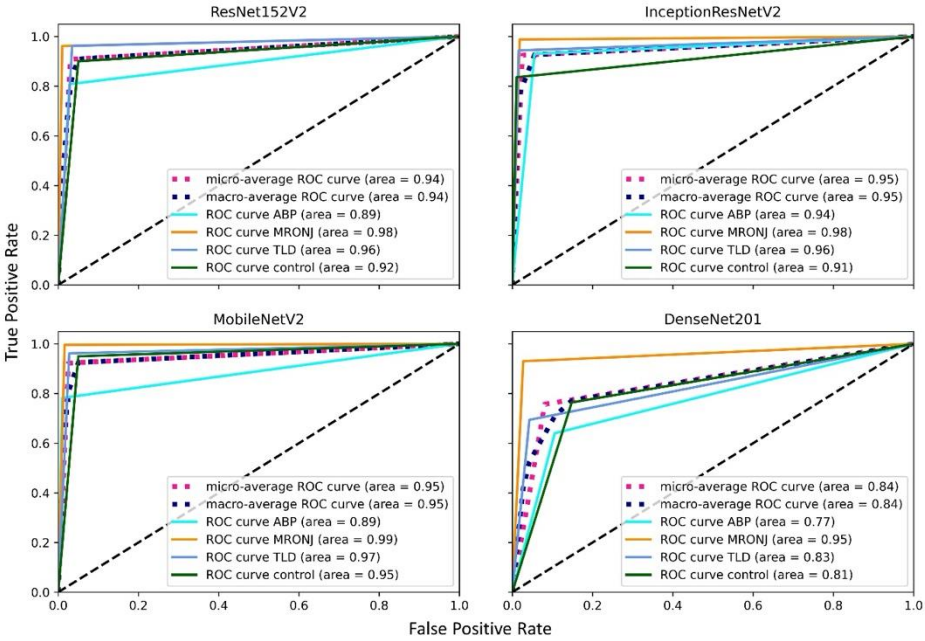

Figure 3. Receiver operating characteristic curves (ROC) of the models on the test dataset. The Area Under the Curve (AUC) for each class is shown as well as the micro and macro-averages across all classes.

All networks successfully classified all bone patterns and showed no statistical differences in their performance. From them, InceptionResNetV2 has already been used for dental applications, showing favourable results in automatic caries detection (accuracy of 0.87) [38], and in the classification of mesiodens (accuracy 0.92) [39] and teeth (accuracy 0.94) [40]. Nevertheless, one of the main disadvantages of working with very deep networks like this one, is the need for computational power that is not always available in clinical settings and research laboratories [24,41]. For this reason, we tested MobileNetV2, which, while maintaining its complexity, operates with fewer parameters and demands less sophisticated hardware [24]. Our results support the latter as a reliable substitute, as this network achieved the highest average AUC.

Despite achieving high accuracies, most of the misclassifications occurred between the abnormal bone pattern and the control images in all models. Perhaps an explanation lies in the distinctive features of each bone pattern. Most notable are the

contrast differences. For instance, osteonecrosis lesions are bounded by clear radiolucent and radiopaque lines, resulting in high-contrast edges being easier to identify by both the human eye and the networks. On the other hand, abnormal patterns have less pronounced radiopaque areas distributed along the trabeculae which are less distinguishable from the homogeneous surface of the normal bone pattern. Given that the algorithms recognize high-contrast edges as seen in the Grad-CAM, images with normal and abnormal bone patterns become more challenging to classify.

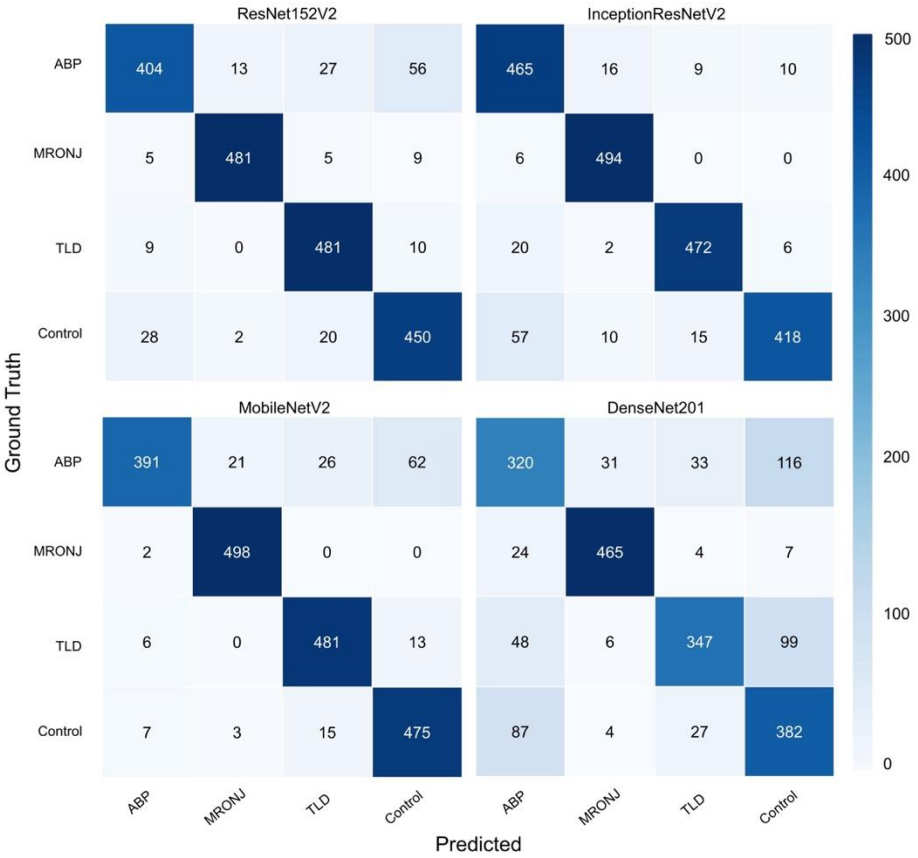

Figure 4. Multiclass confusion matrix of the test dataset (2000 images in total, 500 images for each class) for the four networks (InceptionResNetV2, ResNet152V2, DenseNet201, and MobileNetV2). The diagonal values refer to the correctly classified images (true positives), and the off-diagonal values depict misclassifications (false positives). Elements were colour-mapped according to the maximum and minimum values at the right colour-map bar.

Another explanation may be found in the images selected for each group. In the control group, the images were from a slightly younger population showing mostly sites with a natural dentition and with only 11% of the ROIs involving fully edentulous areas. While 30% of the ROIs in the abnormal bone pattern displayed such condition. When looking at the edentulous areas in the control group, InceptionResNetV2 misclassified 32% of them into ABP. Furthermore, it was seen in the activation maps of several control images that teeth acted as a confounding factor with the area of interest resting on them, suggesting that the indicative feature of the control group was the presence of teeth and rather than the appearance of the bone.

Although there were no significant differences between the CNNs, InceptionResNetV2 was chosen for the development of the automated tool because of its better metrics and performance in ABP classification. While the other categories are important to recognize, they represent less of a challenge for the clinician and our interest lies in the early identification of sclerotic patterns as a risk factor for osteonecrosis [13]. This CNN showed a superior performance in the detection of abnormal patterns due to the presence of large and small kernels at different equivalent depths in its architecture [22], allowing the efficient extraction and merging of globally and locally distributed features, such the mild radiopacities spread over the trabecular bone seen in these images. Additionally, to improve the performance of the model, an expert function could be added to the software where human-supervised corrections can be incorporated to learn from new data and rectify incorrect predictions.

Radiographic findings in patients treated with ARD are not uncommon [5]. In this regard, the color-coding system is of interest when these patients are to undergo tooth extraction, as it allows for easy diagnostic filtering. The presence of a green light or normal bone pattern will indicate a favourable scenario at that tooth extraction site, as this does not increase the likelihood of MRONJ [13,42,43]. Furthermore, while the presence of thickening of the lamina dura is presented as a radiographic and pharmacokinetic marker indicating intake of these drugs [13,44], bone changes related to antiresorptive drugs, such as osteosclerosis, have been associated with an increased risk for MRONJ

[13,42,43]. Finally, a red colour would indicate a settled osteonecrosis lesion in which referral to a specialized clinic for timely treatment is necessary.

Future applications of this diagnostic tool include evaluation prior to implant placement in patients treated with ARDs or early identification of MRONJ prior to bone exposure. It is worth noting that all regions of interest involving osteonecrosis belonged to mature lesions, which showed sequestrum formation, obvious lytic areas and osteosclerosis. Since the radiographic appearance of MRONJ is variable and does not necessarily correlate with clinical staging [45,46], less obvious lesions should be presented to the network to assess possible differences between these and an abnormal bone pattern, given that mild radiographic osteonecrosis lesions are almost indistinguishable from sclerotic or abnormal bone patterns by the human eye. Consequently, some authors have suggested that these sites are latent osteonecrosis lesions that remain unexposed to the oral cavity [13,42,45,47].

Further studies should aim to overcome the limitations of this investigation. To prevent overfitting during training phase and improve the classification performance of the networks, the models should be less generalized by training them with a larger dataset [14]. The dataset of this study was limited as it belonged to only one centre [39] and given that MRONJ has a rather low incidence [9,48]. Although a novel augmentation method was used [20], a variety of data from different panoramic devices and with different scanning parameters are required to prevent biased classification. Moreover, the restricted hardware set up limited the employment of more trainable layers and increasing the batch size. Upgrading and utilizing a more powerful hardware would be imperative to improve the training results. Finally, the Grad-CAM visualisation showed that high contrast and sharp edges attract the highest attention of the models. Through employing feature selection, it would be possible to filter irrelevant or redundant features such as teeth, which are not involved in the classification of bone patterns. Hence, minimizing misclassification of the models.

Conclusion

In the present study, four different CNN architectures successfully classified different mandibular trabecular bone patterns showing reliable potentials for the identification of abnormalities in panoramic radiographs of antiresorptive treated patients. The best network, InceptionResNetV2, was selected for the development of a diagnostic tool. The proposed method is expected to support clinical decision making when alarming trabecular patterns are recognized, thereby minimizing complications with early diagnosis and treatment planning.

Conflicts of interest

Authors declare explicitly that there are no conflicts of interest.

Author Contribution

1. S.B.S: formal analysis, methodology, writing original draft, writing-review & editing.
2. C.M.R: data curation, statistical analysis, writing original draft, writing-review & editing.
3. T.VdW: supervision, writing-review & editing.
4. R.J: conceptualization, supervision, writing-review & editing.

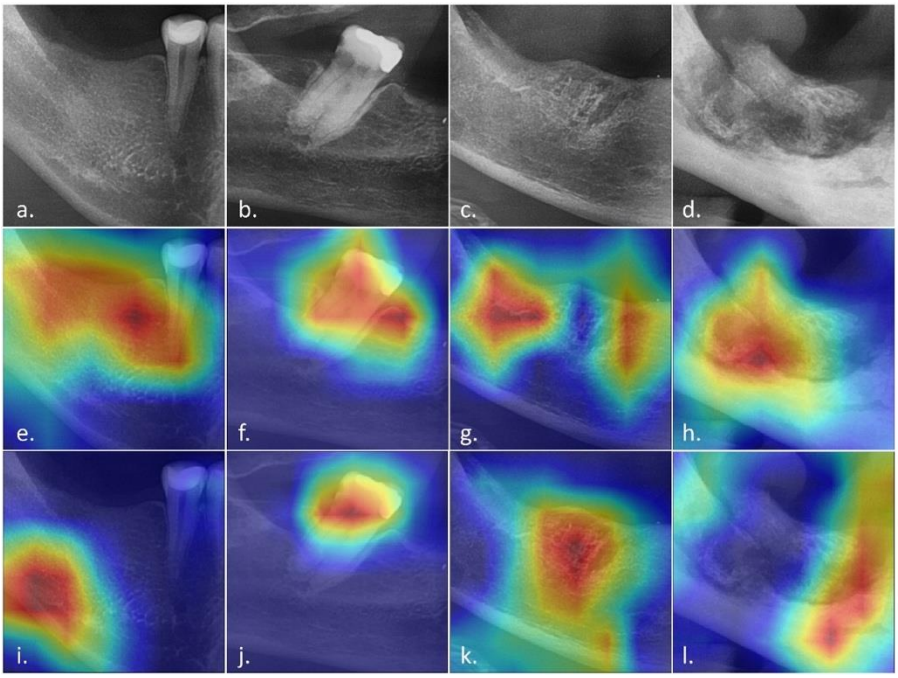

Figure 5. Gradient-weighted Class Activation Mapping (Grad-CAM) of the InceptionResNetV2 (e., f., g., h.) and the MobileNetV2 (i., j., k., l.) for a. control, b. thickened lamina dura (TLD), c. abnormal bone pattern (ABP), and d. osteonecrosis (MRONJ) images. The regions of interest for the algorithm are indicated by means of a warm and cold colour code. Being the warm regions, those in which greater attention was paid to the image features (high-weighted) and the cold regions in which there was less interest (low-weighted).

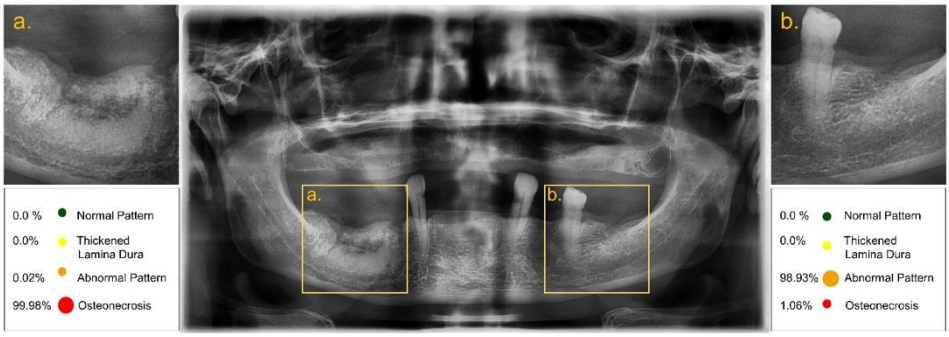

Figure 6. Display of the interface using a panoramic radiograph of a 70-year-old patient presenting clinical bone exposure in the right posterior mandible. No other lesions were observed on the clinical examination. Once the image is imported, manual selection of different regions of interest (a, b) can be performed. The output will show the category to which the selected trabeculated region belongs together with the corresponding probability in the form of a color-coded index.

References

1. Nicolielo LFP, Van Dessel J, Jacobs R, Quirino Silveira Soares M, Collaert B. Relationship between trabecular bone architecture and early dental implant failure in the posterior region of the mandible. Clin Oral Implants Res. 2020 Feb 1;31(2):153–61.

2. Taguchi A, Tanimoto K, Suei Y, Otani K, Wadamoto M, Akagawa Y, et al. Observer agreement in the assessment of mandibular trabecular bone pattern from panoramic radiographs. Dentomaxillofacial Radiology. 1997 Mar;26(2):90–4.

3. Pachêco-Pereira C, Almeida FT, Chavda S, Major PW, Leite A, Guerra ENS. Dental imaging of trabecular bone structure for systemic disorder screening: A systematic review. Oral Dis. 2019 May 17;25(4):1009–26.

4. Lahoud P, Diels S, Niclaes L, Van Aelst S, Willems H, Van Gerven A, et al. Development and validation of a novel artificial intelligence driven tool for accurate mandibular canal segmentation on CBCT. J Dent. 2022 Jan;116:103891.

5. Moreno-Rabié C, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Clin Oral Investig. 2020 Sep 6;24(9):2973–89.

6. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022 May;80(5):920–43.

7. Baron R, Ferrari S, Russell RGG. Denosumab and bisphosphonates: Different mechanisms of action and effects. Bone. 2011 Apr 1;48(4):677–92.

8. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg. 2003 Sep 1;61(9):1115–7.

9. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons Position Paper on Medication-Related Osteonecrosis of the Jaw—2014 Update. Journal of Oral and Maxillofacial Surgery. 2014 Oct;72(10):1938–56.

10. Walton K, Grogan TR, Eshaghzadeh E, Hadaya D, Elashoff DA, Aghaloo TL, et al. Medication related osteonecrosis of the jaw in osteoporotic vs oncologic patients-quantifying radiographic appearance and relationship to clinical findings. Dentomaxillofac Radiol. 2019 Jan;48(1):20180128.

11. Gaêta-Araujo H, Ferreira Leite A, de Faria Vasconcelos K, Coropciuc R, Politis C, Jacobs R, et al. Why do some extraction sites develop medication-related osteonecrosis of the jaw and others do not? A within-patient study assessing radiographic predictors. Int J Oral Implantol (Berl). 2021 Mar 16;14(1):87–98.

12. Kubo R, Ariji Y, Taniguchi T, Nozawa M, Katsumata A, Ariji E. Panoramic radiographic features that predict the development of bisphosphonate-related osteonecrosis of the jaw. Oral Radiol. 2018 May 12;34(2):151–60.

13. Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, et al. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Dec 4;12(1):11280.

14. Yamashita R, Nishio M, Do RKG, Togashi K. Convolutional neural networks: an overview and application in radiology. Insights Imaging. 2018 Aug 22;9(4):611–29.

15. Leite AF, Gerven A Van, Willems H, Beznik T, Lahoud P, Gaêta-Araujo H, et al. Artificial intelligence-driven novel tool for tooth detection and segmentation on panoramic radiographs. Clin Oral Investig. 2021 Apr 26;25(4):2257–67.

16. Vranckx M, Van Gerven A, Willems H, Vandemeulebroucke A, Ferreira Leite A, Politis C, et al. Artificial Intelligence (AI)-Driven Molar Angulation Measurements to Predict Third Molar Eruption on Panoramic Radiographs. Int J Environ Res Public Health. 2020 May 25;17(10):3716.

17. Kuwada C, Ariji Y, Fukuda M, Kise Y, Fujita H, Katsumata A, et al. Deep learning systems for detecting and classifying the presence of impacted supernumerary teeth in the maxillary incisor region on panoramic radiographs. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020 Oct;130(4):464–9.

18. Kwon O, Yong TH, Kang SR, Kim JE, Huh KH, Heo MS, et al. Automatic diagnosis for cysts and tumors of both jaws on panoramic radiographs using a deep convolution neural network. Dentomaxillofac Radiol. 2020 Dec 1;49(8):20200185.

19. Shaheen E, Leite A, Alqahtani KA, Smolders A, Van Gerven A, Willems H, et al. A novel deep learning system for multi-class tooth segmentation and classification on cone beam computed tomography. A validation study. J Dent. 2021 Dec 1;115:103865.

20. Buslaev A, Iglovikov VI, Khvedchenya E, Parinov A, Druzhinin M, Kalinin AA. Albumentations: Fast and Flexible Image Augmentations. Information. 2020 Feb 24;11(2):125.

21. He K, Zhang X, Ren S, Sun J. Deep Residual Learning for Image Recognition. In: 2016 IEEE Conference on

Computer Vision and Pattern Recognition (CVPR). IEEE; 2016. p. 770–8.

22. Szegedy C, Ioffe S, Vanhoucke V, Alemi A. Inception-v4, Inception-ResNet and the Impact of Residual Connections on Learning. 31st AAAI Conference on Artificial Intelligence, AAAI 2017. 2016 Feb 23;4278–84.

23. Huang G, Liu Z, Van Der Maaten L, Weinberger KQ. Densely Connected Convolutional Networks. In: 2017 IEEE Conference on Computer Vision and Pattern Recognition (CVPR). IEEE; 2017. p. 2261–9.

24. Sandler M, Howard A, Zhu M, Zhmoginov A, Chen LC. MobileNetV2: Inverted Residuals and Linear Bottlenecks. In: 2018 IEEE/CVF Conference on Computer Vision and Pattern Recognition. IEEE; 2018. p. 4510–20.

25. Chollet F, Others. Keras: the Python deep learning API. GitHub [Internet]. 2015; Available from: https://keras.io/

26. Russakovsky O, Deng J, Su H, Krause J, Satheesh S, Ma S, et al. ImageNet Large Scale Visual Recognition Challenge. Int J Comput Vis. 2015 Dec 11;115(3):211–52.

27. Bradski G. The OpenCV Library. Dr Dobb's Journal of Software Tools. 2000;

28. Hunter JD. Matplotlib: A 2D Graphics Environment. Comput Sci Eng. 2007;9(3):90–5.

29. Harris CR, Millman KJ, van der Walt SJ, Gommers R, Virtanen P, Cournapeau D, et al. Array programming with NumPy. Nature. 2020 Sep 17;585(7825):357–62.

30. Abadi M, Barham P, Chen J, Chen Z, Davis A, Dean J, et al. TensorFlow: A System for Large-Scale Machine Learning. In: 12th USENIX Symposium on Operating Systems Design and Implementation (OSDI 16). Savannah, GA: USENIX Association; 2016. p. 265–83.

31. Grandini M, Bagli E, Visani G. Metrics for Multi-Class Classification: an Overview. 2020 Aug 13;

32. Selvaraju RR, Cogswell M, Das A, Vedantam R, Parikh D, Batra D. Grad-CAM: Visual Explanations from Deep Networks via Gradient-Based Localization. Int J Comput Vis. 2020 Feb 11;128(2):336–59.

33. Pedregosa F, Varoquaux, Gael Gramfort A, Vincent M, Thirion B, Grisel O, Blondel M, et al. Scikit-learn: Machine Learning in Python. Journal of Machine Learning Research. 2011;12(85):2825–30.

34. Sukegawa S, Yoshii K, Hara T, Matsuyama T, Yamashita K, Nakano K, et al. Multi-Task Deep Learning Model for Classification of Dental Implant Brand and Treatment Stage Using Dental Panoramic Radiograph Images. Biomolecules. 2021 May 30;11(6):815.

35. Sivasundaram S, Pandian C. Performance analysis of classification and segmentation of cysts in panoramic dental images using convolutional neural network architecture. Int J Imaging Syst Technol. 2021 Dec 17;31(4):2214–25.

36. Yang H, Jo E, Kim HJ, Cha I ho, Jung YS, Nam W, et al. Deep Learning for Automated Detection of Cyst and Tumors of the Jaw in Panoramic Radiographs. J Clin Med. 2020 Jun 12;9(6):1839.

37. Estai M, Tennant M, Gebauer D, Brostek A, Vignarajan J, Mehdizadeh M, et al. Deep learning for automated detection and numbering of permanent teeth on panoramic images. Dentomaxillofacial Radiology. 2022 Feb 1;51(2):51.

38. Estai M, Tennant M, Gebauer D, Brostek A, Vignarajan J, Mehdizadeh M, et al. Evaluation of a deep learning system for automatic detection of proximal surface dental caries on bitewing radiographs. Oral Surg Oral Med Oral Pathol Oral Radiol. 2022 Aug;134(2):262–70.

39. Ahn Y, Hwang JJ, Jung YH, Jeong T, Shin J. Automated Mesiodens Classification System Using Deep Learning on Panoramic Radiographs of Children. Diagnostics. 2021 Aug 15;11(8):1477.

40. Rajee M V, Mythili C. Dental Image Segmentation and Classification Using Inception Resnetv2. IETE J Res. 2021 Aug 31;1–17.

41. Thompson NC, Greenewald K, Lee K, Manso GF. The Computational Limits of Deep Learning. 2020 Jul;

42. Nicolatou-Galitis O, Papadopoulou E, Vardas E, Kouri M, Galiti D, Galitis E, et al. Alveolar bone histological necrosis observed prior to extractions in patients, who received bone-targeting agents. Oral Dis. 2020 Feb 3;26(5):odi.13294.

43. Arce K, Assael LA, Weissman JL, Markiewicz MR. Imaging findings in bisphosphonate-related osteonecrosis of jaws. J Oral Maxillofac Surg. 2009 May;67(5):75–84.

44. Klingelhöffer C, Klingelhöffer M, Müller S, Ettl T, Wahlmann U. Can dental panoramic radiographic findings serve as indicators for the development of medication-related osteonecrosis of the jaw? Dentomaxillofac Radiol. 2016 May;45(5):20160065.

45. Bedogni A, Fusco V, Agrillo A, Campisi G. Learning from experience. Proposal of a refined definition and staging system for bisphosphonate-related osteonecrosis of the jaw (BRONJ). Oral Dis. 2012 Sep 22;18(6):621–3.

46. Hamada H, Matsuo A, Koizumi T, Satomi T, Chikazu D. A simple evaluation method for early detection of bisphosphonate-related osteonecrosis of the mandible

using computed tomography. Journal of Cranio-Maxillofacial Surgery. 2014 Sep;42(6):924–9.

47. Saia G, Blandamura S, Bettini G, Tronchet A, Totola A, Bedogni G, et al. Occurrence of bisphosphonate-related osteonecrosis of the jaw after surgical tooth extraction. J Oral Maxillofac Surg. 2010 Apr;68(4):797–804.

48. Khan AA, Morrison A, Hanley DA, Felsenberg D, McCauley LK, O'Ryan F, et al. Diagnosis and Management of Osteonecrosis of the Jaw: A Systematic Review and International Consensus. Journal of Bone and Mineral Research. 2015 Jan 1;30(1):3–23.

GENERAL DISCUSSION, CONCLUSIONS, AND FUTURE PERSPECTIVES

GENERAL DISCUSSION, CONCLUSIONS, AND FUTURE PERSPECTIVES

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GENERAL DISCUSSION

From the first report of bisphosphonate-related osteonecrosis by Marx in 2003[1] until the end of 2019, about 3000 articles were published on Medication-Related Osteonecrosis of the Jaws (MRONJ). From the start of this Ph.D. in February 2020 until October 2023, more than 1000 new studies were released into the public domain. The topics studied around this pathology were diverse, including therapeutic, diagnostic, risk factor, animal, and pathophysiology studies, among others. However, despite this progress, the treatment of MRONJ continues to be challenging, with no single approach proving to be universally effective. Furthermore, there remains a significant unmet clinical need for reliable methods to identify patients at high risk of MRONJ or for the early detection of the condition, which are critical for preventing its onset and mitigating its severity. While imaging appears to be an attractive and non-invasive tool to fill these voids, of all these published investigations, less than 20% used any diagnostic imaging in their methodology.

Thus, given the latter and the reasons identified in the present introduction, this doctoral thesis aimed to use two- and three-dimensional radiographic images to identify risk factors for MRONJ. Specific objectives were set, whose main results and implications are discussed hereafter.

Clinical and radiographic findings in patients under antiresorptive drugs

In chapters 1 to 4, data were collected from 105 patients with cancer and 168 patients with osteoporosis, who had imaging examinations prior to dental extractions. All these patients had been treated with antiresorptive drugs (ARDs), had absence of clinically exposed bone, and were compared against a control group. The purpose of these comparisons was to identify clinical and radiographic findings associated with the use of antiresorptive drugs.

Oncologic patients taking ARDs exhibited several notable differences compared to the control group, including a thicker lamina dura, seen in both panoramic and CBCT assessment [2,3], a widened periodontal ligament space [3], osteosclerotic and

osteolytic regions in the trabecular pattern [3], and sequestrum formation [3], which were seen in the three-dimensional assessment. Furthermore, in the two-dimensional postoperative evaluation, patients with cancer showed more frequent osteosclerotic and mixed-density trabecular bone patterns, visibility of alveolar sockets, persistence of lamina dura, and sequester formation than the control group [2].

Similarly, osteoporotic patients displayed significantly more thickening of the lamina dura than the control group, observed in both panoramic and CBCT assessments [4,5]. In the postoperative assessment, heterogeneous bone patterns (i.e., mixed osteolytic and osteosclerotic areas) [4], sequestrum formation [4], along with persistence of the alveolar socket and lamina dura, were found more often in the ARD-treated group. [4].

The use of ARDs, irrespective of the dose, leads to a clear radiographic sign: thickening of the lamina dura. Our research also reveals that high doses of ARDs are accompanied by additional findings affecting the trabecular bone pattern. We can hypothesize that high doses of drugs induce enhanced bone changes, yet it should not be forgotten that these changes may also be associated with dental infections [6–8]. Even so, it seems plausible to assume that patients undergoing ARD with dental infections might present more frequent bone reactions, such as condensing osteitis. In terms of postoperative changes, there appear to be no major differences between high and low doses of ARDs, but this could be due to the development of MRONJ. The pathology remains one, and when it occurs, it is accompanied by a set of radiographic signs. These radiographic signs have been previously described in lesions with clinical bone exposure and include osteolysis, cortical bone erosion, sequestrum formation, osteosclerosis, and periosteal reaction [9–11].

Another inherent characteristic of the use of antiresorptive drugs, observed in both cancer and osteoporotic patients, is the time taken to achieve mucosal healing at the extraction site. Identifying mucosal healing as the *"epithelial continuity obtained by granulation of the extraction socket with no fistula connected to the underlying bone"* [12,13], both groups of patients took significantly longer than the two weeks on average

from the control group to exhibit this clinical sign [3,5]. Prior investigations, which studied the alveolar socket remodeling under normal conditions, stated that mucosal lining of the exposed post-extraction socket is expected within two weeks [14–17]. We noted this clinical sign on average 14 weeks postoperatively in ARD-treated oncologic patients [3] and 4 weeks in osteoporotic patients [5].

Recognizing that antiresorptive drugs have unique mechanisms of action, some authors have explored potential differences in the radiographic appearance of osteonecrosis associated with bisphosphonates (BRONJ) and denosumab (DRONJ) [18]. Prior results had shown BRONJ exhibiting more often sequester formation and cortical bone osteolysis and DRONJ presenting less frequent radiographic signs in cases where bone exposure was present [19]. In the oncologic cohort, even before the manifestation of exposed bone, sites exposed to bisphosphonates showed significantly more osteosclerosis, while those exposed to denosumab showed no significant features [3]. Contrarily, no specific features were recognized in the osteoporotic cohorts using any medication type [5]. Thus, it cannot be concluded that bisphosphonates or monoclonal antibodies are associated with specific radiographic manifestations.

Risk factors and early radiographic signs of osteonecrosis

Another aim of the research carried out in chapters 1 to 4 was to identify clinical and radiographic risk factors for early detection of osteonecrosis. For this purpose, comparisons were made between ARD-treated patients and tooth extraction sites that did and did not develop MRONJ.

In the oncologic sample it was observed that MRONJ occurred more frequently when patients were on extended courses of ARD treatment [2], had multiple tooth extractions [3], were smokers [3], had shorter drug holidays [3], in teeth with absent and incomplete endodontic fillings with caries, widened periodontal ligament space and/or periapical lesions [2], and where a bone pattern presenting osteosclerotic and/or osteolytic areas [2], sequestrum formation [3], or periosteal reaction was present [3]. Upon tooth extraction, sites developing MRONJ presented postoperatively with mixed

osteosclerotic and osteolytic patterns, bone sequestrum formation, persistence of the alveolar socket, and resorption pattern in the form a crater-like defect [2].

Complementarily, osteoporotic patients presented a greater susceptibility to osteonecrosis when being males [4], smokers [4], undergoing treatment with corticosteroids [5], having mandibular tooth extractions [4], of multi-rooted teeth [4,5], in sites with osteolytic or osteosclerotic trabecular bone patterns [4], when tooth extractions involved furcation involvement, root remnants, or untreated caries lesions [4], and where pre-operative sequester formation was present [5]. In the postoperative assessment, sites that developed osteonecrosis exhibited more often an osteosclerotic or osteolytic bone pattern, crater-like defects, and sequester formation [4].

Perhaps one of the most relevant findings of this thesis is that the formation of bone sequestrum should be considered a pathognomonic sign of MRONJ in any patient treated with ARDs, since this sign was visible only in ARD-treated patients, most of whom subsequently had bone exposure[2–4] or histological evidence of necrosis [5]. This sign and the presence of periosteal reaction highly suggest MRONJ even without bone exposure.

During these years of research, it has become evident that there is a need for a universal diagnostic and stratification method for MRONJ incorporating both clinical and radiographic aspects [20,21]. With the current classification of the American Association of Maxillofacial Surgeons [9], there is ambiguity in those patients who present with symptoms related to dental infection and at the same time show radiographic signs of osteonecrosis such as sequestrum formation or periosteal reaction in the absence of bone exposure. Notably, Nicolatou-Galitis et al. found necrotic bone in biopsies derived from ARD-treated patients during dental extractions who did not present clinical evidence of exposed bone [22]. Thus, we may have misdiagnosed the pain initially thought to be of dental origin from an undiagnosed osteonecrosis [2]. However, it is not clear when MRONJ starts in an infected and stressed environment due to ARD use, and it is plausible that infection and necrosis are causing symptoms at the same time in the same location. In these situations, images are a great help. Our two-

dimensional studies showed that sequential pre-operative and postoperative panoramic radiographs allowed visualization of bony trabecular changes [2,4]. Differences were noted in both cancer and osteoporotic patients treated with ARDs, where pre-operative bone patterns other than normal showed more frequent postoperative osteosclerotic, lytic or mixed bone patterns, which in turn were associated with MRONJ development.

Although in the statistical analyses of the oncologic group, evidence was found that cessation of bisphosphonate or monoclonal antibody administration for a prolonged period had some protective effect on osteonecrosis [3], the measure cannot be recommended in patients undergoing this treatment and requiring tooth extractions. Tooth extractions often come with a degree of urgency, and patients are unable to wait the time needed for the bone to recover from the effect of the drug. Especially when considering that the half-life of bisphosphonates can be up to 10 years [23]. Thus, the patient can hardly avoid the procedure for such a long time. In contrast, interrupting treatment may be feasible in the case of denosumab but risking a larger rebound effect of the underlying oncological/non-malignant disease. For this reason, the prospect of fractures and the advancement of metastatic conditions might justify the continuation of treatment, even in light of the risk of MRONJ[24].

Radiographic features associated to a poor prognostic outcome of MRONJ

Part 2 of the thesis aimed to identify clinical and tomographic prognostic factors for conservative and surgical treatment of MRONJ and to investigate whether any were associated with lesion relapse [25]. In chapter 5, we studied 115 patients treated with ARDs who developed 143 MRONJ lesions. Of these, 40 patients received conservative treatment, and 75 were treated with surgery.

Risk factors for a poor outcome after conservative treatment were identified as clinical stage 3 according to the AAOMS [9], the presence of teeth in or immediately adjacent to the lesion, osteosclerosis extending to more than one-third of the maxilla or mandible, and the presence of bone sequestrum involving the sinus or nasal cortical or the mandibular canal. While for surgical treatment, success was highest for early

stages of MRONJ and in patients who were not being treated with bisphosphonates for an average of 21 months. Furthermore, radiographic risk factors for a poor outcome in operative treatment were the absence of sequestrum formation and the presence of periosteal reaction. Unfortunately, none of the radiographic features were shown to have an association with lesion relapse in either conservative or surgical treatment [25].

Upon identification of radiographic features that facilitate prognostic assessment at the time of starting conservative treatment or performing surgery, it is important to consider that MRONJ lesions and their imaging findings can change over time [26]. In this thesis, we evaluated the initial characteristics of the lesion and its relation to the prognosis, but lesions are not static, and changes could affect treatment outcomes. In this regard, diagnostic imaging is indispensable, and there is no guideline for how often to acquire new images in patients with MRONJ. A first radiographic image at the time of diagnosis is strongly recommended [20,27] and further radiographic examination will depend on the patient's evolution. An example of these clinical and radiographic changes in MRONJ lesions can be seen in Figure 1 and 2, where the case of a patient with MRONJ treated initially conservatively and subsequently with a sequestrectomy is described, achieving a successful outcome.

Yasui et al. studied radiographic prognostic risk factors through follow-up CT scans in patients with MRONJ treated with antibiotics, antiseptic mouthwashes, and sequestrectomy. They identified that lesions with bone sequestrum formation took significantly less time to heal than their counterpart, while patients treated with chemotherapy took significantly longer [26]. Sequestrum formation was previously reported as taking a median of 8 months to be formed [28]. The latter is a natural response of the body to expel deceased and infection-prone tissue, and in patients treated with chemotherapy this organic response may be diminished [26,29].

The debate now arises on whether to wait, when possible, for bone sequestrum to form, as is believed to be the natural course of this pathology [30]. In this way, extensive and unnecessary surgery would be avoided. Besides, following our results, its presence indicates a favorable outcome [25]. However, not everyone agrees

that conservative treatment should be continued indiscriminately [9,31,32]. Moreover, some authors recommend surgical interventions because of their high success rates [33,34], regardless of the lesion's characteristics. Also, our results show more frequent lesion resolution at earlier stages. This is undoubtedly a topic that should be further investigated, bearing in mind the radiographic appearance of the MRONJ lesion because it seems unreasonable to recommend surgery based purely on the clinical aspect of the lesion.

AI as an aid for MRONJ risk factor identification

The third and final part of this thesis aimed to develop and validate a tool for the automated classification of normal, affected and osteonecrosis mandibular trabecular bone patterns in panoramic radiographs using convolutional neural networks (CNNs). This project intended to consolidate the results of Part 1 (Chapters 1 to 4) in a didactic and automated way.

For this purpose, 402 panoramic radiographs were collected from 376 patients, divided into controls, thickening of the lamina dura, bone changes associated with antiresorptive drugs, and lesions evidencing osteonecrosis. Once the images were labelled and classified, four neural networks were selected to perform the categorization task. The best results were achieved by InceptionResNetV2 with an accuracy of 96%, which was used to develop a desktop classification tool. The result was presented as a color-coded clinical risk index intended as a simple scale for clinicians to determine the referral and treatment needs of individual patients undergoing treatment with ARD [35].

This color-coded system enhances diagnostic precision when considering tooth extractions in ARD-treated patients. A green light or normal bone pattern signifies a low risk of MRONJ [36–38], making tooth extraction safer. The presence of a thickened lamina dura serves as a marker for drug intake [36,39], while the development of osteosclerosis is associated with an increased MRONJ risk [36–38]. Lastly, images showing cortical bone erosion, sequestrum formation, and osteolytic areas indicate an

established osteonecrosis lesion, necessitating immediate referral to a specialized clinic for timely treatment [35].

Since the development of our study, similar investigations using machine-learning approaches have been published [40,41]. An exploratory study on CBCT compared the classification of normal trabecular patterns from a control group with osteosclerosis, osteonecrosis and normal bone patterns from MRONJ patients. The algorithm could not only classify all patterns with high accuracy (97%) but also found significant differences between normal bone patterns associated with control and ARD-treated patients [40]. Complementarily, Wilkat et al. automated the assessment of pre-operative MRONJ lesions and studied its correlation with volume defect, MRONJ stage, number of surgeries needed, and invasiveness of the procedure. As results, they found a directly proportional relationship between the lesion's volume and the severity of the studied parameters [41].

These investigations highlight the power of artificial intelligence to recognize patterns that are less apparent to the human eye [40]. Indicating its potential as one of the paths to early recognition of MRONJ and treatment assessment or, at the very least, a useful point-of-care diagnostic aid for clinicians. Potential applications for this developed algorithm include its integration into medical imaging reading stations, providing a support system for healthcare providers. In addition, it could be used to aid shared decision making with ARD-treated patients when discussing possible treatment options.

Considerations

All data used in the publications related to this thesis are of retrospective origin. This limits the extrapolation of results due to surgical variability, missing data in clinical records, variations in protocols over time, and because data comes from a single center experience. Nonetheless, given the low incidence of osteonecrosis, this is an appropriate methodology to achieve an adequate sample size for comparative and longitudinal studies. Besides, UZ Leuven is a reference center for osteonecrosis, hence,

there is a higher concentration of affected patients, which facilitates the conduct of studies on the subject.

Another point to note is that University Hospital Leuven applies a preventive protocol for patients treated with antiresorptive drugs who require tooth extractions. This protocol was implemented circa 2014 and established the prescription of prophylactic antibiotics before surgery and the application of L-PRF during the procedure. Under ideal circumstances, all selected patients should receive the same protocol. For this reason, comparisons between patients who did and did not receive preventive measures were also considered during the statistical analyses in chapters 1 to 4. Yet, most patients who met the inclusion criteria had treatments after 2014, mainly because the quality of the radiographs available in previous years did not allow for the correct assessment of the tooth to be extracted and the surrounding structures.

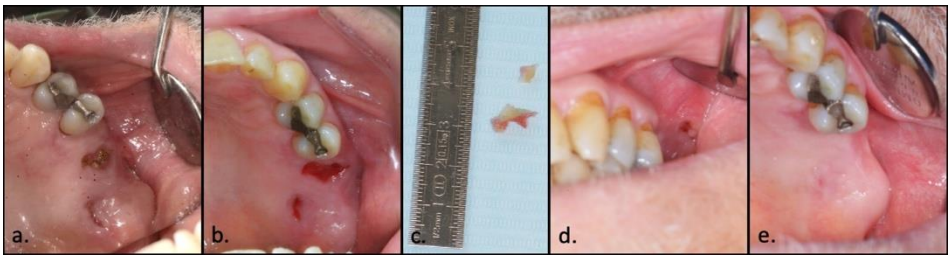

Figure 1. A 73-year-old patient with a history of metastasized prostate cancer treated with denosumab presented with a lesion in the left posterior maxillary region. He had had periodontal extractions of the first and second molars, which despite the use of prophylactic antibiotics and leucocyte and platelet-rich plasma, did not heal. The first image (a) depicts the initial state of the lesion showing bone exposure at three sites (distal second premolar, palate, and vestibule) in the absence of mobility, pain, and infection. CBCT images of the initial lesion are shown in Figure 2. Doxycycline 100mg daily was prescribed and periodic follow-ups were scheduled. A year later (b, c) two bone fragments presented mobility and were painlessly removed without anesthesia during consultation (c). However, 4 months later, a vestibular fistula persisted, probing to the bone, and associated with suppuration (d). A new CBCT was taken, which showed formation of bone sequestrum at that site (Figure 2). A sequestrectomy was performed under local anesthetic and one month later mucosal healing was observed (e). The patient has remained free of bone exposure in recent follow-ups.

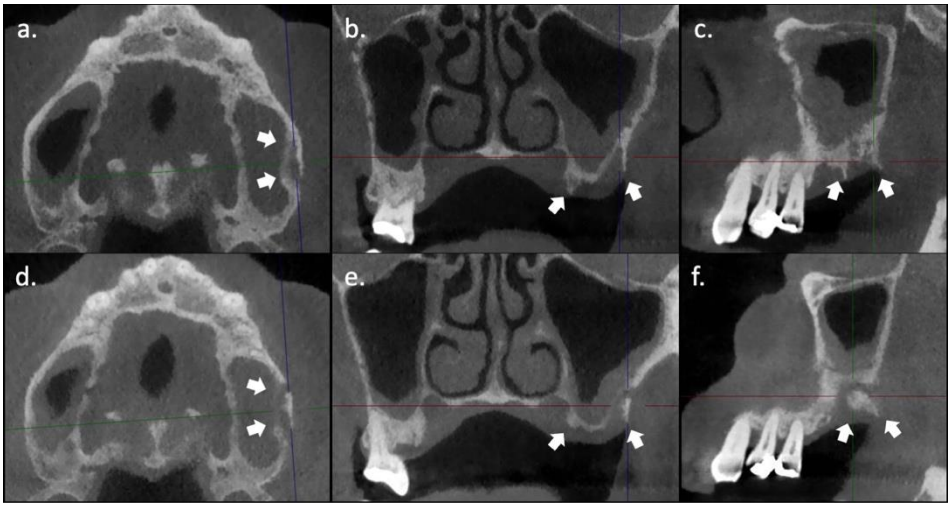

Figure 2. CBCT slices corresponding to patient described in Figure 1. The first CBCT (a, b, c) was acquired at the time of diagnosis and the second (d, e, f) 16 months after. Areas of interest are pointed out with white arrows. In the second examination, sequestrum formation can be seen, which was not present in the initial evaluation.

CONCLUSIONS

The use of two- and three-dimensional radiographic images to identify risk factors related to osteonecrosis is undoubtedly indispensable for the clinical management of patients treated with antiresorptive drugs. Through the present work, it is possible to conclude that radiographic techniques provide valuable information to identify early changes influenced by antiresorptive drugs, local risk factors in patients "at risk", early diagnosis of latent lesions, and to aid in the prognosis of conservative and surgical treatment of MRONJ.

Concretely, the thickening of the lamina dura and a prolonged time to achieve postoperative alveolar mucosal healing was identified as an effect of both high- and low-dose antiresorptive drugs in patients undergoing tooth extractions.

In terms of risk factors for MRONJ following dental extractions, an elevated risk was identified in oncological patients when they had received more prolonged ARDs treatment, had multiple tooth extractions, were smokers, had shorter drug holidays, in teeth with absent and incomplete endodontic fillings with caries, widened periodontal ligament space and/or periapical lesions, and where a bone pattern presenting osteosclerotic and/or osteolytic areas, sequestrum formation, or periosteal reaction was present. Similarly, the following risk factors were identified in osteoporotic patients: males, smokers, treatment with corticosteroids, mandibular tooth extractions, multi-rooted teeth, in sites with osteolytic or osteosclerotic trabecular bone patterns, when tooth extractions involved furcation involvement, root remnants, or untreated caries lesions, and where pre-operative sequester formation was present.

Nevertheless, it should be noted that some of these signs may correspond to latent MRONJ lesions, as bone sequestrum formation and periosteal reaction were identified as radiographic signs highly suggestive of MRONJ.

Regarding clinical and radiographic prognostic factors for treating MRONJ, conservative treatment demonstrated poor results in stage 3 lesions, with tooth involvement, or sequestrum formation. In contrast, surgical treatment showed worse results also in stage 3 lesions, when bisphosphonates were discontinued for a short period, and in cases with absence of sequestration formation and presence of periosteal reaction.

Lastly, the preliminary study employing artificial intelligence was able to classify trabeculated bone patterns using four different convolutional neural network architectures, showing reliable potential for identifying abnormalities in panoramic radiographs of antiresorptive treated patients. The best network, InceptionResNetV2, which had an accuracy of 96%, was selected for developing a diagnostic tool and is expected to support clinical decision-making when alarming trabecular patterns are recognized, thereby minimizing complications with early diagnosis and treatment planning.

FUTURE PERSPECTIVES

The present studies identified risk factors for the development of MRONJ, radiographic indicators of latent lesions, and prognostic signs for conservative and surgical treatment. With these factors in mind, the next step would be incorporating medical imaging, such as panoramic radiographs and CBCT, in diagnosing and treating osteonecrosis and in preventive protocols for this pathology.

To begin with, a diagnostic and staging system that uses medical imaging to provide an accurate picture of disease extent is imperative. This will not only allow earlier and finer stage identification but will also help tailor treatment strategies, facilitating timely interventions and improving the efficacy of current treatment protocols, ensuring better patient outcomes and an improved standard of care.

In patients about to start treatment with ARDs, I strongly suggest obtaining a CBCT for the correct identification of all foci of infection and their timely treatment. In addition, preventive measures such as smoking cessation should be encouraged, dental check-ups should be carried out at least twice a year, and diagnostic imaging should be repeated annually once ARD treatment is started. The annual radiographic check-up can be carried out with a panoramic radiograph and, if necessary, with a CBCT, depending on the patient's requirements. The acquisition of these images will allow comparisons with the baseline status as well as the identification of early bone changes that will correspond to MRONJ risk sites. It is further advised to refrain from acquiring images in less than six months for the sole purpose of a control, as given the bone physiology, few changes will be visible, and this examination will consist of unnecessary irradiation for the patient.

In patients at risk who are to undergo surgical procedures, two- and three-dimensional radiographs are of great help in recognizing the effects of antiresorptive drugs and local risk factors for MRONJ. Furthermore, according to our results on tooth extractions, we can expect a longer healing time when under ARDs. Efforts should focus on exploring the potential of adjunctive therapies for both the prevention and

treatment of osteonecrosis. The latter include, among others, the use of platelet-rich plasma and lasers during invasive procedures in patients at risk or as treatment of established lesions.

Recognizing that there are bone changes associated with the use of antiresorptive drugs has led to the need for identifying risk factors for osteonecrosis or signs of latent MRONJ lesions. Future research should be oriented towards combining histopathology with imaging. For example, in patients requiring tooth extraction, a biopsy could be performed during surgery and combined with the imaging findings, a correlation could be explored. A first attempt by Ristow et al. correlated a set of radiographic bone changes in patients undergoing tooth extractions with necrosis observed in bone biopsies acquired peri-operatively [42].

Today, magnetic resonance imaging (MRI) is emerging as a viable alternative to x-rays in dental applications. Our systematic review uncovered limited but promising findings, indicating MRI's efficacy in detecting early bone changes with a reduced biological impact. It is worthwhile conducting research to explore the appearance of osteonecrosis and its early stages in different context. A practical starting point is utilizing historical oncologic images, considering the frequent examinations undergone by these patients as suggested by Wongratwanich et al 2021 [43].

Concerning the AI diagnostic tool, it can be applied to assess patients receiving ARDs before tooth extractions, implant placement, and for early MRONJ detection prior to bone exposure. Given MRONJ's varying radiographic appearance, the network would be crucial in distinguishing subtle lesions from abnormal bone patterns. Therefore, to unlock its full potential, research should focus on training the models on a larger, more diverse dataset to enhance classification performance. Particularly, with not only mandibular mature lesions displaying distinct characteristics of MRONJ, but also with less evident lesions in both maxillary and mandibular locations. Since this study was constrained by a single-center dataset and the relatively low incidence of MRONJ, incorporating data from multiple sources with diverse scanning parameters is essential to reduce classification bias [44].

References

1. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg. 2003 Sep 1;61(9):1115–7.

2. Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, et al. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280.

3. Moreno Rabie C, Cavalcante Fontenele R, Oliveira Santos N, Nogueira Reis F, Van den Wyngaert T, Jacobs R. Three-dimensional clinical assessment for MRONJ risk in oncologic patients following tooth extractions. Dentomaxillofacial Radiology. 2023 Oct 24;

4. Moreno-Rabié C, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, et al. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8;

5. Moreno-Rabie C, Cavalcante Fontenele R, Oliveira Santos N, Nogueira-Reis F, Van den Wyngaert T, Jacobs R. Key Insights into Antiresorptive Drug Use and Osteonecrosis in Osteoporotic Patients Undergoing Tooth Extractions: a clinical and CBCT assessment. 2023;

6. Gaêta-Araujo H, Ferreira Leite A, de Faria Vasconcelos K, Coropciuc R, Politis C, Jacobs R, et al. Why do some extraction sites develop medication-related osteonecrosis of the jaw and others do not? A within-patient study assessing radiographic predictors. Int J Oral Implantol (Berl). 2021 Mar 16;14(1):87–98.

7. Ueda N, Nakashima C, Aoki K, Shimotsuji H, Nakaue K, Yoshioka H, et al. Does inflammatory dental disease affect the development of medication-related osteonecrosis of the jaw in patients using high-dose bone-modifying agents? Clin Oral Investig. 2021 May 14;25(5):3087–93.

8. Fedele S, Porter SR, D'Aiuto F, Aljohani S, Vescovi P, Manfredi M, et al. Nonexposed variant of bisphosphonate-associated osteonecrosis of the jaw: a case series. Am J Med. 2010;123(11):1060–4.

9. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022 May;80(5):920–43.

10. Wilde F, Heufelder M, Lorenz K, Liese S, Liese J, Helmrich J, et al. Prevalence of cone beam computed tomography imaging findings according to the clinical stage of bisphosphonate-related osteonecrosis of the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Dec;114(6):804–11.

11. Treister NS, Friedland B, Woo SB. Use of cone-beam computerized tomography for evaluation of bisphosphonate-associated osteonecrosis of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 May;109(5):753–64.

12. Shudo A, Kishimoto H, Takaoka K, Noguchi K. Long-term oral bisphosphonates delay healing after tooth extraction: a single institutional prospective study. Osteoporosis International. 2018 Oct 1;29(10):2315–21.

13. Lesclous P, Cloitre A, Catros S, Devoize L, Louvet B, Châtel C, et al. Alendronate or Zoledronic acid do not impair wound healing after tooth extraction in postmenopausal women with osteoporosis. Bone. 2020 Aug 1;137.

14. Amler MH. The time sequence of tissue regeneration in human extraction wounds. Oral Surg Oral Med Oral Pathol. 1969 Mar;27(3):309–18.

15. Araújo MG, Silva CO, Misawa M, Sukekava F. Alveolar socket healing: What can we learn? Periodontol 2000. 2015 Jun 1;68(1):122–34.

16. Saia G, Blandamura S, Bettini G, Tronchet A, Totola A, Bedogni G, et al. Occurrence of bisphosphonate-related osteonecrosis of the jaw after surgical tooth extraction. J Oral Maxillofac Surg. 2010 Apr;68(4):797–804.

17. Migliorati CA, Saunders D, Conlon MS, Ingstad HK, Vaagen P, Palazzolo MJ, et al. Assessing the association between bisphosphonate exposure and delayed mucosal healing after tooth extraction. The Journal of the American Dental Association. 2013 Apr 1;144(4):406–14.

18. Querrer R, Ferrare N, Melo N, Stefani CM, Dos Reis PED, Mesquita CRM, et al. Differences between bisphosphonate-related and denosumab-related osteonecrosis of the jaws: a systematic review. Support Care Cancer. 2021 Jun 2;29(6):2811–20.

19. Pichardo SEC, Broek FWT, Fiocco M, Appelman-Dijkstra NM, van Merkesteyn JPR. A comparison of the cone beam computed tomography findings in medication-related osteonecrosis of the jaws related to denosumab versus bisphosphonates: an observational pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020 Apr 1;129(4):411–7.

20. Bedogni A, Fusco V, Agrillo A, Campisi G. Learning from experience. Proposal of a refined definition and staging system for bisphosphonate-related osteonecrosis of the jaw (BRONJ). Oral Dis. 2012 Sep 22;18(6):621–3.

21. Hamada H, Matsuo A, Koizumi T, Satomi T, Chikazu D. A simple evaluation method for early detection of bisphosphonate-related osteonecrosis of the mandible using computed tomography. J Craniomaxillofac Surg. 2014 Sep;42(6):924–9.

22. Nicolatou-Galitis O, Papadopoulou E, Vardas E, Kouri M, Galiti D, Galitis E, et al. Alveolar bone histological necrosis observed prior to extractions in patients, who received bone-targeting agents. Oral Dis. 2020 Jul 3;26(5):955–66.

23. Lin JH. Bisphosphonates: A review of their pharmacokinetic properties. Vol. 18, Bone. Bone; 1996. p. 75–85.

24. Liu C, Xiong YT, Zhu T, Liu W, Tang W, Zeng W. Management of Tooth Extraction in Patients Taking Antiresorptive Drugs: An Evidence Mapping Review and Meta-Analysis. J Clin Med. 2022 Dec 28;12(1):239.

25. Moreno Rabie C, García-Larraín S, Contreras Diez de Medina D, Cabello-Salazar I, Cavalcante Fontenele R, Van den Wyngaert T, et al. How does the clinical and tomographic appearance of MRONJ influence its treatment prognosis? Dentomaxillofacial Radiology. 2023 Oct 23;

26. Yasui T, Nagamine H, Tanaka K, Kimura M, Karube T, Kawana H, et al. Treatment outcomes and time to healing of medication-related osteonecrosis of the jaw based on image findings. Dentomaxillofacial Radiology. 2023 May 16;20220352.

27. Campisi G, Bedogni A, Fusco V. Raccomandazioni clinico-terapeutiche sull'osteonecrosi delle ossa mascellari (ONJ) farmaco-relata e sua prevenzione. Versione 2. Palermo University Press; 2020. 1–234 p.

28. Ferlito S, Puzzo S, Palermo F, Verzì P. Treatment of bisphosphonate-related osteonecrosis of the jaws: presentation of a protocol and an observational longitudinal study of an Italian series of cases. British Journal of Oral and Maxillofacial Surgery. 2012 Jul;50(5):425–9.

29. Van den Wyngaert T, Claeys T, Huizing MT, Vermorken JB, Fossion E. Initial experience with conservative treatment in cancer patients with osteonecrosis of the jaw (ONJ) and predictors of outcome. Annals of Oncology. 2009 Feb 1;20(2):331–6.

30. Barragan-Adjemian C, Lausten L, Ang DB, Johnson M, Katz J, Bonewald LF. Bisphosphonate-Related Osteonecrosis of the Jaw: Model and Diagnosis with Cone Beam Computerized Tomography. Cells Tissues Organs. 2009 Dec;189(1–4):284–8.

31. Kaibuchi N, Hoshi K, Yamazaki A, Miyamoto-Sangu N, Akagi Y, Okamoto T. The progress of medication-related osteonecrosis of the jaw with conservative initial treatment: A 12-year retrospective study of 129 patients. Bone Rep. 2021 Jun 1;14:101072.

32. Ristow O, Ruckschloss T, Muller M, Berger M, Kargus S, Pautke C, et al. Is the conservative non-surgical management of medication-related osteonecrosis of the jaw an appropriate treatment option for early stages? {A} long-term single-center cohort study. J Craniomaxillofac Surg. 2019;47(3):491–9.

33. Kawaoka Y, Kojima Y, Sawada S, et al. Periosteal reaction as a risk factor for poor outcomes after surgical treatment for medication-related osteonecrosis of the jaw: A retrospective analysis of 205 surgeries. J Oral Maxillofac Surg Med Pathol. 2020 Jan 1;32(1):8–13.

34. De Cicco D, Boschetti CE, Santagata M, Colella G, Staglianò S, Gaggl A, et al. Medication-Related Osteonecrosis of the Jaws: A Comparison of SICMF–SIPMO and AAOMS Guidelines. Diagnostics. 2023 Jun 21;13(13):2137.

35. Baseri Saadi S, Moreno-Rabié C, van den Wyngaert T, Jacobs R. Convolutional neural network for automated classification of osteonecrosis and related mandibular trabecular patterns. Bone Rep. 2022 Dec 1;17:101632.

36. Moreno-Rabié C, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, et al. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Dec 4;12(1):11280.

37. Nicolatou-Galitis O, Papadopoulou E, Vardas E, Kouri M, Galiti D, Galitis E, et al. Alveolar bone histological necrosis observed prior to extractions in patients, who received bone-targeting agents. Oral Dis. 2020 Feb 3;26(5):odi.13294.

38. Arce K, Assael LA, Weissman JL, Markiewicz MR. Imaging findings in bisphosphonate-related osteonecrosis of jaws. J Oral Maxillofac Surg. 2009 May;67(5):75–84.

39. Klingelhöffer C, Klingelhöffer M, Müller S, Ettl T, Wahlmann U. Can dental panoramic radiographic findings serve as indicators for the development of medication-related osteonecrosis of the jaw? Dentomaxillofac Radiol. 2016 May;45(5):20160065.

40. Gürses BO, Alpoz E, Şener M, Çankaya H, Boyacıoğlu H, Güneri P. A support vector machine-based algorithm to identify bisphosphonate-related osteonecrosis throughout the mandibular bone by using cone beam computerized tomography images. Dentomaxillofacial Radiology. 2023 Apr;52(4).

41. Wilkat M, Singh DD, Lutz I, Möllmann H, Gellrich NC, Rana M. Use and Evaluation of a Computer-Assisted Examination Method for the Diagnosis and Analysis of Medication-Related Osteonecrosis of the Jaw.

Craniomaxillofac Trauma Reconstr. 2021 Mar;14(1):36–42.

42. Ristow O, Schnug G, Smielowksi M, Moratin J, Pilz M, Engel M, et al. Diagnostic accuracy comparing OPT and CBCT in the detection of non-vital bone changes before tooth extractions in patients with antiresorptive intake. Oral Dis. 2023 Apr 21;29(3):1039–49.

43. Wongratwanich P, Shimabukuro K, Konishi M, Nagasaki T, Ohtsuka M, Suei Y, et al. Do various imaging modalities provide potential early detection and diagnosis of medication-related osteonecrosis of the jaw? A review. Dentomaxillofac Radiol. 2021 Sep 1;50(6):20200417.

44. Ahn Y, Hwang J, Jung YH, Jeong T, Shin J. Automated mesiodens classification system using deep learning on panoramic radiographs of children. Diagnostics. 2021 Aug;11(8):1477.

SUMMARY

The main objective of this doctoral thesis was to use two- and three-dimensional radiographic images for the identification of risk factors for medication-related osteonecrosis of the jaws (MRONJ). It was hypothesized that the use of radiographic images is an essential diagnostic tool for early diagnosis and treatment selection of MRONJ. This general objective and hypothesis were subdivided into three parts including six chapters where specific secondary objectives were set.

Part 1 investigated the radiographic findings associated to the use of antiresorptive drugs (ARDs) and risk factors for MRONJ in patients undergoing tooth extractions. For that purpose, four retrospective longitudinal case control studies were set up in oncologic and osteoporotic patients and using panoramic radiographs and cone beam computed tomography (CBCT) (**Chapters 1 to 4**). The main results of these investigations show that thickening of the lamina dura is an indicator of antiresorptive drug use and a prolonged time to achieve postoperative alveolar mucosal healing is expected on both high and low doses of antiresorptive drugs.

An increased risk for MRONJ after tooth extraction was identified in oncological patients with one or more risk factors. The identified risk factors include: longer ARD treatment, multiple tooth extractions, smoking, a shorter "drug holiday", teeth with absent and incomplete endodontic fillings with caries, widened periodontal ligament space and/or periapical lesions, and osteosclerotic and/or osteolytic bone pattern, sequestrum formation or periosteal reaction. Similarly, the following risk factors were identified in osteoporotic patients: men, smoking, treatment with corticosteroids, mandibular tooth extractions, multi-rooted teeth, osteolytic or

osteosclerotic trabecular bone patterns, tooth extractions accompanied by furcation involvement, root remnants or untreated carious lesions, and preoperative sequestrum formation.

Lastly, it should be noted that some of these signs may correspond to latent MRONJ lesions, as bone sequestrum formation and periosteal reaction were identified as radiographic signs highly suggestive of MRONJ.

Part 2 studied the clinical and tomographic prognostic factors for conservative and surgical treatment of MRONJ. This part consisted of **Chapter 5**, which was a retrospective cohort study including patients treated with at least one administration of ARDs, a diagnosis of MRONJ stage 1, 2, or 3 according to the American Association of Oral and Maxillofacial Surgeons (AAOMS), and a CBCT of the MRONJ lesion.

Regarding the results, advanced stage lesions showed a worse therapeutic prognosis in both treatments. Particularly in the conservative treatment, lesions with tooth involvement or sequestrum formation were also associated to lack of healing. In contrast, surgical treatment showed worse results when bisphosphonates were discontinued for a short period, and in cases with absence of sequestration formation and presence of periosteal reaction.

Part 3 developed and validated a tool for the automated classification of normal, affected, and osteonecrosis mandibular trabecular bone patterns in panoramic images using convolutional neural networks. This last part included **Chapter 6**, where four pre-trained convolutional neural networks were used for automatic classification of bone patterns showing normal, thickened lamina dura, abnormal trabeculae, and osteonecrosis cases.

All networks detected and correctly classified mandibular bone patterns with optimal performance metrics and showed no statistical differences in their performance. The best network was chosen for the development of the automated tool

due to its slight better metrics and better response in the abnormal bone pattern classification, reaching an accuracy of 96%. The output of this tool indicated in a simple manner the category to which the selected trabeculated region belongs together with the corresponding probability in the form of a color-coded index.

To summarize, the use of two- and three-dimensional radiographic imaging to identify risk factors for osteonecrosis is undeniably essential for the clinical management of patients receiving antiresorptive medicines. This research has showed that radiographic techniques provide valuable information for identifying early changes influenced by antiresorptive drugs, local risk factors in "at risk" patients, early diagnosis of latent lesions, and supporting the prognosis of conservative and surgical treatment of MRONJ.

SAMENVATTING

Het hoofddoel van dit onderzoek was om twee- en driedimensionale radiografische beelden te gebruiken voor de identificatie van risicofactoren voor medicatiegerelateerde osteonecrose van de kaken (MRONJ). De hypothese was dat het gebruik van radiografische beelden een essentieel diagnostisch hulpmiddel is voor vroegtijdige diagnose en behandelselectie van MRONJ. Deze algemene doelstelling en hypothese werden verder verdeeld met specifieke secundaire doelstellingen.

Deel 1 onderzocht de radiografische bevindingen in verband met het gebruik van antiresorptieve geneesmiddelen (ARDs) en risicofactoren voor MRONJ bij patiënten die tandextracties ondergingen. Daarvoor werden vier retrospectieve longitudinale case-controlonderzoeken opgezet bij oncologische en osteoporotische patiënten en met gebruik van panoramische röntgenfoto's en cone beam computertomografie (CBCT) **(hoofdstukken 1 tot 4)**. De belangrijkste resultaten van deze onderzoeken tonen aan dat verdikking van de lamina dura een indicator is voor het gebruik van antiresorptieve geneesmiddelen en dat een langere tijd tot postoperatieve alveolaire mucosale genezing wordt verwacht bij zowel hoge als lage doses van medicatie.

Een verhoogd risico voor MRONJ na tandextractie werd geïdentificeerd bij oncologische patiënten met één of meerdere risicofactoren. De geïdentificeerde risicofactoren betreffen: langere ARD-behandeling, meerdere tandextracties, roken, een kortere "drug holiday", tanden met afwezige en incomplete endodontische vullingen met cariës, verbrede parodontale ligamentruimte en/of periapicale laesies, en osteosclerotische en/of osteolytische botpatroon, sekestrumvorming of periosteale reactie. Op dezelfde manier werden de volgende risicofactoren geïdentificeerd bij osteoporotische patiënten: mannen, roken, behandeling met corticosteroïden,

mandibulaire tandextracties, tanden met meerdere wortels, osteolytische of osteosclerotische trabeculaire botpatronen, tandextracties gepaard met furcatiebetrokkenheid, wortelresten of onbehandelde cariëslaesies, en preoperatieve sekwestervorming.

Verder moet worden opgemerkt dat sommige van deze tekenen overeen kunnen komen met latente MRONJ-laesies, aangezien botsekwestrumvorming en periostale reactie werden geïdentificeerd als radiografische tekenen die zeer suggestief zijn voor MRONJ.

Deel 2 bestudeerde de klinische en tomografische prognostische factoren voor conservatieve en chirurgische behandeling van MRONJ. Dit deel bestond uit **hoofdstuk 5**, een retrospectieve cohortstudie met patiënten die werden behandeld met ten minste één toediening van antiresorptieve geneesmidelen, een diagnose van stadium 1, 2 of 3 MRONJ volgens de American Association of Oral and Maxillofacial Surgeons en een CBCT van de MRONJ laesie.

Wat de resultaten betreft, vertoonden laesies in een geavanceerd stadium een slechtere therapeutische prognose bij beide behandelingen. In het bijzonder bij de conservatieve behandeling werden laesies met tandbetrokkenheid of sequestrumvorming ook geassocieerd met een gebrek aan genezing. Daarentegen liet chirurgische behandeling slechtere resultaten zien wanneer bisfosfonaten voor een korte periode werden gestaakt en in gevallen met afwezigheid van sekwestrumvorming en aanwezigheid van periostale reactie.

In **deel 3** werd een hulpmiddel ontwikkeld en gevalideerd voor de automatische classificatie van normale, aangetaste en osteonecrose mandibulaire trabeculaire botpatronen in panoramische beelden met behulp van convolutionele neurale netwerken. Dit laatste deel omvatte **hoofdstuk 6,** waarin vier voorgetrainde convolutionele neurale netwerken werden gebruikt voor automatische classificatie van

botpatronen met normale, verdikte lamina dura, abnormale trabeculae en osteonecrose.

Alle netwerken detecteerden en classificeerden mandibulaire botpatronen correct met optimale prestatiecijfers en vertoonden geen statistische verschillen in hun prestaties. Het beste netwerk werd gekozen voor de ontwikkeling van het geautomatiseerde hulpmiddel vanwege de iets betere metriek en betere respons bij de classificatie van abnormale botpatronen, waarbij een nauwkeurigheid van 96% werd bereikt. De uitvoer van dit hulpmiddel geeft op een eenvoudige manier de categorie aan waartoe het geselecteerde trabeculaire gebied behoort, samen met de corresponderende kans in de vorm van een kleurgecodeerde index.

Samenvattend is het gebruik van twee- en driedimensionale radiografische beeldvorming om risicofactoren voor osteonecrose te identificeren ontegenzeggelijk essentieel voor het klinische beheer van patiënten die antiresorptieve geneesmiddelen krijgen. Dit onderzoek heeft aangetoond dat radiografische technieken waardevolle informatie bieden voor het identificeren van vroege veranderingen die worden beïnvloed door antiresorptieve geneesmiddelen, lokale risicofactoren in patiënten "at risk", vroege diagnose van latente laesies en ondersteuning van de prognose van conservatieve en chirurgische behandeling van MRONJ.

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Isadora Cabello Salazar: Contributed to the data collection for Chapter 5.

Jens Borremans: Contributed to the data collection for Chapter 1.

Laurence Lapauw: Contributed to the data collection for Chapter 1 and proofread the corresponding manuscript.

Marte Berckmans: Contributed to the data collection for Chapter 5.

Nele Van Assche: Acted as external jury member during the evaluation of the thesis manuscript.

Nicolly Oliveira Santos: Performed three-dimensional radiographic observations for Chapters 3 and 4 and proofread these manuscripts.

Reinhilde Jacobs: Set the foundations for the current project. She closely followed-up on the progress of the thesis and papers. She proofread every article and chapter in this thesis. Her expertise in dentomaxillofacial imaging and related research provided valuable insights substantially improving the quality of the present project.

Rocharles Cavalcante Fontenele: Performed three-dimensional radiographic observations for Chapters 3 and 4 and proofread the manuscripts belonging to Chapter 3, 4, and 5.

Santiago García Larraín: Performed three-dimensional radiographic observations for Chapter 5.

Silke Bergans: Contributed to the data collection for Chapter 1.

Soroush Baseri Saadi: Identified, customized, tested, and validated the convolutional neural networks used in Chapter 6. He also contributed to the writing of this article.

Tim Van den Wyngaert: He closely followed-up on the progress of this thesis and papers. He proofread every article and chapter in this thesis. His understanding of osteonecrosis provided valuable insights which allowed to refine the reporting of the results, substantially improving the quality of the present project.

Wim Coucke: Acted as statistical advisor throughout the preparation of this thesis. He performed the statistical analyses of Chapters 1 and 2.

PERSONAL CONTRIBUTION

Catalina Moreno is the first author of all thesis chapters and corresponding articles since she conceptualized the projects, asked for ethical approval, gathered, organized, analyzed all clinical and radiographic data, and performed the radiographic observations. Furthermore, she also wrote all peer-reviewed papers. Her supervisors, Prof. dr. Reinhilde Jacobs and Prof. dr. Tim Van den Wyngaert, as well as all her co-authors, provided significant support as specifically mentioned under scientific acknowledgements.

CONFLICT OF INTEREST

The author of this doctoral thesis has no conflict of interest to declare.

CURRICULUM VITAE

Catalina Moreno Rabie was born in Concepción, Chile, in 1992. She obtained her bachelor's and master's degree in dentistry in 2016 at the Universidad de los Andes, Chile. During her final year of dentistry, she completed a clinical and research internship at KU Leuven, where she studied anatomical variations in the retromolar area on CBCT. Between 2017 and 2018 she worked as a general dentist. Within this period, she also completed a course in dental emergency management organized by the emergency unit of the Barros Luco Trudeau healthcare complex and the University of Chile. In 2019, she obtained her diploma in the Postgraduate studies in Advanced Medical Imaging at KU Leuven (2018 - 2019, *summa cum laude*), the thesis topic was on guided endodontics under the supervision of dr. Andrés Torres and prof. Reinhilde Jacobs.

Between 2020 and 2024, Catalina worked as a doctoral researcher in biomedical sciences at KU Leuven under the supervision of prof. dr. Reinhilde Jacobs and prof. dr. Tim Van den Wyngaert. Specifically, her thesis topic investigated the effects of antiresorptive drugs on the jaw bones, possible risk factors for the development of medication-related osteonecrosis of the jaws (MRONJ), and the prognostic risk factors for this pathology. In September 2021, she was awarded the second prize in the Robert Frank Senior Clinical Science Award at the CED-IADR/NOF Oral Health Research Congress (Brussels, Belgium), and in July 2023, the first prize in the Research Award Competition at the IADMFR World Tour Congress (Brussels, Belgium).

CONTRIBUTIONS TO (INTER)NATIONAL CONFERENCES AND ACADEMIC TASKS

CONFERENCES AND LECTURES

16-18/09/2021	Continental European Division of the International Association for Dental Research (CED-IADR) Oral Health Research Congress. Oral presentation: “Radiographic Predictors of MRONJ in Patients Undergoing Tooth Extraction”. (Second Prize CED-IADR Robert Frank Clinical Research Competition)
08-10/06/2022	18th European Congress of DentoMaxilloFacial Radiology (ECDMFR) Attendee
10/11/2022	Oral Health Sciences (OHS) Department Day, Dentistry, KU Leuven Poster presentation: “Convolutional neural network for automated classification of osteonecrosis and related mandibular trabecular patterns”.
17/02/2023	International Association of DentoMaxilloFacial Radiology (IADMFR) webinars: February 2023 Oral presentation: “Radiographic signs of antiresorptive drug therapy and osteonecrosis of the jaws”.
05-08/07/2023	International Association of DentoMaxilloFacial Radiology (IADMFR): World Tour Congress Research Award Presentation: “How does the radiographic appearance of MRONJ influence its conservative and surgical prognosis?”. (First prize in the research award competition) Oral presentation: “The art of artificial intelligence”.
27/09/2023	Taller de Casos Clínicos Bucomaxilofacial y Medicina Oral 2023, Universidad de Chile Oral presentation: “Signos radiográficos del tratamiento con fármacos antirresortivos y de la osteonecrosis de los maxilares”.

ACADEMIC TASKS

2020-2024 Mentor of 2 biomedical and 1 engineering student during their thesis year.

Supervision of 11 (bio)medical, dental, and specialization students:

- 3 lab rotation students
- 5 student researchers
- 3 dentomaxillofacial radiologist in training

REVIEWER

BMC Oral Health

Dentomaxillofacial Radiology

Scientific Reports

LIST OF PUBLICATIONS

PUBLICATIONS OF DOCTORAL RESEARCH CHAPTERS

1. **Moreno-Rabié C**, Gaêta-Araujo H, Oliveira-Santos C, Politis C, Jacobs R. Early imaging signs of the use of antiresorptive medication and MRONJ: a systematic review. Vol. 24, Clinical Oral Investigations. Springer; 2020. p. 2973–89.

2. **Moreno-Rabié C**, Lapauw L, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, van den Wyngaert T, Jacobs R. Radiographic predictors for MRONJ in oncologic patients undergoing tooth extraction. Sci Rep. 2022 Jul 4;12(1):11280. doi: 10.1038/s41598-022-15254-y.

3. **Moreno Rabie C**, Cavalcante Fontenele R, Oliveira Santos N, Nogueira Reis F, Van den Wyngaert T, Jacobs R. Three-dimensional clinical assessment for MRONJ risk in oncologic patients following tooth extractions. Dentomaxillofac Radiol. 2023 Oct 24:20230238. doi: 10.1259/dmfr.20230238.

4. **Moreno-Rabié C**, Gaêta-Araujo H, Ferreira-Leite A, Coucke W, Gielen E, Van den Wyngaert T, Jacobs R. Local radiographic risk factors for MRONJ in osteoporotic patients undergoing tooth extraction. Oral Dis. 2023 Jan 8. doi: 10.1111/odi.14496.

5. **Moreno-Rabié C**, Fontenele RC, Oliveira Santos N, Nogueira-Reis F, Van den Wyngaert T, Jacobs R. Key Insights into Antiresorptive Drug Use and Osteonecrosis in Osteoporotic Patients Undergoing Tooth Extractions: a clinical and CBCT assessment. *Under revision.*

6. **Moreno Rabie C**, García-Larraín S, Contreras Diez de Medina D, Cabello-Salazar I, Cavalcante Fontenele R, Van den Wyngaert T, Jacobs R. How does the clinical and tomographic appearance of MRONJ influence its treatment prognosis? Dentomaxillofac Radiol. 2023 Oct 23:20230304. doi: 10.1259/dmfr.20230304.

7. Baseri Saadi S, **Moreno-Rabié C**, van den Wyngaert T, Jacobs R. Convolutional neural network for automated classification of osteonecrosis and related mandibular trabecular patterns. Bone Rep. 2022 Oct 29;17:101632. doi: 10.1016/j.bonr.2022.101632. (Shared first authorship)

OTHER PUBLICATIONS IN THE FIELD

1. **Moreno-Rabie C**, Scharager-Lewin D, Aranguiz V. Uso de dentífricos y colutorios para el control de la hipersensibilidad dentinaria en adultos: una revisión narrativa. Rev clínica periodoncia, Implantol y Rehabil oral. 2018 Dec;11(3):177–83.

2. **Moreno Rabie C**, Vranckx M, Rusque MI, Deambrosi C, Ockerman A, Politis C, et al. Anatomical relation of third molars and the retromolar canal. Br J Oral Maxillofac Surg. 2019 Oct 1;57(8):765–70.

3. **Moreno-Rabié C**, Torres A, Lambrechts P, Jacobs R. Clinical applications, accuracy and limitations of guided endodontics: a systematic review. Int Endod J. 2020 Feb 23;53(2):214–31.

4. Vranckx M, Lauwens L, **Moreno Rabie C**, Politis C, Jacobs R. Radiological risk indicators for persistent postoperative morbidity after third molar removal. Clin Oral Investig. 2021 Jan 3;1–10.

5. Vrielinck L, **Moreno-Rabie C**, Schepers S, Van Eyken P, Coucke W, Politis C. Peri-zygomatic infection associated with zygomatic implants: A retrospective longitudinal cohort study. Clin Oral Implants Res. 2022 Apr;33(4):405-412.

6. Vrielinck L, **Moreno-Rabie C**, Coucke W, Jacobs R, Politis C. Retrospective cohort assessment of survival and complications of zygomatic implants in atrophic maxillae. Clin Oral Implants Res. 2023 Feb;34(2):148-156.

7. Torres A, De Bie E, **Moreno-Rabié C**, Strijbos O, Peeters V, Pedano MS, Lambrechts P, Jacobs R. How accurate is targeted endodontic microsurgery? An in-vitro study. 2023. *Manuscript in preparation.*

8. Coropciuc R, **Moreno-Rabié C**, DeVos W, Van de Casteele E, Marks L, Lenaerts V, Coppejans E, Lenssen O, Coopman R, Walschap J, Nadjmi N, Jacobs R, Politis C, Van den Wyngaert T. Navigating the complexities and controversies of medication-related osteonecrosis of the jaw (MRONJ): A critical update and consensus statement. Acta Chirurgica Belgica. 2023. DOI: 10.1080/00015458.2023.2291295