Risk reduction strategies for patients at risk of medication-related osteonecrosis of the jaws undergoing dental extractions.

Submitted in fulfilment for the conditions governing candidates for the degree of

Doctor of Philosophy

University College London

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Declaration

I, Roberto Sacco confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed…………………………………..

Date …………………………………….
Abstract

In 2003, a case of osteonecrosis of the jaw related with medicine was documented for the first time. In the past, osteonecrosis of the jaw was thought to be caused by intravenous and oral bisphosphonates; but, as time has progressed, the number of drugs that have been linked to this severe side effect has expanded. Over the last two decades, a significant amount of research has been conducted with the goal of better understanding the aetiopathogenesis, epidemiology, clinical symptoms, and management of MRONJ. Extraction procedures performed in the dentist's practice have long been cited as one of the primary risk factors for the progression of MRONJ. The objective of this doctoral study was to analyse typical MRONJ risk reduction techniques in patients who were taking anti-resorptive medication treatment and to comprehend the effects these strategies had on high-risk patients as well as low-risk patients. There were three different studies done:

1) A systematic review of comparative risk reduction studies reporting any type of dental extraction protocols in patients exposed to antiresorptive and antiangiogenic drug therapy;

2) A retrospective secondary care service evaluation of patients considered to be at high risk taking antiresorptive drugs for bone metastatic cancer reasons;

3) A retrospective primary care service evaluation of patients considered to be at low risk taking antiresorptive drugs for bone metabolic disorders.

The findings of Study 1 revealed that none of the oral surgery modified procedures had any proof to minimize the risk of osteonecrosis in patients who were receiving antiresorptive and antiangiogenic medication therapy. According to the results of Study 2, minimally invasive surgical procedures have a detrimental influence on patients with cancer, and they actually increase the risk of developing MRONJ. The results of Study 3 suggested that minimally invasive surgical procedures with adjuvant antibacterial medication treatment did not reduce significantly the MRONJ frequency in patients who were regarded to have a low risk of bone necrosis. This highlighted that these protocols were ineffective. The results of this PhD thesis have shown that the impact of minimally invasive surgical methods on the reduction of MRONJ are
counterproductive in some cases. To investigate the possibility of a reduction in MRONJ, additional comparative study is being recommended.
Impact statement

Although medication-related osteonecrosis of the jaw (MRONJ) is an uncommon side effect connected with the intake of anti-resorptive and anti-angiogenic medications (ranging from 0.02% to 5% according to the type of drugs, patient’s medical condition and route of administration), it has a major adverse influence on the quality of life of individuals who have been diagnosed with the condition. Because of their poor oral health, many oral-specific complaints that are often connected with pain, and difficulties with speaking, patients who are afflicted by MRONJ have been shown to have a low quality of life, according to the findings of the study that has been conducted. As a result, developing effective preventive actions to lessen the impact of the occurrence or find other solutions is of the utmost significance. The overarching goal of this study and subsequent PhD was to further our understanding of osteonecrosis of the jaw related with medicine and raise more people’s awareness of the condition. All the articles published during the deep scanning of the literature, were created as a result of the discoveries from this dissertation and were published in a variety of medical and dentistry journals. The systematic review that was provided in chapter 5 of this PhD helped to add to a better understanding of the lack of research and information linked with any osteonecrosis of the jaws preventative plan. The experiences of patients receiving oral surgery treatments in secondary and primary care in relation to this adverse occurrence were recorded in two different chapters (6 and 7). Indeed, the findings are incredibly helpful for a variety of applications, including oral health and research. In the course of this PhD research, certain opportunities to build interventional types of strategies have been evaluated. Unfortunately, without a positive answer to the study’s question. However, the study is yielding some encouraging results; nevertheless, further high-quality research must be conducted in order to examine the possibility of preventing or at least reducing the risk of MRONJ.
Scientific Output

Publications


Acknowledgments

I would like to dedicate this thesis to my parents, brother and extended family for providing the support and strength in pursue my dreams. I also wish to thank my parents for their generous financial assistance throughout my education. I also would like to dedicate this achievement to my life partner Ilaria and my son Dario whose continual love and support has been invaluable. Thank you for the sacrifices you have made on my behalf. Thanks to my colleagues and friends at the Eastman Dental Institute for your assistance, discussion, and fun times. Between them they inspired and motivated me, enabled me to work productively, and allowed me to develop as an independent researcher.
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<tr>
<td>AAOMS</td>
<td>America Association of Oral and Maxillofacial Surgeons</td>
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<tr>
<td>AF</td>
<td>Atrial fibrillation</td>
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<tr>
<td>ALD</td>
<td>Alendronate</td>
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<tr>
<td>APR</td>
<td>Acute phase response</td>
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<td>ATLL</td>
<td>Adult T-cell leukemia/lymphoma</td>
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<td>ATP</td>
<td>Adenosine triphosphate</td>
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<td>BC</td>
<td>Breast cancer</td>
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<td>BMAs</td>
<td>Bone-modifying agents</td>
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<td>BMD</td>
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<td>BMP-2</td>
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<td>BMPs</td>
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<td>BMU</td>
<td>Basic Multicellular Unit</td>
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<td>BPs</td>
<td>Bisphosphonates</td>
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<td>BRC</td>
<td>Bone remodelling compartment</td>
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<td>Bisphosphonate related osteonecrosis of the jaw</td>
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<td>Btl</td>
<td>Bottle</td>
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<tr>
<td>C-group</td>
<td>Control group</td>
</tr>
<tr>
<td>c-Kit</td>
<td>Receptor tyrosine kinase</td>
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<td>CBCT</td>
<td>Cone beam computed tomography</td>
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<td>CCS</td>
<td>Case-control studies</td>
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<td>CENTRAL</td>
<td>Cochrane Central Register of Controlled Trials</td>
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<td>CI</td>
<td>Confidence intervals</td>
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<td>CKC</td>
<td>Clear cell kidney cancer</td>
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<td>CLO</td>
<td>Clodronate</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<tr>
<td>CT</td>
<td>Computerised tomography</td>
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<tr>
<td>CTX</td>
<td>Carboxy-terminal telopeptide cross-linked type 1 collagen</td>
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<td>DKK-1</td>
<td>Dickkopf-1</td>
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<td>DM</td>
<td>Diabetes mellitus</td>
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<td>Dmab</td>
<td>Denosumab</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>DPT</td>
<td>Dental orthopantomography</td>
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<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<tr>
<td>EGFRs</td>
<td>Epidermal growth factor receptor</td>
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<tr>
<td>ERK</td>
<td>Extracellular signal-regulated protein kinases</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FGF</td>
<td>Fibroblastic growth factor</td>
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<td>FGFRs</td>
<td>Fibroblastic growth factor receptors</td>
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<td>FHH</td>
<td>Familial hypocalciuric hypercalcemia</td>
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<td>FIHPT</td>
<td>Familial isolated primary hyperparathyroidism</td>
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<tr>
<td>FPP</td>
<td>Farnesyl diphosphate synthase</td>
</tr>
<tr>
<td>FPS</td>
<td>Farnesyl pyrophosphate synthase</td>
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<td>GAG</td>
<td>Glycosaminoglycans</td>
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<td>GGPP</td>
<td>Geranylgeranyl diphosphate synthase</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte/macrophage-colony stimulating factor</td>
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<tr>
<td>GPRD</td>
<td>General Practice Research Database</td>
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<tr>
<td>GTPases</td>
<td>Guanosine triphosphatases</td>
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<td>HD</td>
<td>Hodgkin’s disease</td>
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<td>HMG-CoAR</td>
<td>3-hydroxy-3-methyl- glutaryl-CoA reductase</td>
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<td>HPT-JT</td>
<td>Hyperparathyroidism-jaw tumor syndrome</td>
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<td>HRA</td>
<td>Health Research Authority</td>
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<tr>
<td>HTLV-1</td>
<td>Human T-lymphotropic virus type 1</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Insulin-like growth factors</td>
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<td>IGF</td>
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<td>IgH</td>
<td>Immunoglobulin heavy chain class</td>
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<td>Interleukin</td>
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<td>IL-1</td>
<td>Interleukin 1</td>
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<td>Interleukin 11</td>
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<td>IL-6</td>
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<tr>
<td>IM</td>
<td>Intramuscular</td>
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<tr>
<td>INPLASY</td>
<td>International platform of registered systematic review and meta-analysis protocols</td>
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<tr>
<td>IO</td>
<td>Inorganic matrix</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>LPL</td>
<td>Lipoprotein-related protein</td>
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<td>M-CSF</td>
<td>Macrophage colony-stimulating factor</td>
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<td>MABs</td>
<td>Monoclonal antibodies</td>
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<td>MAP</td>
<td>Mitogen activated protein kinase</td>
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<tr>
<td>mCRC</td>
<td>Metastatic colorectal cancer</td>
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<tr>
<td>MEN</td>
<td>Multiple endocrine neoplasia</td>
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<tr>
<td>MGUS</td>
<td>Monoclonal gammopathy of undetermined significance (MGUS)</td>
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<td>MIP</td>
<td>Macrophage Inflammatory Proteins</td>
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<td>Matrix metallopeptidase-13</td>
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<td>MMP-9</td>
<td>Matrix metallopeptidase-9</td>
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<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>mRCC</td>
<td>Metastatic renal cell carcinoma</td>
</tr>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MRONJ</td>
<td>Medication related osteonecrosis of the jaw</td>
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<tr>
<td>MSCs</td>
<td>Mesenchymal stem cells</td>
</tr>
<tr>
<td>MTC</td>
<td>Medullary carcinoma of the thyroid</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian target of rapamycin inhibitors</td>
</tr>
<tr>
<td>MTX</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>NCPs</td>
<td>Non-collagenous proteins</td>
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<tr>
<td>Nd:YAG</td>
<td>Neodymium-doped yttrium aluminum garnet</td>
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<tr>
<td>NHL</td>
<td>Non-Hodgkin’s lymphoma</td>
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<tr>
<td>NR</td>
<td>Not reported</td>
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<tr>
<td>NSCLC</td>
<td>Non-squamous non-small cell lung cancer</td>
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<td>NSHPT</td>
<td>Neonatal severe primary hyperparathyroidism</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>OC</td>
<td>Osteocalcin</td>
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<tr>
<td>OI</td>
<td>Osteogenesis imperfecta</td>
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<tr>
<td>OM</td>
<td>Organic matrix</td>
</tr>
<tr>
<td>ON</td>
<td>Osteonectin</td>
</tr>
<tr>
<td>ONJ</td>
<td>Osteonecrosis of the jaw</td>
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<tr>
<td>OP</td>
<td>Osteoporosis</td>
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<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>OPN</td>
<td>Osteopontin</td>
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<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
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<td>OSM</td>
<td>Osteomyelitis</td>
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<td>PAM</td>
<td>Pamidronate</td>
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<td>PBAs</td>
<td>Protein biological agents</td>
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<tr>
<td>PC</td>
<td>Prostate cancer</td>
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<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
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<td>PDGFRs</td>
<td>Platelet-derived growth factor receptors</td>
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<tr>
<td>PET/CT</td>
<td>Positron emission tomography with computed tomography</td>
</tr>
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<td>PFS</td>
<td>Progression free survival</td>
</tr>
<tr>
<td>PG-E2</td>
<td>Prostaglandin-E2</td>
</tr>
<tr>
<td>PGs</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>PI3K/mToR</td>
<td>Phosphoinositide 3-kinase/mammalian target of rapamycin</td>
</tr>
<tr>
<td>PIGF</td>
<td>Placenta growth factors</td>
</tr>
<tr>
<td>PMO</td>
<td>Post-menopausal osteoporosis</td>
</tr>
<tr>
<td>PNET</td>
<td>Pancreatic neuroendocrine tumours</td>
</tr>
<tr>
<td>PRF</td>
<td>Platelet-rich fibrin</td>
</tr>
<tr>
<td>PRGF</td>
<td>Plasma rich in growth factors</td>
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<tr>
<td>PRGs</td>
<td>Proteoglycans</td>
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<tr>
<td>PRISMA</td>
<td>Preferred Reporting Items for Systematic Reviews and Meta-analyses</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>PTHrP</td>
<td>Tumour derived parathyroid hormone-related protein</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor activator of nuclear factor-kB</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator of nuclear factor kappa-b ligand</td>
</tr>
<tr>
<td>RCTs</td>
<td>Randomised control trials</td>
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<td>Abbreviation</td>
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CHAPTER 1: OSTEOLOGY

1.1 Bone Structure and Function

The bone is a high-calcified tissue with different types of mechanical and metabolic functions. It is indeed a dynamic type of tissue, which undergoes persistent remodelling mediated by cells, cytokines and hormones. Histologically, the bone is a specialised connective tissue while, embryologically it derives from the mesenchyme tissue (Taichman, 2005). The bone can be classified according to its anatomy (long bones, flat bones) and its structure (cortical or cancellous) (Taichman, 2005).

Bone is a composite material whose extracellular matrix consists of mineral (65%), water (10%), lipids (1%), and organic material (25%), the latter being predominantly formed by type I collagen (90%), non-collagenous proteins and proteoglycans retaining water (10%) (Boskey, 2003). Moreover, it contains cells and water. The organic matrix is predominantly composed (90%) of Type I collagen, while the remaining part (10%) by non-collagenous glycoproteins (osteocalcin, osteonectin, osteopontin and bone sialoprotein). These components have both mechanical and metabolic functions, and the composition and architectural features vary with age (Boskey & Coleman, 2010). The inorganic matrix is made of hydroxyapatite, Ca₁₀(PO₄)₆(OH)₂, which is accumulated on the collagen and gives strength to the tissue (Jee, 2001; Clarke, 2008). The bone also contains blood and lymphatic vessels which, together with nervous supply, penetrate the bone tissue via Haversian and Volkmann’s canals (Figure 1.1 and Figure 1.2).
Figure 1.1 Micro-structure of mature bone. Reproduced from Gray’s Anatomy: The anatomical basis of clinical practice, 41th ed., Editor-in-chief: Standring S, Page 89, Copyright Elsevier, 2016.

Figure 1.2 Hierarchical structure of bone from the macro- (left panel) to the sub-nanostructure (right panel) and related in situ micrographs using electronic microscopy. Reproduce from Gasser and Kneissel, 2017

Bone tissue cells play an active and important role in bone remodelling. The bone tissue is constantly modified during the life of an individual. This is because of biomechanical force changing as well as repairing and removing
old and damage bone with a new and mechanically stronger tissue. According to any specific biological needs, bone tissue can be compact (cortical bone) or trabecular (cancellous bone). Cortical bone is a dense type of tissue which surrounds the marrow areas. Typically, the architect of this type of bone is mainly formed by secondary Haversian systems, which consist of a central canal carrying a vascular bundle (blood vessels and nerves) surrounded by multiple layers of concentric lamellae. Whereas, trabecular bone is formed by a network of trabecular portions interposed in the bone marrow compartment (Boskey, 2003). An external periosteal surface and internal endosteal surface are present in all the cortical bone tissues. Except the joints, where bone is lined with articular cartilage, the periosteum is a fibrous connective tissue sheath that covers the outer cortical surface of bone. Periosteum is known to contain blood vessels, nerve fibres, osteoblasts, and osteoclasts in it. It is also essential for appositional development and fracture healing.

The endosteum is a structure that protects the inner layer of cortical and cancellous bone, as well as blood vessel canals (Volkmann's canals) in bone. In addition to the aforementioned, bone could well be macroscopically grouped into two types, depending on the architecture of collagen that forms the osteoid tissue:

- woven bone
- lamellar bone,

Woven bone seems to have a random configuration of collagen fibers, whereas lamellar bone seems to have a consistent parallel orientation of collagen forming sheets (lamellae) (Weiner & Wagner, 1998). Because of the alternating orientations of collagen fibrils, lamellar bone has a higher mechanical strength compared to woven bone. As a result, woven bone is more fragile to mechanical stimulus than lamellar bone (Currey, 2002; Burr & Akkus, 2014).

From the histological point of view, woven bone is formed when osteoblasts quickly create osteoid. This happens in all foetal bones and during fracture healing, but the resultant woven bone is replaced by a process known as
remodelling, which involves the deposition of more robust lamellar bone (Currey, 2002; Burr & Akkus, 2014).

Bone is a multidimensional hard tissue material with a microscopic structure that is arranged in a hierarchical order (Rho et al., 1998). The bone biomechanical behaviour is determined by how the elements of each levels of this structural hierarchy interact among each others. (Rho et al., 1998; Currey, 2002; Burr & Akkus, 2014).

Mineral is deposited fast as an undefined calcium phosphate combined with huge quantities of calcium carbonate during mineralization, which occurs in humans within the initial 3 weeks following osteoid formation by the osteoblast. Approximately 60-70% of total mineralization is achieved through this procedure. While bone typically matures throughout the subsequent phase of secondary calcification, crystals increase in size to a final size of 40 Å~ 3 Å~ 7.5 nm, becoming more plate like and crystalline, and orienting themselves parallel to each other and to the collagen fibrils. The calcium carbonate concentration is reduced during final bone maturation, which can take more than a year in humans, but it still plays a vital role in the preservation of acid-base homeostasis (Burr & Akkus, 2014).

As described before, the organic part of bone is mainly formed of type I collagen (~90%), non-collagenous polypeptides (NCPs), proteoglycans, cytokines, a variety of growth and differentiation proteins (~5%), and lipids (~2%). The peptides found in bone's extracellular environment are commonly divided into two categories: structural proteins (collagen and fibronectin) and proteins with specific activities. The latter regulate collagen fibril diameter, operate as chemical messengers, growth factors, or proteases, or conduct other activities. Type I collagen is the most prevalent protein in the bone matrix, a unique triple helix molecule composed of two identical amino-acid α1-chains and one chemically identical but genetically distinct α2-chain (Bilezikian et al., 2002).

The length of each chain is around 1000 amino acids. The arrangement of collagen fibrils is uneven, leaving holes or gaps that serve as areas for mineralisation and crystallization (Figure 1.2) During both primary and secondary mineralization, the "holes" serve as places for the deposition of bone mineral plates. In general, collagen fibres and mineral crystals do have
preference configuration that changes from layer to layer in mature bone, giving bone its characteristic lamellar structure that is best viewed under polarised light. This fibre orientation ensures the maximum collagen density per area of tissue (Weiner & Wagner, 1998; Bilezikian et al., 2002).

These lamellae are normally parallel to one other when bone is deposited over a flat surface, like the periosteum of trabecular bone, or concentric when bone is formed on a surface that surrounds a blood vessel, such as a Harvesian system or osteons in cortical bone. Thus, cortical bone has principally an osteonal architecture, consisting of a blood vessel core surrounded by concentric lamellae measuring up to 2 mm in length. A vast network of osteocyte cells connects the numerous osteons, allowing for the exchange of nutrients and biomechanical stimuli through the cortical bone surfaces. There is no preferred orientation of collagen fibres when bone is created rapidly, as it is during growth. Woven bone is a form of bone tissue in which collagen fibres are lightly packed and irregularly arranged. Except in pathological circumstances, woven bone is virtually non-existent in the mature bones (Weiner & Wagner, 1998).

NCPs account for 10-15% of overall bone protein levels and around 2% of total bone weight. They have critical functions in the structure of the extracellular environment, in the coordination of mineral-matrix and cell signaling pathways, and in the regulation of bone mineralization. The NCPs most important are:

- osteocalcin
- osteonectin
- osteopontin

Taking up between 10 and 20% of the total protein in a human bone, osteocalcin (OC) is the most prevalent protein found in the bone matrix that is not collagenic. Mature osteoblasts are secreting OC. 1,25(OH)2 vitamin D3 stimulates OC synthesis whereas parathyroid mone inhibits it (PTH). The protein similarly recruits osteoclasts, which helps to control bone density. (Chen et al., 2004).

Osteonectin (ON) is a bone-specific protein with a molecular weight of 32,000 Dalton that binds across both hydroxyapatite and collagen. When ON binds to insolubilized type I collagen, the resulting complex links synthetic apatite
crystals and free calcium ions. The ON complexes can activate mineral phase formation from metastable stable salt solutions. ON is a skeletal tissue specific protein that binds the bone mineral and collagen phases, perhaps beginning active mineral deposition in healthy bone (Termine et al., 1981).

Osteopontin (OPN) is a heavily phosphorylated sialoprotein which is a major component of calcified tissue's mineralized extracellular matrix. OPN is a protein complex that is expressed by a range of cells, such macrophages, endothelial cells, smooth muscle cells, and epithelial cells. OPN participates in a variety of biological activities (Sodek et al., 2000).

Bone development, wound healing, immune responses, cancer, bone resorption, and calcification all include this protein. Both physiological and pathological mineralization are closely regulated by OPN. Osteoclasts and osteoblasts—bone remodelling cells—express OPN in normal bone tissue. Osteoclast-derived OPN reduces hydroxyapatite production during bone mineralization (Sodek et al., 2000).

Proteoglycans are common macromolecules with a core protein linked sulfated glycosaminoglycan (GAG) chains. Proteoglycans (PRGs) organise bone extracellular matrix via their various forms and location. These proteoglycans include mostly chondroitin-6-sulfate. (Mania et al., 2009).

Bone proteoglycans, due to their structural diversity, play a wide range of roles in this specific tissue. These roles include: (1) acting as crucial structural elements in the supramolecular organisation of other extracellular matrix macromolecules; (2) acting as co-receptors for cytokines, several growth and differentiation factors (such as TGF) and osteoprotegerin (OPG); (3) they take part in mechanisms that control bone formation, resorption and homeostasis; (4) they are responsible for the strength of the tissue and inhibit mineralization (Sodek et al., 2000).

The regulation of osteoclastogenesis and bone resorption is an important function of prostaglandins (PGs) in bone biology.

In recent years, it seems more evident that the pathophysiological bone remodelling aspect is coordinated by the molecular triad osteoprotegerin (OPG) - receptor activator of nuclear factor-kB ligand (RANKL) - receptor activator of nuclear factor-kB (RANK). (Theoleyre et al., 2004).
OPG is a member of the tumour necrosis factor (TNF) receptor family and has three structural domains that influence its biological functions, including a heparin-binding domain that may interact with proteoglycans. OPG is thought to be a decoy receptor that prevents RANKL, a member of the tumour necrosis factor family required for osteoclast differentiation, from binding to its transmembrane protein RANK receptor, consequently inhibiting the end stage of osteoclastic differentiation and causing apoptosis in mature osteoclasts (Lacey et al., 1998).

1.1.1 Skeletal Morphogenesis and Development

Ossification is the mechanism through which osteoblasts produce new bone. Two critical mechanisms mediate this process (Brighton & Hunt, 1991):

1. Intramembranous-ossification
2. Endochondral ossification

Intramembranous ossification is required for foetal bone tissue growth and development. Intramembranous ossification is a process that predominantly forms flat type of bones (including mandible and maxilla). The processes of intramembranous ossification follows particular phases such as: ossification centre creation, mineralization, trabeculae generation, and periosteum development. Mesenchymal stem cells (MSCs) and, eventually, osteoblasts stimulate the process of intramembranous ossification.

Membranous ossification, which is basically the primary calcifications of a vascular connective tissue, occurs at specific sites defined as ossification centres. MSCs multiply across the vasculature network in these ossification centres. Once the proliferation process begins, the MSCs differentiate into mature cells called osteoblasts, which form osteoid in the aggregate's core. Once the osteoid (not-mineralized tissue) is formed, it begins to crystallize, resulting in a calcific osteoid centre comprising osteocytes and functional osteoblasts. Later on, the colony of entrapped osteoblasts is replaced by vascular gaps (spongy layer), which reduce gradually. Following that, the surrounding tissue condenses and creates a fibro-vascular periosteum layer around the edge. The periosteum is produced, and newly generated bone
continues to develop in the form of trabeculae. The increased quantity of trabeculae creates a network known as woven bone, that will ultimately be substituted by lamellar bone. Endochondral ossification originates in long bones and vice versa. This process involves hyaline cartilage at first, which is subsequently replaced by mineralized bone tissue. The development of the primary ossification centre, the development of the secondary ossification centre, and the production of articular cartilage and the epiphyseal plate are all steps of endochondral ossification. The initiation of the endochondral ossification occurs in particular cartilage region termed "primary ossification centres". These centres emerged throughout foetal growth phase and are in charge of long and short bone diaphysis creation (Brighton & Hunt, 1986; Marotti et al 1992; Clarke, 2008).

Secondary ossification begins after birth and is accountable for the development of lengthy bone epiphyses as well as flat bone borders. The major centre of ossification, which begins generally in the middle section of the diaphysis, is where the initial region of ossification occurs. At this stage of ossification, a series of processes occurs: creation of periosteum and bone collar, mineralization of stroma, infiltration of periosteal bud, and production of trabeculae. During apoptosis, chondrocytes in the central centre of ossification cease secreting collagen and proteoglycans and begin depositing alkaline phosphatase, which is a crucial step in the process. Chondrocytes regulate the expression of vascular endothelial growth factor (VEGF). Blood tubes induced by VEGF expression transport hematopoietic and osteoprogenitor cells. The network of blood vessels generates the periosteal bud, which mature osteoblasts utilise to start depositing osteoid, which is eventually turned into bone trabecula (Marotti, 1996).

Similar to the main ossification centre, the secondary ossification centre begins in the epiphysis of the long bones. Epiphyseal plate describes the cartilage located between the main and secondary ossification centres. This cartilage stimulates the development of new cartilage, which is ultimately substituted by bone. The region where the main and secondary ossification centres converge is known as the "epiphyseal line." Under the periosteum, the formation of new bone tissue causes the diaphysis to grow in diameter. In the internal cavity, osteoclasts continue to breakdown bone until its maximum thickness is
reached. When the bone is fully developed, epiphyseal and metaphyseal calcification will merge and growth will cease (Eriksen, 1986; Eriksen et al., 1994) (Figure 1.3).

**Figure 1.3** Intramembranous and Endochondral Ossification models. Usha K and Nandeesh BN. Physiology of Bone Formation, Remodeling, and Metabolism. Reproduce from Fogelman et al, 2012.

The bone performs critical metabolic activities. It serves as a reservoir for vital ions such as calcium and phosphorus in the body. Furthermore, the bone serves as a reservoir for certain cytokines and growth hormones, like insulin-like growth factor (IGF), transforming growth factor- (TGF-), and bone morphogenetic proteins (BMPs), which may be released during the remodelling process. Furthermore, bone buffers blood pH by storing or releasing alkaline salts (Raisz, 1993; Raisz, 1999). Bone cells (osteoblasts, osteoclasts, and osteocytes) coordinate bone metabolism, which is influenced by a variety of substances such as hormones, vitamins, growth factors, and cytokines. One of this is the parathyroid glands hormone (PTH). Serum calcium is the primary regulating signal for PTH secretion. The same is true for thyroid C cells, which release calcitonin in case of calcium imbalance. Serum phosphate decreases calcium concentration, but environmental phosphate increases vitamin D (1,25-Dihydroxyvitamin D) synthesis. As a result, serum phosphate may affect PTH synthesis. These hormones and vitamins also interact with the activity of organs such as the kidney and the intestines, allowing the release or uptake
of calcium and phosphate absorption. Growth factors and cytokines are thought to have a role in local bone tissue formation via modulating cellular activities (differentiation and proliferation). Insulin-like growth factors I and II (IGF I and II), transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMP), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), granulocyte/macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF), and tumour necrosis factor (TNF), are the most significant cytokines involved in bone remodelling. However, the interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 11 (IL-11), prostaglandins (PGs), and leukotrienes have a principal and active role in bone healing and remodelling (Marotti, 1988; Mohan & Baylink, 1991; Fraher, 1993, Canalis E et al., 2003). Cytokines are important factors that are indispensable for the normal physiological process in a human body. Indeed, they elicit highly pleiotropic activities, and they have the ability to induce a wide spectrum of functional responses in a diverse range of cells. Table 1 summarises the functions of growth factors and cytokines in local bone remodelling.

<table>
<thead>
<tr>
<th>Growth factors</th>
<th>Stimulate bone formation</th>
<th>Stimulate bone resorption</th>
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<tr>
<td>BMP-2, BMP-4, BMP-6, BMP-7, IGF-I, IGF-II TGF-β, FGF, and PDGF</td>
<td>TNF, EGF, PDGF, FGF, M-CSF, and GM-CSF</td>
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<tr>
<td>Cytokines</td>
<td>IL-4, IL-13 and IFN</td>
<td>IL-1, IL-6, IL-8, IL-11 and PG</td>
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Table 1.1 Factors implicated in bone remodelling

1.1.2 Bone Cells and Function

The cells that reside in bone tissue play a vital function in bone tissue maintenance and remodelling (bone turnover). The bone tissue is predominantly populated by four kinds of cells:

- **Osteoblasts:** responsible for forming the bone via deposition of new organic matrix (OM).
- Osteoclast: responsible for bone resorption
- Osteocyte: responsible for bone maintenance
- Bone lining cells: responsible in the early stage of bone resorption.

Osteoblasts are responsible for bone production. When originally deposited and not yet mineralized, the extracellular matrix formed by osteoblasts is known as osteoid, but it is later mineralized by the build up of calcium phosphate in the form of hydroxyapatite. This process yields the strong yet lightweight material (composed of both organic and inorganic components) that is the primary substance of bone tissue. Following osteoblasts' activity synthesis, they may undergo apoptosis, get entrapped within mineralizing bone matrix and become osteocytes, or "inactive" lining cells that remain on inactive bone surfaces. Bone lining cells have a flattened appearance with very few cellular components, indicating a low metabolism rate. (Figure 1.4).

![Figure 1.4](image)

**Figure 1.4** Osteoblastogenesis and fate: scheme illustrating osteoblastogenesis and the main transcription factors governing proliferation and differentiation of osteoblast precursors. Reproduced from Gasser and Kneissel, 2017

However, these seemingly resting cells are in intimate interaction with matrix-embedded osteocytes via tight junctions and participate in calcium transfers between both the extracellular matrix components and the bone marrow compartment, and they are able to reactivated in response to PTH in a short period of time to play an important role to local bone formation processes (Dobnig & Turner, 1995; Kim et al., 2012).
Active osteoblasts contain a highly basophilic cytoplasm, many mitochondria, and classic intracytoplasmic features, including a very well endoplasmic reticulum with engorged compartment. All of these characteristics are compatible with their ability to create a high number of extracellular peptides. Osteoblasts are oriented in the way that the area of the phospholipid bilayer membrane is in intimate contact with the bone surface. MSCs may differentiate not only in osteoblast but could potentially evolve in other cells such as into chondrocytes, osteoblasts, myoblasts, and or adipocytes (Caplan & Bruder 2001; Jiang et al., 2002). Key transcription factors influence this type of differentiation. Sox-5, Sox-6, and Sox-9 all play a role in chondrocytes expression. MSCs progenitors, preosteoblasts, osteoblasts, bone lining cells, and osteocytes are all members of the osteoblast-lineage cells. Hedgehogs, BMPs, TGF-β, PTH, and Wingless-related integration site (WNTs) are some of the cytokines implicated in osteoblast formation (Rosen, 2013). Osteocalcin, osteopontin, osteonectin, bone sialoprotein, alkaline phosphatase, and a significant amount of type I collagen are just some of the many extracellular proteins that are produced by osteoblasts throughout the matrix synthesis process (Rosen, 2013). IGF-I and IGF-II, TGF-β, and BMPs are only some of the cytokines that osteoblasts secrete (Rosen, 2013). These important factors are essential for the proper formation and function of osteoblasts, and they are usually deposited in the calcified bone matrix. Along with calcium and phosphate, the bone matrix stores growth factors, it is believed that IGF-1 released by osteoblasts acts as controlled system of osteoblastic cell activity. (Canalis, 2009). Bone morphogenetic proteins (BMPs) are members of the transforming growth factor beta (TGF-β) superfamily. They were first discovered as the active substances and capable of inducing bone formation in ectopic locations (Urist, 1965; Urist et al., 1979). They play an essential function in the repair of injuries and are synthesized and retained in the skeleton. (Chen et al., 2004; Gazzerro & Canalis, 2006).

The bone morphogenetic proteins like BMP-2, BMP-4, and BMP-7 induce MSCs to become osteogenic cells, which in initiates endochondral ossification (Yamaguchi et al., 1991). One of the most express cytokines in bone matrix is represented by the TGF-β. TGF-β plays a crucial role in skeletal development and have both beneficial or harmful effects on bone formation.
depending on the specific circumstances and dose (Janssens et al., 2005; Maeda et al., 2004). TGF-β interact by using a pathway very similar to BMPs. However, upon binding to its specific receptors, TGF-β induces activation of the mothers against decapentaplegic homolog 2 and 3 (Smad2 and Smad3) (Feng & Derynck 2005; Massague et al., 2005).

During the phase of matrix deposition, tiny gaps termed lacunae become encased with osteoblast progenitors, which thereafter give rise to osteoblasts. Over 90% of the bone cells are osteocytes, making them the most prevalent cell type in the bone tissue, whereas osteoblasts account for just 4-6% and osteoclasts for only 1-2% (Rosen, 2013). Within the bone tissue, the osteocytes are found in a regular pattern in the mineralized matrix and communicate to each other via dendrite-like processes in a network of micro-canals (canaliculi) that radiate towards the surface (Rosen, 2013). Osteocytes are specialised cells in the bone matrix that are able to sense subtle changes in physical signals, such as strain or fluid flow, respond to variations in peaks of circulating factors including hormones and ions, and amplify these signals, ultimately resulting in an interconnected physiological adaptation of the skeleton to modifications (Schaffler et al., 2014). Bones may alter their structure and microarchitecture via a process termed modelling, which can include either bone resorption or creation. Studies in vivo suggest that osteocytes may detect fatigue-induced microdamage and signal neighbouring osteoclast progenitor cells to begin the complicated remodelling process that ultimately leads to the replacement of damaged bone (Verborgt et al. 2002).

Between 5% and 20% of osteoblasts differentiate into osteocytes during the bone-formation process. At the beginning of the process, when the cytoplasmic structures of the osteoblast are being developed, the osteoblast cytoplasm decreased by approximately 30%, and at the end, when the osteocyte has matured, by about 70%. Sclerostin, which is encoded by the SOST gene, is a marker for the differentiated intracellular osteocyte (Balemans et al., 2001; Poole et al., 2005). Bone mass is negatively regulated by this secreted protein. For proper bone homeostasis, it functions as an antagonist of conventional WNTs signalling in the osteoblastic lineage (Baron & Kneissel, 2013). In humans and other animals, its absence or suppression leads to
increased bone mass because of its effect on both bone production and bone resorption.

Bone resorption process is performed by osteoclasts, which are multinucleated, large type of cells. From a structural point of view, these cells are formed by multiple nuclei surrounded by a multitude of mitochondria, endoplasmic reticulum, and a large Golgi apparatus (Rosen, 2013). Energy generation and protein synthesis take place in these cells due to the presence of vesicles, lysosomes, and vacuoles. Osteoclasts are essentially a monocyte-macrophage that specialises in destroying bone (Suda et al., 1999). Two cytokines—macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL)—are essential for osteoclastogenesis (Suda et al., 1999; Boyle et al., 2003). Marrow stromal cells and osteoblasts generate both a membrane-bound and a soluble version of each protein. Thus, non-hematopoietic cells in the bone marrow are essential for the recruitment of osteoclasts from their mononuclear precursor. The primary osteoclastogenic cytokine needed for precursor cell priming is RANKL, a member of the TNF family that was first found as a secreted protein of activated T cells (Weitzmann et al., 2000).

In bone, RANKL produced by osteoblast-lineage cells including osteocytes binds to the RANK receptor, which is expressed in osteoclast progenitors and mature osteoclasts. RANKL expression is upregulated in osteoblast-lineage cells by stimulators of osteoclastogenesis such as parathyroid hormone (PTH), PTH-related protein (PTHRP), prostaglandin-E2 (PG-E2), interleukin-1 (IL-1), and the active form of vitamin D3. However, a soluble version of the TNF receptor is expressed by osteoblasts and osteocytes and acts as a decoy receptor for RANKL to suppress osteoclastogenesis (Kostenuik & Shalhoub 2001). Differentiation and activation of osteoclasts are regulated by the ratio of RANKL to osteoprotegerin (OPG) in osteoblast-lineage cells (Boyle et al., 2003; Rosen, 2013). Active bone-resorbing osteoclasts demonstrate cellular polarisation with the apical membrane comprised of the clear zone (or sealing zone) and the ruffled border and a basolateral plasma membrane (Nakamura, 2007). The resorption of bone tissue occurs in two stages: first, the mineralized matrix is dissolved, and then the organic matrix is broken down by enzymes. (Figure 1.5).
Figure 1.5 Osteoclast ultrastructure/resorption: schematic representation of a resorbing osteoclast showing the sealing zone (SZ), ruffled border (RB), transition zone (TZ), nuclei, and resorption lacuna. Reproduce from Gasser and Kneissel, 2017

The ruffled border is generated beneath the firmly sealed clear zone. Carbonic anhydrase, which transforms CO2 and H2O into H+ and HCO3− (Gay & Mueller, 1974), and a vacuolar -type H+ -ATPase work together to acidify the confined Howship's lacuna (Blair et al., 1989). A pH of 4.5 induces localized hydroxyapatite demineralisation, exposing the organic matrix, which is mostly composed of type I collagen. A variety of interesting enzymes such as cathepsin K, matrix metallopeptidase-9 (MMP-9), and matrix metallopeptidase-13 (MMP-13) degrade the demineralized extracellular organic components (Gelb et al., 1996; Rosen, 2013). Endocytosed protein fragments are carried in vesicles to lysosomes, where they are either destroyed or transferred to the basolateral surface, where they seem to release their contents into the extracellular space (Salo et al. 1997). The Rho family of small GTPases is essential for actin cytoskeleton remodelling in several types of cells, including osteoclasts. Messages from tyrosine kinase receptors activate small GTPases, which bind GTP and transmit to the cytoskeleton to conduct their particular actions after binding to bone. Rho signalling seems to primarily affect cell adhesion through actin ring formation and a constitutively active isoform of the GTPase, which increases podosome development, osteoclast movement, and bone turnover (Jaffe & Hall, 2005). Following the end of bone resorption,
osteoclasts initiate programmed cell death, often known as apoptosis. The signals that cause osteoclast apoptosis are yet unknown. High levels of extracellular calcium released by osteoblasts, osteoclast detachment, and a reduction of pro-survival cytokines (M-CSF and RANKL) have all been thought to be possible explanations. The activation of extracellular signal-regulated protein kinases (ERK) is required for osteoclast life, and TNF- and IL-1 postpone osteoclast death through ERK expression. Several steroid hormones have been identified as regulating osteoclast development, activity, and survival. Estrogens and androgens decrease the synthesis of the osteoclastogenic cytokines IL-1 and IL-6, respectively, and oestrogens also cause osteoclast death (Rosen, 2013).

1.1.3 Bone Modelling and Remodelling

Early bone formation and bone modelling affect the size and form of a bone. Bone resorption and production must be separated in this process; bone is dissolved from one anatomical region and newly bone is synthesized at another. An important significant aspect of modelling is the preservation of bone morphology during linear development (Seeman, 2003; Burr & Akkus, 2019). The bulk of bone remodelling is accomplished by skeletal maturity, however the process may continue in adulthood, for example, in a dynamic response to mechanical loads and exercise, as well as in some disease (e.g. renal disease) (Krahl et al., 1994; Burr & Akkus, 2019).

- The bone remodelling cycle
The remodelling cycle is a process where the skeleton manages its own maintenance and repair. This process also offers the ability for convenient access to important minerals such as phosphate and calcium, which are needed for important physiological activities (Mori & Burr, 1993; Bentolila et al., 1998). The bone remodelling activity is a strictly controlled process in which aged and damaged bone tissue is replaced with new and healthy tissue. Biologically, the cycle occurs inside a Basic Multicellular Unit (BMU), which is formed by osteoclasts, osteoblasts, and a capillary blood vessels (Frost, 1990). The BMU's composition and structure vary based on whether it is found in
cancellous or cortical bone. The BMU appears on the trabecular bone surface and is known as Howship's lacunae. In contrast, in cortical bone, osteoclasts within the BMU eliminate defective bone, following which differentiated osteoblasts lay down new bone concentrically to provide a vascular supply inside the Haversian canal (Manolagas, 2018). In both cases, the BMU is surrounded by cells that define the bone remodelling compartment (BRC). The BRC offers a distinct remodelling region with intimate anatomical connections between osteoclasts and osteoblasts cells (Eriksen, 2010).

- **Key steps in the remodeling cycle – Cellular and molecular mechanisms**

The remodeling cycle occurs in a highly regulated way with five steps:

- Activation
- Resorption
- Reversal
- Formation
- Termination

Respectively, this process takes 120–200 days in both cortical and cancellous bone (Agerbaek et al, 1991). Osteocytes are mainly coordinate the bone remodeling by regulating the activity of osteoclast and osteoblast differentiation and thus bone resorption and formation as per Figure 1.6.

![Figure 1.6 Schematic diagram of the bone remodeling cycle different phases; Reproduce from - Kenkre and Bassett, 2018](image_url)

- **Activation**
In this phase, the precursor cell lines for osteoclasts are recruited from the bloodstream and activated. The bone surface is exposed as the lining cells detach from the underlying bone area to form a canopy over the resorbing region (Burr & Akkus, 2019). Resorption pits are isolated from the surrounding bone by multi-nucleated pre-osteoclasts, which are formed when several mononuclear cells fuse to create pre-osteoclasts and adhere to the bone matrix. The beginning of bone remodelling process is the first crucial step, guaranteeing that only takes place if it is necessary. Osteocytes transmit signals to other cells through their vast network of dendritic structures, commencing the process of selective remodelling, which is the removal of a particular region of damaged or old bone (Mori & Burr, 1993; Goldring, 2015). When bone matrix is damaged, osteocytes undergo apoptosis, which triggers the release of signaling cascade substances that promote local angiogenesis and the mobilization of osteoclast and osteoblast precursors, respectively (Chen et al., 2015a; Atkins & Findlay, 2012). In contrast, non-targeted remodelling does not happen at a particular spot but rather as a result of systemic changes in hormones like PTH, giving access to bone calcium reserves (Burr & Akkus, 2019).

- **Resorption**

Osteocytes also play a role in regulating the differentiation and activity of osteoclasts. Adhesion to the bone surface, creation of a sealing area, and production of a ruffled border occur from the osteoclast's reorganised microstructure which give a significantly increased secretion surface area. At first, osteoclasts release ions, created by Carbonic Anhydrase II, into the targeted area to degrade bone tissue. Maintaining electro-neutrality is a direct result of the hydrogen-ATPase pumping hydrogen into lacunae, which is connected to chloride transport through a channel (Silver et al., 1988; Burr & Akkus, 2019). Proteases like cathepsin K and matrix metalloproteinases then break down the collagen-rich matrix of the bone tissue (Delaisse et al., 2003). The osteoclasts' planned death puts an end to the resorption phase and prevents excessive resorption (Xing & Boyce, 2005).
• **Reversal**

The transition from bone resorption to bone synthesis, known as the reversal phase, is poorly understood. However, two major phenomena are suggested. Before any new bone matrix can be deposited on a newly resorbed bone surface, additional signalling occurs to link resorption and creation, preventing any bone loss (Howard et al., 1981; Sims & Martin, 2014). Osteoblastic cells remove unmineralized collagen matrix from the bone surface, and subsequently deposit a non-collagenous mineralized matrix "cement-line" to improve osteoblastic adhesion. Bone resorption and creation occur in response to a signal that is not completely understood at this time. However, the cells of the reversal phase very certainly participate in either the transmission or reception of these stimuli (Everts et al., 2002; Raggatt & Partridge, 2010). Evidence suggests that the Ephrin receptor class and their membrane-bound ligand, found on osteoblasts, might be the basis of the coupling aspect by communicating with osteoclasts directly or indirectly via the secretion of cytokines like IL-6. Matrix-derived factors including BMP-2, TGF-β, and IGF may also play a role in other signalling pathways (Sims & Martin, 2015; Matsuo & Otaki, 2012).

• **Formation**

There are two distinct phases of bone formation. Firstly, the osteoid matrix, which is rich in type 1 collagen, is synthesised and secreted by osteoblasts. Secondly, osteoblasts are involved in controlling the mineralization of osteoid matrix (Burr & Akkus, 2019). Bone mineralization, in which hydroxyapatite crystals are formed between collagen fibrils, is a complicated process whose control is only partially understood. However, it is understood that local regulator factors of mineralization, such as pyrophosphate and non-collagenous proteins like osteopontin, as well as systemic modulation of calcium and phosphate levels, all contribute to the overall effect. Tissue nonspecific alkaline phosphatase and ectonucleotide pyrophosphatase play key roles in controlling the inorganic pyrophosphate to phosphate ratio, which regulates the mineralization process (Anderson, 2003).
• **Termination**
When calcification is concluded, osteoblasts could transform into bone-lining cells, or become encased inside the bone matrix and ultimately develop into osteocytes or undergoing an apoptotic process. The osteocytes in this phase play an important role in signalling the completion of remodelling by secreting factors against the osteogenesis, especially antagonists of the Wnt pathway such as SOST (Bonewald, 2012; Burr & Akkus, 2019).

• **Major signalling pathways**
To ensure balanced resorption and creation, the remodelling cycle is closely controlled. While systemically secreted proteins have a regulatory function, the concept that remodelling takes place at many, independent locations all at the same time suggests that local control is essential for establishing this fine equilibrium. As a result, two important signalling pathways have been identified as key aspects: the RANKL/RANK/OPG and WNT transduce signals, which are generated both systemically and locally. Because of their regulatory function in influencing the balance and timing of bone resorption and production throughout the remodelling cycle, they are potentially relevant targets for pharmaceutical therapies in bone disease (Burr & Akkus, 2019).

• **RANKL/RANK/OPG signalling pathway**
Understanding the control of osteoclastogenesis throughout the remodelling cycle was significantly advanced by the 1990s discovery of the RANKL/RANK/OPG signalling pathway, which served as the therapeutic target for the development of the new antiresorptive denosumab (Boyce & Xing, 2007). Prior to the activation of RANKL, a suitable quantity of M-CSF is necessary. This M-CSF is secreted by osteocytes and osteoblasts and increases RANK synthesis (Arai et al., 1999). The interaction of RANKL to its receptor and RANK on osteoclastic progenitor cells promotes osteoclast maturation, stimulation, and longevity (Kong et al., 1999a; Yasuda et al., 1998; Takayanagi et al., 2002; Kearns et al., 2008). While RANKL may be synthesized by osteoblasts, osteocytes, and chondrocytes, it is hypothesized that osteocytes from within the bone matrix could detect changes in
physical stress and microdamage, hence inducing osteoclastogenesis through RANKL synthesis at the start of the bone remodelling process (Xiong et al., 2015; Nakashima et al., 2011). OPG works as a decoy receptor for RANKL and was identified prior to the discovery of RANK/RANKL signalling pathway. Osteoblasts and osteocytes have the ability to suppress osteoclastic bone resorption by suppressing and inhibiting RANKL from binding to RANK (Nakashima et al., 2011; Simonet et al., 1997). Thus, the RANKL:OPG ratio plays an important role in the control of bone resorption, bone mass, and skeletal integrity, and it is influenced by a variety of systemic variables (Boyce et al., 2012) Figure 1.7.

**Wnt signalling**

The Wnt signalling pathway was found as a primary modulator of osteoblastic bone development in a study of uncommon clinical disorders with excessive bone mass phenotypes were expressed (Figure 1.8). Cytoplasmic b-catenin is targeted for deterioration in the absence of Wnt. When Wnt is present, it attaches to a dual receptor complex that comprises a seven transmembrane domain receptor and co-receptors Lipoprotein receptor-related proteins (LPL 5 or 6). This prevents the complex from functioning, resulting in the buildup of cytoplasmic b-catenin. The b-catenin protein subsequently migrates to the nucleus, where it activates target gene transcription, resulting in osteoblast
proliferation and differentiation (Clevers & Nusse, 2012). Loss of function mutations of the LPL-5 co-receptor like in individuals with osteoporosis-pseudoglioma syndrome result in decreased Wnt signalling and osteoblastic bone production, causing a low bone density phenotype (Clevers & Nusse, 2012). The secreted Wnt inhibitor was discovered via research into the unusual high bone density illnesses such as sclerosteosis and Van Buchem disease. These hereditary disorders are linked to SOST gene loss of function mutations. Osteocytes release SOST gene proteins, which inhibit Wnt signalling by attaching to the co-receptors LPL 5 or 6 (Clevers & Nusse, 2012; Williams, 2014). During the bone remodelling process, osteocyte activation of Wnt-inhibitors reduces, allowing osteoblastic bone synthesis to be carried on following bone resorption. While in the termination phase, freshly created osteocytes get entrapped inside the bone matrix, which determine the re-expression of Wnt inhibitors, resulting in the termination of bone formation (Clevers & Nusse, 2012; Williams, 2014).

Figure 1.8 Schematic illustration of Wnt signaling pathway. Reproduce from - Kenkre and Bassett, 2018

- **Endocrine regulation of the bone remodelling cycle**

**PTH:** Depending on the extent of exposure, PTH might have directly opposite implications for bone remodelling. Indeed, PTH increases osteoclast activity and serves as an important physiological process in controlling the calcium homeostasis. Furthermore, the long-term exposure to PTH caused by neoplasms or parathyroid dysfunction (e.g. parathyroid adenoma or hyperplasia in primary hyperparathyroidism) leads to hypercalcaemia, bone
loss, and elevated bone fracture risk (Stein et al., 2013). Persistent elevated concentration of PTH in the blood stream causes both cortical and cancellous bone loss, with cortical bone suffering the most. The regulation of the OPG-RANKL-RANK signalling pathway by PTH causes catabolic consequences. Continuous PTH stimulates osteoclastogenesis by increasing RANKL and inhibiting OPG in osteocytes and osteoblasts (Silva & Bilezikian, 2015). Monocyte chemoattractant protein 1, which is involved in osteoclast precursor recruitment and development, is similarly enhanced in response to excessive PTH and is considered to have a pathological role in individuals with primary hyperparathyroidism (O'Brien et al., 2008). Intermittently given PTH, on the other hand, is employed as an anabolic agent in the treatment of osteoporosis. Intermittent activation of PTH receptors promotes bone growth by modulating Wnt signalling. Increased canonical Wnt signalling promotes osteoblastogenesis, target gene expression, and bone production (O'Brien et al., 2008; Li et al., 2014).

**Vitamin D.** 1,25(OH)2 vitamin D influence calcium and phosphate intestinal absorption, which are important minerals during bone formation and mineralization. Currently, 1,25(OH)2 vitamin D physiological activities are not fully understood. Several investigations have shown vitamin D receptor (VDR) expression in osteoclast and osteoblast progenitor cells, as well as in osteocytes, indicating that vitamin D may have direct impacts in bone physiology (Lanske et al., 2014; Wang et al., 2014; van Driel & van Leeuwen, 2014). VDR expression has been seen in human osteoclast precursors, although investigations in adult osteoclasts have shown inconsistent results (Zarei et al., 2016). In vitro investigations study showed expression of the vitamin D-activating enzyme 1-hydroxylase in human osteoblasts and osteoclasts, as well as mRNA expression in osteocytes, indicating that vitamin D activity in skeletal cells may be regulated locally (Lanske et al., 2014; van Driel et al., 2006). Initial investigations in VDR-deficient animals revealed that dietary calcium consumption alone could repair their dysfunctional skeletal phenotype, indicating that any direct activities of vitamin D in skeletal cells are likely to be restricted. (Panda et al., 2004). Consistent with this finding, no obvious skeletal phenotype was seen in mice given a regular diet after cell-
specific ablation of the VDR in the mature osteoblast/osteocyte lineage. Although high dosage 1,25(OH)2vitamin D produced hypercalcaemia and hypomineralization in these animals, partial resistance suggests a function for the osteoblast VDR in this process. Furthermore, transgenic osteoblast-specific VDR over-expression boosted bone mass and strength owing to increased osteoblastic bone production and decreased osteoclastic resorption, whereas osteoblast-specific VDR deletion resulted in a slight increase in trabecular bone volume in older animals (Lieben et al., 2012). These results support a fundamental function for the intestinal VDR in controlling calcium availability for skeletal calcification, but they also imply that vitamin D may potentially have direct activities in skeletal cell membranes. (Panda et al., 2004; Lieben et al., 2012).

**Calcitonin.** Calcitonin is secreted in the thyroid's follicular C-cells, although its functional purpose is unknown, its activity reduces bone resorption. Studies have found that calcitonin at pharmacological doses, lower the osteoclast number, secretory activity, and ruffled border development by acting on the osteoclast calcitonin receptor (Zaidi et al., 2002; Carter & Schipani, 2006). Calcitonin-deficient in preclinical studies, on the other hand, have enhanced bone formation. At physiological concentrations, calcitonin suppresses the effects of sphingosine-1-phosphate, a connecting factor that links bone formation to resorption (Zaidi et al., 2002; Carter & Schipani, 2006).

**Thyroid hormone.** Thyrotoxicosis is a well-known source of secondary osteoporosis, and it is linked to enhanced osteoblastic bone production as well as increased osteoclastic activity. Thyroid hormones increase osteoblast development and mineralization, although it is unknown if thyroid stimulating hormone have any direct effect on osteoclasts. Prolonged thyroid hormone depletion causes impairment of bone remodelling cycle, resulting in poor bone turnover and a greater bone mass. Hyperthyroidism, on the other hand, promotes bone turnover, shortens the bone remodelling cycle, and causes inactivation of osteoblastic and osteoclastic activity, leading to a 10% loss of bone every remodelling cycle. (Bassett & Williams, 2016).
**Growth hormone and insulin-like growth factor 1.** Growth hormone (GH) promotes the production of insulin-like growth factor 1, which increases bone turnover by boosting both osteoblastic bone development and osteoclastic bone loss. Despite this, increase of osteoblastic bone expression prevails, the result is in a little net gain in bone mass. In GH shortage, bone resorption surpasses bone growth, eventually leading to osteoporosis. (Olney, 2003).

**Glucocorticoids.** Glucocorticoids induce osteoporosis at elevated serum dosages. This is due to a reduced osteoblast development and function with an increased apoptosis turnover (Weinstein et al., 1998). Glucocorticoids, on the other hand, enhance osteoclastic osteoclast activity by decreasing OPG and boosting RANKL expression in osteoblasts while decreasing RANK expression in osteoclasts. However, the increased bone resorption is only temporary, as long-term glucocorticoid therapy reduces osteoclast levels and activity (Mitra, 2011). Glucocorticoids, on the other hand, have been proven to have an anabolic impact on bone turnover at physiological doses (Mitra, 2011; Henneicke et al., 2014).

**Sex hormones.** Postmenopausal osteoporosis is defined by a disruption in the bone remodelling cycle, with increased osteoclastic bone activity compared to osteoblastic bone deposition, resulting in net bone loss. As a result, oestrogen reduces bone turnover by decreasing osteoclast quantity and activity and boosting osteoclast death through the oestrogen receptor-α (Krassas & Papadopoulou, 2001). Oestrogen also suppresses osteoblast and osteocyte death, allowing bone production to continue while limiting bone remodelling (Ribot et al., 2006; Khosla et al., 2012). Aromatase enzymes transform androgens to oestrogens, and adrenal steroids are the main source of oestrogens in postmenopausal women. Women who use aromatase inhibitors or have low aromatase expression are more likely to develop osteoporosis. Similarly, aromatase is necessary for male bone mass. It has been shown that oestrogen levels, rather than androgen concentrations, dictate bone mass in the ageing adult males’ population (Ribot et al., 2006; Khosla et al., 2012). Androgens, like oestrogens, promote net bone development by increasing bone formation while suppressing degradation. Low levels in males increase the rate
of remodelling, which is related to testosterone aromatizing less oestrogen. A lack of oestrogen or androgen causes an increase in bone turnover (Manolagas et al., 2013).

- **Paracrine regulation of the bone remodelling cycle**

  **Growth factors**: Both TGF-β and BMPs belong to the TGF superfamily and are found in bone. Canonically, they communicate through (Smad-dependent) and/or (Smad-independent) pathways. They stimulate the production of runt-related transcription factor (Runx) 2, the primary osteoblast transcription factor necessary for the induction of osteoblast development. TGF-β has also been linked to the synchronization of resorption and bone production by stimulating mesenchymal stem cell migration to resorptive regions (Tang et al., 2009; Bruderer et al., 2014).

  **Prostaglandins**: Prostaglandins control bone resorption and formation locally through numerous related receptors. Nonetheless, the precise involvement of prostaglandins in the bone remodelling cycle is unknown. For example, PG-E2 is a powerful bone resorption stimulant that is considered to work by raising the RANKL/OPG ratio to promote osteoclastogenesis. However, PG-E2 increases bone formation by stimulating osteoblast proliferation and differentiation (Raisz, 1999).

  **Cytokines**: Cytokines like IL-1 and IL-6, as well as tumour necrosis factor-α (TNF-α), may induce osteoclastogenesis, whilst others like IL-4 and gamma interferon can suppress it. These cytokines have a significant role in the pathogenesis of osteoporosis in postmenopausal women. Oestrogen insufficiency increases IL-1, IL-6, and TNF-α, which induces an increase in RANKL expression as well as osteoclastogenesis and bone loss (Roodman, 1993).

1.1.4 Bone Healing Pattern

Bone repair is still largely an unexplained sequence of numerous biological activities. For bone induction and conduction, it requires intracellular and extracellular biochemical signalling. It is a multilevel type of mechanism with a
defined temporal and environmental order (Einhorn, 1998; Sandberg et al., 1993). Molecular systems that govern the creation of bone tissue during embryogenesis are reproduced during bone healing (Ferguson et al., 1999). Many local and systemic regulatory factors, such as developmental and differentiation factors, hormones, cytokines, and extracellular matrix, engage with a variety of cell types, including bone tissue and cartilage forming primary cells and even muscle mesenchymal cells participants were selected at the injured area or from the bloodstream.

- **Biology of fracture healing**

Bone tissue healing is a complicated, but well-staged process. The regeneration mechanism that starts in initial response to injury and results in optimum bone repair with a functional restoration. The normal embryogenesis route is recapitulated throughout the repair process, with the synchronized involvement of various types of cells (Ferguson et al., 1999). The cortex, periosteum, bone marrow, and external surrounding tissues all play a part in the healing process to different degree, based on various specifications existing at the damaged tissues such as signalling pathways, hormones and nutrients, pH, oxygen concentration, the electrical environment, and the structural strength that has been achieved (Carter et al., 1996; Ferguson et al., 1999). Fracture healing, like all other repair processes, begins with the activation of an immunological response (Einhorn et al., 1995). A haematoma forms and inflammatory reaction ensues at this first stage. Cytokines, platelets, BMPs, and MSCs are key participants during the early inflammatory phase. Proinflammatory cells release IL-1, IL-6, and TNF-α, which have a chemotactic impact on other inflammatory cells as well as the attraction of mesenchymal cells (Kon et al., 2001). Cho et al showed a peak in IL-1 and -6 expression one day after fracture, followed by a fast drop until day three to near low or undetectable (Cho et al., 2002). Platelets, when triggered by thrombin and sub - endothelial collagen, produce PDGF and TGF-β, which aid in the onset of fracture healing (Bolander, 1992). These factors promote mesenchymal cell migration, activation, and proliferation, as well as revascularization, chemotactic stimuli of acute inflammatory cells, and platelet aggregation. BMPs are simultaneously secreted from the bone matrix and generated by recruited
primary mesenchymal cells (Bostrom, 1998). MSCs multiply and differentiate into a chondrogenic or osteogenic lineage during the next several days (Shea et al., 2003). Angiogenesis occurs at this early stage of events, and it is required for the regeneration cascade to progress further. FGF, VEGF, and angiopoietins 1 and 2 govern vascular ingrowth into the growing callus (Lieberman et al., 2002; Gerstenfeld et al., 2003). Angiopoietic 1 is thought to be produced during the early stages of fracture healing, while VEGF is thought to be induced later on, mostly during endochondral and bone development (Gerstenfeld et al., 2003). Table 1.2 summarised the cascade of cellular and molecular signalling events.

<table>
<thead>
<tr>
<th>Time</th>
<th>Cellular events</th>
<th>Molecular aspects</th>
</tr>
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| Day 1   | - Haematoma formation, inflammation
- Recruitment of mesenchymal cells
- Osteogenic differentiation of MSCs from bone marrow | Cytokines: IL-1, IL-6, TNF-a released by inflammatory cells; PDGF, TFG-beta released from degranulating platelets; BMP-2 expression and restricted to day 1 expression of GDF-8 |
| Day 3   | - MSCs proliferation begins
- Proliferation and differentiation of preosteoblasts and osteoblasts in regions of the ossification
- Angiogenesis begins | Decline of cytokines levels; Expression of TGF-β2, -β3, GDF-10, BMP-5, -6; Angiopoietin-1 is induced |
| Day 7   | - Peak of cell proliferation between days 7 and 10
- Chondrogenesis and endochondral ossification begins (days 9—14 maturation of chondrocytes) | Peak of TGF- β2 and - β3 expression;
Expression of GDF-5 and probably GDF-1 |
| Day 14  | - Cessation of cell proliferation in intramembranous ossification, but osteoblastic activity continues
- Mineralization of the soft callus, cartilage resorption, and woven bone formation
- Neo-angiogenesis which infiltrates along new mesenchymal cells
- Phase of most active osteogenesis until day 21 | Decreased levels of expression for TGF- β2, GDF-5, and probably GDF-1; Expression of BMP-3, -4, -7, and -8; VEGFs expression;
Second increase of IL-1 and TNF-a which continues during bone remodelling |
| Day 21  | Woven bone remodelled and subsequently replaced by lamellar bone | Decreased expression of TGF- β1 and TGF- β3, GDF-10, and BMPs (2—8) |

Table 1.2 Cellular events and molecular signalling expression during fracture healing (Kon et al., 2001; Gerstenfeld et al., 2003)

- **Dental Socket Healing**

Many animal researches have been conducted to investigate the physiological mechanisms that occur during extraction socket healing, as well as their chronological sequence (Lin et al., 1994; Cardaropoli et al., 2003; Sato & Takeda 2007). A blood clot immediately fills the alveolar socket, which is soon replaced by granulation tissue Figure 1.9 (Pagni et al., 2012; Christopher, 1942).
The blood clot typically fills up the socket completely to the wound's soft tissue edges. Thus, the blood clot is in close contact with portions of the damaged periodontal ligament, which contains a considerable number of mesenchymal cells, fibres, and blood vessels. Following that, erythrocytes are lysed by coagulative necrosis. This process begins near the edge of the socket, and the blood clot is supplanted by granulation tissue. To cover the healed socket, the epithelial mucosa migrates all over the granulation tissue (Mangos, 1941). Because the epithelial cells identify this inflammatory tissue as a connective tissue, cellular migration occurs along its surface. The remnant primary fibres of the damaged periodontal tissues, which are perpendicular to the surface of the bone tissue wall and embedded in the bundle bone, help to produce a bone matrix towards the extraction socket's centre. The bone matrix partially substitutes periodontal ligament fibres along with the remanent blood clot and granulation tissue. The active production and accumulation of collagen fibres to generate the bone matrix forecasts calcified tissue formation. Several osteoclasts are present at this time in the marrow gaps inside the bundle bone covering the socket as well as in the Volkmann canals, supporting the socket remodelling process. The gradual deposition of an osteoid matrix and its increasing calcification inside the socket parallels the gradual loss of the periodontal ligament and bundle bone. From the lingual to the buccal regions, the mineral apposition rate, mineralizing surface, and mineral production rate
all decrease. Resorption of the bundle bone causes connection between the bone marrow areas of nearby interdental septa and the newly produced woven bone in the socket in various places. The woven bone trabeculae migrate from the old bone of the socket towards the centre of the socket and are often connected with the formation of newly created blood vessels. The presence of bone matrix is gradually restricted to the core section of the socket as woven bone develops. This time frame corresponds to the extraction socket's greatest mineralized tissue composition (Lin et al., 1994; Cardaropoli et al., 2003; Sato & Takeda 2007). Osteoclasts are present on the socket walls up to surface of the native lamellar bone, but the osteoclastic resorbing activity involves also the trabeculae of newly formed woven bone, indicating that the process of modeling/remodeling of the newly formed bone has begun. Most of the woven bone in the socket apical of the bridge is replaced with lamellar bone and bone marrow. Concomitantly, collagen fibers from the lining mucosa become inserted in the new "cortical" bone and, hence, a periosteum—like structure is established. At this stage of healing, the entire region of the extraction socket apical to the socket sealing bridge is characterized by a high content of well-organised bone marrow and trabeculae of lamellar bone. Socket repair after tooth extraction has also been studied in humans and bone organization and architecture is often not completed at 24 weeks after tooth extraction (Claflin 1936; Mangos 1941; Christopher 1942; Amler et al., 1960; Boyne 1966; Trombelli et al., 2008).

1.2 Bone Targeting Disease

A broad range of medical diseases that influence bone strength may be classified as primary or secondary skeletal illness. As a result, these disorders predispose individuals to an increased risk of fracture. This section of the dissertation identifies the most frequent diseases that impact bone quality and need antiresorptive and/or antiangiogenic medication treatment.

1.2.1 Osteoporosis

Osteoporosis (OP) is a metabolic disorder characterised by a reduction in the quantity of properly mineralized bone as well as a disruption in bone micro-
architecture, notably of the cancellous bone. As a result, OP should be considered as the result of a unique imbalance in bone remodelling, which results in net bone loss because the quantity of bone resorbed by osteoclast activity does not equal the extent of bone formed by osteoblasts for a variety of causes (Heaney et al., 1978). OP is a complex condition that, although most often encountered in women, may affect any age group. The most frequent kinds of osteoporosis are shown below.

- **Postmenopausal Osteoporosis**
  Women who are oestrogen deficient are vulnerable to this type of osteoporosis. The majority of cases of primary osteoporosis in female are postmenopausal or senile. Postmenopausal osteoporosis is distinguished by trabecular bone mass loss, which results in most likely spine and radius fractures. A deficiency of oestrogen and vitamin D is regarded to be the primary pathophysiology component in postmenopausal osteoporosis (Heaney et al., 1978). Oestrogen insufficiency is the most important mechanism of the rapid phase of bone loss. As previously mentioned, oestrogen suppresses both the development and stimulation of osteoclasts, leading to a discrepancy between bone resorption and formation processes (Scharla et al., 1990). An increase in the rate at which bone tissue remodelling cycles are commenced worsens this remodelling imbalance. Oestrogen operates partly via the osteoblast by enhancing the biosynthesis of osteoprotegerin or IGF, and partly through the bone marrow microenvironment via monocytes by decreasing the production of IL-1 and TNF-α (Eriksen et al., 1988). As a consequence, greater cytokine production in response to oestrogen deficiency may lead to quicker bone loss. Oestrogen deficiency increases the vulnerability of the skeletal system to parathyroid hormone and maybe other resorption-inducing chemicals, aggravating the remodelling deficit (Cosman et al., 1993). Skeletal calcium outflow into extracellular fluids increases urinary calcium excretion, but intestinal calcium absorption decreases (Heaney et al., 1978).

- **Osteoporosis in Men**
  Despite the fact that osteoporotic fractures are less common in men than in women, osteoporosis is nevertheless a dangerous condition. The aetiology of
osteoporosis in males is often complicated, and the majority of osteoporotic men have such risk factors. A small percentage of the two-thirds of men with osteoporosis have a variety of risk factors that contribute to bone loss and frailty, such as other medical conditions, medicines, or lifestyle choices (Marcus et al., 2001; Amin & Felson, 2001). Alcoholism, corticosteroid usage, and hypogonadism are the most severe. Aside from the previously mentioned risk factors, a significant number of osteoporotic males have an idiopathic condition.

- **Idiopathic osteoporosis:** Osteoporosis of unclear origin may appear in males of any range of age, however it is particularly severe in younger men. The condition is distinguished by a slow rate of bone growth. Several potential causes have been proposed. However, genetic causes are the most likely. The particular genes that cause this kind of osteoporosis are unknown (Vanderschueren et al., 2000).

- **Hypogonadism:** Sex hormones are definitely crucial for skeletal health and development in males, particularly for adult bone health maintenance (Vanderschueren et al., 2000). Hypogonadism is linked with poor bone density; hypogonadism appears to accelerated bone remodelling and quick bone turnover, and testosterone supplementation enhances bone mass in hypogonadal males. Androgen deprivation treatment for prostate cancer is one of the common causes of severe hypogonadism; in this circumstance, bone loss is fast, and the incidence of fractures is obviously raised. Men's hormonal efficiency and androgens levels drop with age, and this reduction may be a major risk factor for age-related bone loss and fracture incidence. The strength of this alliance is still somewhat unknown (Khosla et al., 2002; Orwoll, 2003).

- **Systemic inflammatory diseases related to osteoporosis**
  The RANKL/OPG proportion is the primary factor in encouraging osteoclast formation, and as a consequence, this balance is crucial for bone mass regulation (Boyle et al., 2003; Walsh et al., 2006). T-cell expression in inflammatory conditions may result in elevated RANKL overexpression.
Cortisol, which are often used to regulate underlying inflammatory state, lower osteoblast quantity and function while also impairing OPG synthesis (Hofbauer et al., 1999). The combination of these phenomena (RANKL/OPG ratio and glucocorticoids to modulate the inflammatory response) results in considerable bone loss. These pathologies, which are mentioned further below, are considered essential causes of osteoporosis.

- **Inflammatory arthritis**: Rheumatoid arthritis (RA) is a symmetrical polyarthritic systemic inflammatory condition. This condition is characterised by four basic types of degenerative skeletal remodelling: isolated peripheral articular erosions, subchondral bone resorption, peri-articular osteopenia, and systemic osteoporosis. RA causes an increase in RANKL production from activated T cells and synovial fibroblasts that is not associated with a rise in OPG, resulting in local bone loss (articular erosions) and global bone loss (osteoporosis). The tendency of the synovial pathology in RA to promote osteoclast-mediated bone demineralization may be linked to the creation of a broad range of products by cells inside the inflamed tissue with the ability to attract osteoclast progenitors and encourage their maturation and function (Schett., 2011). These include chemokines as well as RANKL, IL-1, IL-6, IL-11, IL-15, IL-17, monocyte colony-stimulating factor, TNF-α, PG, and PTH (Walsh et al., 2005; Schett, 2011). Diarra et al. finding's provided insights on the process behind the dysregulation of bone resorption and production in this kind of inflammatory arthritis. They found that cells in inflammatory RA tissue generated dickkopf-1 (DKK-1), an inhibitor of the wingless WNT-signaling pathway, which is important for osteoblast-mediated bone production. TNF-α was also shown to be a significant activator of DKK-1 by Diarra et al., implicating this pro-inflammatory agent in both bone production and bone degradation (Diarra et al., 2007).

- **Inflammatory bowel diseases**: The pathophysiology that leads to osteoporosis in illnesses such as Crohn's disease is multifactorial. Secondary osteoporosis is caused by proinflammatory cytokines such as IL-6, IL-1, and TNF-α, as well as gastrointestinal impaired absorption.
related to disease severity or intestinal ablation, corticosteroid use, and starvation. Individuals with Crohn's disease are undoubtedly young, and the precise association with a plethora of possible harmful skeletal variables and an increased risk of fractures and osteoporosis remains unknown (Kong et al., 1999b; Gravallese et al., 2000; Romas et al., 2002).

- **Chronic obstructive pulmonary disease**
  Pro-inflammatory mediators, notably TNF-α, are the primary reason behind the pathophysiology of chronic obstructive pulmonary disorder (COPD) (Franciosi et al., 2006). Elevated inflammatory biomarkers suggest not only the seriousness of pulmonary disease but also an increased likelihood of comorbidities, most notably cardiovascular disease, diabetes, and osteoporosis (Gan et al., 2004; Sevenoaks et al., 2006). During the initial year following diagnosis, a significant frequency of osteoporosis was identified among 2,699 COPD patients from the United Kingdom based study (Soriano et al., 2005). According to research, bone mass loss seems to be connected with greater elimination of bone collagen protein degradation products, indicating a protein catabolic condition which may lead not just to bone loss, but also to loss of muscle mass and function, as well as continuous impairment (Bolton et al., 2004). An associated component is the continuous use of systemic corticosteroids, which increases the incidence of bone fractures compared to non-users (McEvoy et al., 1998).

- **Other causes of secondary osteoporosis**
  Numerous systemic disorders have been shown to raise the possibility of developing osteoporosis and, as a result, the risk of bone fragility.

  - Diabetes mellitus (DM) associated to osteoporosis: The DM related with OP is one of reduced bone cycle, with reduced bone formation being the primary cause of bone turnover (Goodman and Hori., 1984; Bouillon et al., 1995). Insulin has an anabolic impact on bone tissue, and its drop in type 1 diabetes may result in decreased bone synthesis, leading to a reduction in IGF-1 levels. In vitro studies have
also shown that prolonged exposure to high plasma glucose level, causes osteoblast malfunction, and that poor metabolic regulation has a significant detrimental influence on bone density (Goodman & Hori., 1984).

1.2.2 Mastocytosis associate to osteoporosis: In all form of this disease, the position relative of the mast cell to bone remodelling surfaces, as well as the production by this cell of a significant number of inflammatory mediators and cytokines able of regulating bone turnover, results in skeletal involvement ranging from serious osteolysis to severe osteosclerosis, with osteoporosis being among the most commonly occurring pathology in all aspects of mastocytosis (Valent et al., 2007; Barete et al., 2010). The use of corticosteroids also hastens bone loss. Bone marrow mastocytosis is a substantial "occult" source of secondary osteoporosis, approximatly 9% of men result affected by "idiopathic osteoporosis" (Brumsen et al., 2002).

1.2.2 Parathyroid disorder

The parathyroid gland is constituted of both solid and follicular tissue. Chief cells, Oxyphil cells, transitional oxyphil cells, and large clear cells are constituted the parathyroid gland solid parenchyma. The chief cells are in charge of PTH synthesis, accumulation, and secretion. PTH is released by the parathyroid chief cells as a result of a decrease in the quantity of unbound calcium in the bloodstream. Thyroid hormone is a polypeptide of 84 amino acids (Brewer et al., 1972; Cinti & Sbarbati, 1995). A membrane-bound calcium-sensing receptor with some unusual properties detects a decrease in the quantity of circulating calcium. Even with a relatively high quantity of circulating calcium, a minor decrease in serum calcium causes an almost rapid and detectable elevation in PTH levels. As a result, the calcium receptor must possess a low calcium affinity, otherwise it would be continually saturated (Ganong, 2005). PTH's major purpose is to enhance the amount of free, calcium levels in the plasma, which is important for numerous physiological
activities. PTH also reduces serum phosphate, which is a consequence. Indeed, its activities are carried out by both directly and indirectly processes. The first is dependent on the presence of the PTH receptor and its signaling pathways, while the latter is primarily related to enhanced vitamin D activation (Kifor et al., 2002; Ganong, 2005). Diseases of the parathyroid glands are an essential factor in mineral metabolism disorders. These conditions are caused by either excessive or insufficient PTH secretion. Pathological parathyroid gland diseases are generally categorised in (Rosen et al., 2013):

- Hypoparathyroidism
- Primary hyperparathyroidism
- Secondary hyperparathyroidism

**Hypoparathyroidism**
This might be either congenital or acquired, and it can be caused by reduced functioning of the gland, abnormal PTH synthesis and secretion, or a decreased tolerance to PTH. Genetic disorders that causing absence of the gland are most frequently associated to the DiGeorge sequence aberration, which entails chromosome 22q11 micro-deletion (Hiéronimus et al., 2006). Other genetic disorders cause an abnormal type of PTH, and inherited calcium-sensing receptor abnormalities may block PTH (Marx, 2000). This may impair renal calcium reuptake, leading to hypercalcuria and hypocalcaemia (Rosen et al., 2013). Iatrogenic hypoparathyroidism is the most prevalent cause. It is caused by the injury or removal of the parathyroid glands or their blood supply during a thyroidectomy or other neck surgery. The occurrence is determined by many variables, including the degree of resection, the surgeon's expertise, retro-sternal extension of the thyroid, malignancy, failure to locate one of the parathyroids intra-operatively, and the existence of Graves' disease (Shoback, 2008). Other acquired causes of hypoparathyroidism include severe hypomagnesaemia, which might be caused by gastrointestinal disorders (Rosen et al., 2013), and heavy metal accumulation within the parathyroid parenchyma, such as iron in haemochromatosis and copper in Wilson's disease.
• **Primary hyperparathyroidism**

Primary hyperparathyroidism is defined as an excess of PTH generation in a patient with normal serum calcium levels. Excess circulating PTH causes hypercalcaemia as a result of increased vitamin D activation, increased renal reuptake and intestinal calcium absorption, and excessive bone demineralization. As phosphate excretion in the urine increases, hypophosphataemia develops ([Shoback, 2008](#)). At least 80% of instances of primary hyperparathyroidism are caused by a single parathyroid adenoma ([Marx, 2000](#)). The remaining 15-20% are mostly related to parathyroid gland hypertrophy. Some of the inherited types of primary hyperparathyroidism, such as familial hyperparathyroidism and parathyroid malignancy, are among the extremely uncommon causes of primary hyperparathyroidism. The latter type accounts for just 0.5% of all instances of primary hyperparathyroidism and is often a slowly growing aggressive tumour that necessitates contralateral thyroidectomy, paratracheal tissue dissection, lymph node and thymus removal, and other procedures ([Bilezikian & Silverberg, 2000](#)). Other parathyroid diseases include hereditary primary hyperparathyroidism, which is marked by hypercalcemia and increased or non-suppressed serum PTH. Multiple endocrine neoplasia (MEN), familial (benign) hypocalciuric hypercalcemia (FHH), neonatal severe primary hyperparathyroidism (NSHPT), hyperparathyroidism-jaw tumour syndrome (HPT-JT), and familial isolated primary hyperparathyroidism (FIHPT) are among the hereditary disorders ([Bilezikian & Silverberg, 2000; Rosen et al., 2013](#)). Multiple endocrine neoplasia (MEN), familial (benign) hypocalciciuric hypercalcemia (FHH), neonatal severe primary hyperparathyroidism (NSHPT), hyperparathyroidism-jaw tumour syndrome (HPT-JT), and familial isolated primary hyperparathyroidism (FIHPT) are among the hereditary disorders ([Rosen et al., 2013](#)).

**MEN**: MEN syndromes are a group of autosomal dominant disorders where the subject develops neuro-endocrine tumours. Tumors may be non-functional or functional, in the latter hormones are secreted. MEN 1 is caused by a deletion in the MEN 1 gene, which is positioned on chromosome 11. Mutations in the c-RET proto-oncogene, which encodes RET, a tyrosine kinase protein linked to cellular membrane receptors, cause MEN 2. The most common cause of
primary hyperparathyroidism in MEN 1 is multi-nodular hyperplasia. A
gastrinoma or other entero-pancreatic tumour is another frequent tumour
identified in MEN 1. MEN 2 is distinguished by medullary thyroid cancer (MTC)
and phaeochromocytoma, and it is further split into MEN 2A and MEN 2B.
Primary hyperparathyroidism occurs in MEN 2A but not in MEN 2B, and MTC
appears later and with less aggressiveness in the former. A third subtype of
MEN 2 is familial medullary thyroid carcinoma, which is characterised by the
presence of thyroid cancer in at least four family members (Bilezikian &
Silverberg, 2000; Falchetti et al., 2008).

- Secondary hyperparathyroidism

PTH levels will rise if hypocalcaemia is caused by a condition other than
hypoparathyroidism, such as malabsorption or calcium or vitamin D
deficiency. Chronic renal failure may cause hyperparathyroidism via a more
complicated disease process, making it more severe and harder to treat than
other types of secondary hyperparathyroidism. (Bilezikian & Silverberg, 2000;
Rosen et al., 2013).

1.2.3 Rickets and Osteomalacia

When the epiphyseal lines have fused, osteomalacia affects adults due to
inadequate mineralization caused by a lack of vitamin D in the diet, whereas in
children this results in rickets. This is the most common vitamin D-related illness
(Steendijk, 1968). The active vitamin D metabolite 1,25-dihydroxyvitamin D
[1,25(OH) 2 D] increases phosphate and calcium uptake from the intestine and
makes calcium and phosphate accessible for calcification. In mild to severe
vitamin D deficiency, a decrease in the calcium concentration stimulates the
parathyroid glands. As a compensatory response, increasing serum PTH
enhances the transformation of 25-hydroxyvitamin D [25(OH)D] to 1,25(OH) 2
D. However, an increase in PTH causes bone resorption. As a result, vitamin
D insufficiency may contribute to bone loss and the development of
osteoporosis (Lips, 2001). Rickets and osteomalacia are linked to muscle
weakness, and it has recently been shown that mild and moderate vitamin D
deficiency may be linked to impaired muscle strength and collapses (Snijder et al., 2006; Wicherts et al., 2007). In vitro and in vivo research has revealed that 1,25(OH)2 D has a variety of actions, including stimulating osteoblasts and longitudinal growth, stimulating immune system development, reducing proliferation and differentiation of several types of cells, promoting insulin response and increasing glucose tolerance (Lips, 2006; Norman, 2006). Vitamin D insufficiency has been linked to autoimmune disorders such as type 1 diabetes and multiple sclerosis, infectious diseases such as TB, type 2 diabetes, cardiovascular disease, numerous kinds of cancer, and depression in recent years (Peterlik & Cross, 2005; Bouillon et al., 2008).

**Vitamin D deficiency:** Children (particularly preterm children), pregnant women, elderly, and non-Western immigrants are at high risk of vitamin D insufficiency. Decreased serum 25(OH)D levels have been recorded in young adults and adolescents, as well as children (Prentice, 2008; Mansbach et al., 2009). Low sun exposure, decreased biosynthesis or ingestion of vitamin D3 (e.g., dark skin tone), micronutrient deficiency (e.g., small-bowel illnesses), or enhanced reduction of 25(OH)D (antiepileptics) may all contribute to this (Munns et al., 2006). While 25(OH)D is the most abundant circulating product and storage of vitamin D, the active form 1,25(OH)2 D is responsible for practically all vitamin D activities. This active metabolite operates as a steroid hormone via VDR. Specific binding of 1,25(OH)2 D to the VDR in osteoblastic lineage cells results in a catabolic or anabolic impact depending on cell maturation, according to preclinical studies (Saji et al., 2010). The primary effect seems to be an increase in RANKL and a decrease in osteoprotegerin, which stimulates osteoclast production, although activation of osteoblastic genes responsible for bone formation, such as osteocalcin and osteopontin, has also been reported (Christakos et al., 2007; Goltzman, 2007). The newly generated bone of the proximal portion does not mineralize in children with vitamin D deficiency, and cartilage proliferation is extended. The growth plate thickens, widens, and becomes uneven. As a consequence, rickets is clinically diagnosed (Steendijk, 1968). The newly produced bone matrix, the osteoid, does not calcified in adults, resulting in osteomalacia. Rickets is defined clinically by slowed longitudinal development, expansion of the epiphyseal area, and painful
swelling surrounding these zones. Osteomalacia is characterised clinically by bone pain, muscle weakness, and difficulty walking (Steendijk, 1968; Rosen et al., 2013).

1.2.4 Paget Disease

Paget's disease is a skeletal localised bone remodelling condition. The disease is characterised by an increased osteoclast-mediated bone turnover, followed by corrective elevations in new bone production, resulting in a disordered featured with mix of woven and lamellar bone at afflicted bone locations. This architectural bone shift result is represented by less compact, more vascular, and more prone to deformation or fracture than healthy bone tissue (Roodman & Windle, 2005). Clinical signs and symptoms will differ across patients according to the number and area of afflicted skeletal sites, in addition to the degree and severity of aberrant bone turnover. Despite the fact that Paget's disorder is the second most prevalent illness after osteoporosis, the mechanisms involved in its development are still being studied. Paget's disease pathogenesis has been linked to both hereditary and environmental variables. Paget's disease is likely to be transmitted from generation to generation in an autosomal dominant form. Between 15% and 30% of Paget's diseased patients have a positive family history of the condition (Siris et al., 1980; Morales-Piga et al., 1995). Several predisposition loci for Paget's disease have been found in recent genome-wide association studies. These include CSF-1 gene variations, RANK gene abnormalities, PML gene permutations, and three additional genetic mutations (Albagha et al., 2010; Albagha et al., 2011). The most common alterations associated with Paget's disease are located in a locus on 5q35-QTER that encodes sequestasome-1 (SQSTM1/p62), an ubiquitin binding protein (Laurin et al., 2002). Sequestasome-1 is essential in the RANK signalling pathway. Increased bone loss predominates in the early stages of Paget's disease, and radiographs show lytic alterations. Following this, the enormous number of osteoblasts available at these places cause a mix of enhanced resorption and relatively closely linked new bone growth. The new bone formed during this phase is aberrant, likely due to the rapid nature of the process. Collagen fibres are distributed erratically rather than linearly, resulting
in much more primitive woven bone. The ultimate result is a "mosaic" pattern of woven bone with irregular pieces of lamellar bone connected in an unorganised manner by multiple cement lines, indicating the extent of earlier regions of demineralization. Abundant fibrous connective tissues and a greater number of blood vessels penetrate the bone tissue, explaining the hypervascular condition of the tissue. Over time, hypercellularity in a site of damaged bone may decrease, resulting in a sclerotic, pagetic mosaic tissue with no signs of active bone turnover, sometimes known as burned-out Paget's disease (Siris et al., 1980; Roodman & Windle, 2005).

1.2.5 Bone Metastatic

Any malignant tumour may metastasise to bone, however the most common metastatic lesions are usually associated to carcinoma type of cancers. Although any type of carcinomas may migrate to bone, the most typical are lung, breast, prostate, kidney, or thyroid carcinomas. In all cancer patients the incidence of bone metastasis has been reported as high of 70%, however this data is likely to be much higher (Ober, 1959). The most frequent tumours in males that are causing bone metastasis are those of the lung and prostate (Duchek et al., 1975). Usually, at the time of first presentation, bone marrow invasion is evident in 5 to 21% of individuals with lung cancer (Anner & Drewinko, 1977). On the other hand, the most frequent neoplasm causing bone metastasis in women is represented by the breast cancer. At the time of diagnosis, up to 21% of individuals will have bone marrow metastatic infiltration (Ingle et al., 1978). One or more destructive lesions appear in a patient with a history of cancer. In this situation, determining the presence of metastatic cancer often affects the course of the illness and necessitates critical treatment choices. After the lung and the liver, the third most frequent site of metastatic carcinoma is the bone. Although carcinomas usually spread to other organs through lymphatics, bone metastases originate as a result of cancer cells spreading hematogenously. The most prevalent sites are the vertical type of bones, notably the spine is one of the common site. Other common region of bone metastatic frequency are the pelvis and proximal femurs. These preferred locations in adults are correlate to the distribution of hematopoietic
marrow. Because the hematopoietic marrow contains open vascular sinuses, tumour cells may readily flow from the blood to the marrow. Native bone cells are always responsible for bone alterations in metastatic cancer. The most common alteration is osteolysis, which is regulated by osteoclasts. PTHrP is the most effective osteoclast important regulator generated by metastatic tumour cells. Cytokines released by cancer cells also stimulate osteoclasts (Mundy, 1987) Among them are different growth factors and PGs. Some squamous carcinomas release IL-1, which activates osteoclasts (Sato et al., 1986). The appearance of metastatic carcinoma in bone may stimulate a tumour development cycle. Osteoclastic bone resorption causes the bone matrix to produce growth factors. These substances, most likely TGF-β and TGF-1, promote tumour development and invasiveness while also activating osteoclasts (Chappard et al., 2011). Patients with metastatic cancer to the bone complain of discomfort. Discomfort may be present exclusively during exercise at first, but it quickly escalates to pain during rest. A pathologic fracture is the most common cause of sudden intense pain, which affects 25% of patients (Koskinen & Nieminen, 1973). The degree of cortical damage is associated to pathologic fracture. The growth model of neoplastic cells is a distinguishing hallmark of metastatic cancer in bone tissue. A fibrous stroma separates the cells, which are organised in compact clusters or lines. Bone metastases always suggest that the malignancy is at an advanced stage (Greenspan & Norman, 1988). As a result, therapy is focused on keeping the remaining patient's life as pleasant as possible. Bisphosphonates, especially pamidronate and zoledronate, have indeed been found to directly influence the treatment of cancer-induced osteolysis. Bisphosphonates have been demonstrated to trigger tumour cell death while also inhibiting tumour cell adherence and invasion (Morgan & Lipton, 2010). These medications successfully break the cycle of bone resorption/tumour stimulation (Lipton, 2008).

1.2.6 Myeloma and Other haematological malignancies

Multiple myeloma (MM) is an incurable plasma cell neoplastic disease characterised by monoclonal para-protein synthesis from terminally
differentiated plasma cells and lytic bone tissue damage. (Hideshima & Anderson, 2002).

MM accounts for roughly 10% of all blood cancers, with a fatality rate that has risen during the last thirty years (Jemalet al., 2001). MM is often followed by an age-dependent pre-malignant malignancy known as monoclonal gammopathy of unknown aetiology (MGUS), which affects around 3% of people over the age of 50. This syndrome is distinguished by the presence of 3 g/dL M-protein, 10% plasma cells in the bone marrow tissue, a residue or lack of urine M-protein, and the absence of anaemia, hypercalcaemia, nephropathy, and lytic bone lesions (Kyle & Rajkumar, 2005). According to epidemiological research, the probability of progression of MGUS to MM is around 1% per year, although the processes causing this change remain unknown (Kyle et al., 2002; Hideshima et al., 2004). These processes are most likely the result of a complex interaction between processes unusual to aberrant plasma cells, such as genetic alterations and the expression of soluble chemokines, which alter the bone-marrow micro - environment, influence angiogenesis, and promote the growth of this abnormal cell population. TNF-α, IL-6, IL-1β, RANKL, and macrophage inflammatory protein-1α are some of the most well-studied chemokines implicated in the pathogenesis of MM (Lust and Donovan, 1999; Kyle & Rajkumar, 2005). Pesticides, toxic substances, and ionising exposure to radiation are among the exogenous conditions linked to MM (Schwartz, 1997; Bertazzi et al., 1999). The processes by which these interactions produce multiple myeloma are, however, unknown. The malignant cell type that makes up multiple myeloma, converted plasma cells or plasmablasts, originate from post-germinal centre B cells (Hideshima et al., 2004). Under normal physiologic conditions, the plasma cell is a differentiated cells B cell that produces immunoglobulin. A B cell's development and maturation is a fairly complicated process. B-cell formation begins in the bone marrow, and extramedullary lymphatic tissues are essential for continued B-cell differentiation. When B lymphocytes are exposed to antigens inside a main lymphatic organ (i.e., a lymph node follicle), they become activated and undergo proliferation and physical hypermutation (Hideshima et al., 2004). These somatically altered B cells undergo immunoglobulin heavy chain class (IgH) switching after leaving the lymphoid organ and before migrating back to
the bone marrow, where additional plasma cell differentiation occurs, culminating in clonal selection (Roodman, 2004b). Non-random chromosomal translocation mistake have a role in the early pathogenesis of MGUS and MM. Five recurrent translocations have been documented in MGUS, MM, and plasma cell leukaemias, all implicating the IgH gene domain on chromosome 14q32. The most common structural abnormalities implicated in the aetiology of multiple myeloma are IgH translocations (Avet-Loiseau et al., 1998). Primary IgH translocations occur early in myelomagenesis and may be the starting event, while secondary translocations persist throughout disease progression (Hideshima et al., 2004). Other characteristics that have been proposed to indicate a greater likelihood of MGUS transition to MM include the existence of an IgA M-protein and an aberrant kappa to lambda serum free light chain proportion (Rajkumar et al., 2004; Roodman, 2004b; Kyle & Rajkumar, 2005). Bisphosphonates (BPs) are the current standard of therapy for MM associated to bone lesions. BPs are the current therapy of choice for managing pain associated with bone disease and preventing skeletal-related events. Treatment with oral clodronate has been demonstrated to minimise the onset of osteolytic lesions, fractures, hypercalcaemia, and bone pain in MM patients (McCloskey et al., 1998; McCloskey et al., 2001). In patients with newly diagnosed MM, in a recently published study, it was found that intravenous zoledronic acid decreased the incidence of skeletal-related events (SREs) and hypercalcaemia, and bone fractures compared to oral clodronate (Morgan et al., 2010). Denosumab (Dmb), a human monoclonal antibody that binds to RANKL with high affinity and specificity, was licensed by the FDA in 2010 for the prevention of SREs associated MM with bone lesions. A recent clinical study found that denosumab slows bone resorption and prevents SREs in individuals who are resistant to BPs treatment (Body et al., 2006; Fizazi et al., 2009). Aside from MM, numerous haematological malignancies may impact bone tissue.

**Adult T-Cell leukemia/lymphoma:** Adult T-cell leukaemia/lymphoma (ATLL) is a CD4 + T cell cancer induced by infection with the human T-lymphotropic virus type 1 (HTLV-1). Lytic bone lesions with hypercalcaemia have been associated with almost 70% of patients affected by ATLL (Taylor et al., 2005). The ATTL
cause hypercalcaemia by promoting osteoclasts development and activation and induces RANKL expression in an autocrine manner on ATLL cells (Okada et al., 2004). ATLL cells express bone remodelling by secreting chemokines such as IL-1, IL-6, TNF-α, and macrophage inflammatory proteins (MIP) 1α/1β. MIP-1α stimulation of integrin-mediated adherence to the endothelium allows circulating ATLL cells to infiltrate a number of organs (Raza et al., 2010). MIP-1α is critical for the chemotaxis of monocytes, osteoclast progenitor cells, and the generation of osteoclastogenic substances IL-6, PTHrP, and RANKL by osteoblasts or stromal cells in ATLL, as it is in MM (Tanaka et al., 2000; Han et al., 2001). Bone involvement has seldom been observed in more conventional types of acute lymphoid leukaemia and is considered to be caused similarly by malignant cell PTHrP synthesis (Inukai et al., 2007).

Non-Hodgkin’s lymphoma (NHL): In NHL bone lesions are uncommon. Only around 10% of NHL cases have bone lesions. Histiocytic and poorly differentiated NHL are the most prevalent histologic subtypes of NHL that present with bone symptoms. PTHrP serum levels are high in NHL subjects with hypercalcaemia, as they are in ATLL (Firkin et al., 1996).

Hodgkin’s lymphoma (HD): Bone lesions in HD are infrequent and rarely seen at diagnosis. Anatomical regions commonly associated to HD are: spine, pelvis, femur, humerus, ribs, sternum, scapula, and base of the skull (Borg et al., 1993). Fibrosis and a heterogeneous inflammatory infiltration with uncommon, unusual cells are common findings in bone biopsies. In HD patients, bone disease may be lytic, blastic, or mixed. Elevated new bone synthesis occurs in areas of osteoclastic activity due to tumour cell promotion of osteoblast activity. Hypercalcemia commonly manifested in HD and is linked with lymphoma cell overproduction of 1,25(OH) 2 vitamin D 3 or PTHrP. (Franczyk et al., 1989; Seymour & Gagel, 1993).

1.2.7 Osteogenesis Imperfecta
The congenital connective tissue condition osteogenesis imperfecta (OI) is characterized by impaired bone fragility and grave osteoporosis.
Dentinogenesis imperfecta, scoliosis, low height, atypically coloured sclera, hearing loss, and connective tissue's laxity are common manifestation of the illness (Rauch & Glorieux, 2004; Cheung & Glorieux, 2008). In the early 80s, OI was linked to genetic abnormalities in type I collagen genes caused by aberration of COL1A1 and COL1A2 genes (Chu et al., 1983). While type I collagen mutation was proven in only certain rare instances (Cohn et al., 1990; Edwards et al., 1992), the presence of recessively inherited subtypes of OI had to be inferred in others where no collagen primary sequence anomalies were obvious. It is known that type I collagen mutations do not cause 10-15% of all OI cases, and some of these are recognised by others due to specific bone phenotypic caratheristics (Glorieux et al., 2002), clinical presentation (Glorieux et al., 2000), or because they have linked to a genetic location that does not comprise type I collagen genes (Labuda et al., 2002). It took a long time to locate the initial gene accountable for certain recessive OI cases (Morello et al., 2006). OI is a rare condition that affects one in every 15,000 to 20,000 births (Forlino et al., 2011). The prevalence of type I OI could range from 2.35 to 4.7 per 100,000 people globally. The majority of the time, it is caused by mutations in the COL1A1 and COL1A2 genes. Recently, many abnormalities associated with OI have been identified (Valadares et al., 2014). According to the International Society of Skeletal Dysplasias, OI is classifiable on inheritance mode and genes implicated as follows (Warman et al., 2010):

a) Type I - Non-deforming (autosomal dominant) - associated to mutations of COL1A1, COL1A2 / X-linked / PLS3
b) Type II - Perinatal (autosomal dominant and recessive) - associated to mutations of COL1A1, COL1A2, CRTAP, LEPRE1, PPIB, BMP1
c) Type III - Progressively deforming (autosomal dominant and recessive) - associated to mutations of COL1A1, COL1A2, CRTAP, LEPRE1, PPIB, FKBP10, SERPINH1, SERINF1, WNT1
d) Type IV - Moderate - (autosomaldominant and recessive) - associated to mutations of COL1A1, COL1A2, CRTAP, FKBP10, SP7, SERPINF1, WNT1, TMEM38B
e) Type V - Calcification of interosseous membrane or hypertrophic callus (autosomal dominant) - associated to mutations of IFITM5

Histopathologically, OI is indicated by abnormalities in collagen type 1 secretion or aberrant collagen secretion, which results in inadequate osteoid formation (Hoyer-Kuhn et al., 2015). Endochondral and intra-membranous ossification are also impacted. Thin, disorganised proliferative and hypertrophic zones, as well as a thinning calcified zone, are characteristic histological findings (Doty & Mathews, 1971).

Other skeletal disorders resembling OI that take place in the differential diagnosis of OI are: Hypophosphatasia (mild to severe bone fragility/deformity; low alkaline phosphatase activity, autosomal recessive, autosomal dominant inheritance, ALPL genetic defect); Idiopathic hyperphosphatasia (severe bone fragility/deformity, raised alkaline phosphatase activity, autosomal recessive inheritance, TNFRSF11B genetic defect); Bruck syndrome (moderate to severe bone fragility/deformity, congenital joint contractures, autosomal recessive inheritance; telopeptide lysyl hydroxylase deficiency); Cole-Carpenter syndrome (severe bone fragility/deformity, craniosynostosis, unknown genetic defect and inheritance) (Rauch & Glorieux, 2004).
CHAPTER 2 BONE TARGETING DRUG THERAPY

2.1 Anti-resorptive medications

BPs are a family of drugs commonly used to treat a range of bone disorders, including bony complications of MM, metastatic malignancy, OP, giant cell tumours, and Paget’s disease (Spardinas et al., 1998; Coleman and McCloskey, 2011). Studies have reported that the Dmab could be more effective than BPs in reducing the incidence of, and delaying the time to, SREs (Peddi et al., 2013). In addition, Dmab can also be used for postmenopausal osteoporosis disease resulting in sustained reduction of bone turnover markers with an increase of bone mineral density (Sutton and Riche, 2012).

2.1.1 Pharmacology: Pharmacodynamics and pharmacokinetic

In the past, BPs were originally called di-phosphonates. They have been known since the middle of the 19th century when they were predominantly used for industrial proposes (mainly in the textile and oil industries) because of their property of inhibiting calcium carbonate precipitation (Fleisch, 2002). Bisphosphonate is a family of drugs, which are stable analogues of inorganic pyrophosphate. Its stability is determined by a carbon atom replacing the oxygen atom that links the two phosphates (Russell, 2011).

BPs have two phosphonate groups that share a common carbon atom (P-C-P). Consequently, BPs are not transformed to metabolites in the body and are excreted unchanged. The two-phosphonate groups have a dual function: they bind to bone mineral and also have an anti-resorptive activity. Modifications of these phosphonate groups can radically decrease the affinity of the BP for bone mineral, as well as reduce biochemical potency. The R1 and R2 side-chains attached to the carbon atom are responsible for a variety of activities, (Fleisch, 2002, Cremers and Papapoulos, 2011), (Figure 2.1).
BPs are classified in two groups according to the presence or not of nitrogen group. The Non-Nitrogen bisphosphonates group, such as etidronate, clodronate and tiludronate, has low anti-resorptive potency and inhibits the osteoclast function via intracellular metabolism to toxic ATP-metabolites (Cremers and Papapoulos 2011; van Beek ER et al., 2003). The Nitrogen bisphosphonates group, such as alendronate, ibandronate, olpadronate, pamidronate, risedronate, and zoledronate, is more potent inhibitors of osteoclastic bone resorption and inhibits farnesyl pyrophosphate synthase (FPS) a key enzyme of the mevalonate biosynthetic route. The suppression of this enzyme causes a disruption in the cytoskeleton organisation of the osteoclast, leading the apoptosis of these cells (Russell, 2011; Cremers and Papapoulos 2011, van Beek ER et al., 2003).

Bisphosphonates can be classified according to the route of administration: oral, intravenous (IV), or intramuscular (IM). Oral bisphosphonates such as, risedronate, alendronate, tiludronate and etidronate, are usually taken weekly. The intravenous bisphosphonate such as pamidronate and zoledronic acid are usually administered monthly. Some other BPs such as ibandronate and clodronate can be administered both orally and intravenously (Russell, 2011).

Under ideal conditions, less than 1% of an oral dose is absorbed and, if this is taken with food, the absorption can be completely blocked. 50% of the absorbed dose binds avidly to bone surfaces; the kidneys excrete the remaining non-bind part. The plasma half-life can vary between five to eleven days according to the bisphosphonate (Lasseter et al., 2005).

The skeletal half-life for the BPs integrated into bone is extremely long (over 10 years) (Russell, 2011).

BPs have two main biological properties: inhibition of calcification, when given at high doses, and inhibition of bone resorption.
These numbers are however subject to changeability according to individuals. Individuals with high bone turnover rates preserve a greater dose in their skeleton and consequently have lower renal excretion rates (Mitchell et al., 2001; Cremers et al., 2003). The mechanisms of action of the BPs are complicated and multifactorial. The side chains influence the binding affinity (R1 side chain) and the anti-resorptive potency (R2 side chain) (Green et al., 1994). Different types of bisphosphonates have differing affinities to bone with the rank order from greatest to least being zoledronate, alendronate, ibandronate, risedronate, etidronate and clodronate (Table 2.1). Once in the bone they directly affect the bone resorption by increasing the apoptosis of osteoclasts. The net effect of this is to reduce bone resorption and bone turnover. Angiogenesis is reduced by depression of blood flow and a marked decrease in vascular endothelial growth factor. Epithelial keratinocytes are also repressed by BPs. The effect of these activities is to reduce healing capacity (Drake et al., 2008; Cheng et al., 2009).

<table>
<thead>
<tr>
<th>Pharmacologic active ingredient</th>
<th>Formulation</th>
<th>Route of administration</th>
<th>Target therapy</th>
<th>Relative Potency</th>
<th>Indication and frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alendronic acid (sodium salt)</td>
<td>Tab 70 mg</td>
<td>Oral</td>
<td>Osteoclast inhibition</td>
<td>1,000</td>
<td>Treatment of osteoporosis (70 mg/week); Treatment and prevention of osteoporosis induced by glucocorticoids (70 mg/week)</td>
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<td></td>
<td>Tab 10 mg</td>
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<tr>
<td>Ibandronic acid (monosodium salt monohydrate)</td>
<td>Tab 50 mg</td>
<td>Oral</td>
<td>Osteoclast inhibition</td>
<td>1,000</td>
<td>Prevention of SREs in breast cancer patients with bone metastases (50 mg/day p.o. or 6 mg every 3–4 weeks iv.)</td>
</tr>
<tr>
<td></td>
<td>Bit 6 mg/6 ml</td>
<td>IV</td>
<td></td>
<td></td>
<td>Treatment of hypercalcemia of malignancy; Treatment of postmenopausal osteoporosis in patients at high risk of fracture (150 mg/4 weeks p.o. or 3 mg every 3 months iv.)</td>
</tr>
<tr>
<td></td>
<td>Tab 150 mg</td>
<td>Oral</td>
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<td></td>
<td>Bit 3 mg/3 ml</td>
<td>IV</td>
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</tr>
<tr>
<td>Neridronate acid (sodium salt)</td>
<td>Bit 25 mg/2 ml</td>
<td>IV/IM.</td>
<td>Osteoclast inhibition</td>
<td>-</td>
<td>Osteogenesis imperfecta (2 mg/kg/3 months); Paget’s bone disease (different schedules)</td>
</tr>
<tr>
<td></td>
<td>Bit 100 mg/8 ml</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pamidronic acid (disodium salt)</td>
<td>Bit 15 mg/5 ml</td>
<td>IV</td>
<td>Osteoclast inhibition</td>
<td>1,000-5,000</td>
<td>Prevention of SREs in breast cancer patients with bone metastases or MM with bone lesions (60–90 mg every 3–4 weeks); Treatment of hypercalcemia of malignancy</td>
</tr>
<tr>
<td></td>
<td>Bit 30 mg/10 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bit 60 mg/10 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bit 90 mg/10 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risedronic acid</td>
<td>Tab 35 mg</td>
<td>Oral</td>
<td>Osteoclast inhibition</td>
<td>1,000</td>
<td>Treatment of postmenopausal osteoporosis (35 mg weekly or 5 mg daily); Treatment and prevention of osteoporosis induced by glucocorticoids (35 mg weekly or 5 mg daily); Treatment of Paget’s disease</td>
</tr>
<tr>
<td></td>
<td>Tab 5 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zoledronic acid (monohydrate)</td>
<td>Bit 4 mg/5 ml</td>
<td>IV</td>
<td>Osteoclast inhibition</td>
<td>10,000+</td>
<td>Prevention of SREs in cancer patients with bone metastases or MM (4 mg every 3–4 weeks); Treatment of hypercalcemia of malignancy</td>
</tr>
<tr>
<td></td>
<td>Bit 5 mg/100 ml</td>
<td>IV</td>
<td></td>
<td></td>
<td>Treatment of osteoporosis (5 mg once per year); Treatment of bone Paget’s disease</td>
</tr>
</tbody>
</table>

Table 2.1 Bisphosphonates: indication, dose and potency.
2.1.2 Bisphosphonate: biochemical mechanism of action

The first generation of bisphosphonate is the non-nitrogenous bisphosphonates. This bisphosphonate family of drug is considered the least effective one. Metabolically, they are incorporated as cytotoxic adenosine triphosphate (ATP) analogues, which deposit in the osteoclasts’s cytoplasm. These analogues inhibit the intracellular ATP-dependent enzymes, instigating the disruption of the mitochondrial membrane potential, hence promoting the osteoclast apoptosis (Frith et al., 2001; Lehenkari et al., 2002).

The nitrogenous bisphosphonates are predominantly deposited in the bone where they inhibit the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAR) biosynthetic pathway, resulting in the inhibition of both the enzyme farnesyl diphosphate (FPP) synthase and geranylgeranyl diphosphate (GGPP) synthase. As results the prenylation of guanosine triphosphatases (GTPases) is impeded (Fisher et al., 1999; Bergstrom et al., 2000).

The GTPases are responsible for cytoskeletal regulation, cell proliferation and apoptosis. Moreover, bisphosphonates inhibit crystal aggregation, and delay crystal dissolution, by binding with calcium in hydroxyapatite crystals. (Fleisch et al., 1969; Bifulco 2005).

2.1.3 Bisphosphonate: cellular effects

The bisphosphonate mechanism of action is expressed at cellular level with significant alteration promoted biochemically. Bisphosphonates control the activities of numerous cells, including bone cells, angiogenesis, and tumourgenesis. A bisphosphonate key action is linked directly to the osteoclasts. The inhibition of osteoclasts is one of the most studied in literature. One of the bisphosphonate cellular effects is to impair the recruitment, differentiation and activity of osteoclast including their apoptosis (Fleisch, 1998; Santini et al., 2003).

During bone resorption, bisphosphonates are released from the bone matrix by acidification, and absorbed by the osteoclasts through endocytosis (fluid phase)
Many studies have reported that osteoclast structural changes in response to bisphosphonates include: severe cytoplasmic retraction, an increased number of nuclei, altered actin rings, fewer vacuoles and fewer microtubules (Selander et al., 1994; Murakami et al., 1995; Endo et al., 1993; Hiroi-Furuya et al., 1999). Changes have been observed in the cytoplasmic organelles, such as: golgi apparatus, rough endoplasmic reticulum and lysosomes, affecting the osteoclast function. Other studies have shown an inhibition of the osteoclastogenesis in response to bisphosphonates. Moreover, it has been suggested that BPs reduce osteoclastic bone adhesion in vitro particularly, affecting the osteoclasts function by reducing the sealing zone and decreasing the size and depth of resorption pits (Cecchini et al., 1987; Hughes et al., 1989; Colucci et al., 1998; Suzuki et al., 2006). The apoptosis is another effect of bisphosphonates on osteoclast function. Such process has been observed both in in-vitro and in an in-vivo studies. However, the mechanism by which this process occurs remains still unclear (Miller & Jee, 1979; Hughes et al., 1995; Selander et al., 1996). Bisphosphonates have also an impact on the osteoblast; it has been reported that they cause impairment on osteoblastogenesis, osteoblast function and osteoblast apoptosis. The effect of these drugs on osteoblasts is mainly dependent on the concentration of the exposure (Tsuchimoto et al., 1994; Endo et al., 1996).

The outcome these studies should be considered carefully, because conducted in-vitro and thus further studied are needed in vivo. These studies have reported that at higher bisphosphonate concentrations, osteoblast function is inhibited. This inhibition includes: cell growth, proliferation, mineralisation, migration and adhesion (Tsuchimoto et al., 1994; Pan et al., 2004; Idris et al., 2008; Walter et al., 2011). Apoptosis of osteoblasts has been linked to bisphosphonates and observed in many studies (Naidu et al., 2008).

In addition, bisphosphonates have been confirmed to inhibit osteocyte apoptosis, both in in-vitro studies and in an animal model (Plotkin et al., 1999; Follet et al., 2007).
One of the most important actions performed by both non-nitrogenous and nitrogenous bisphosphonates is to be holding anti-angiogenic effects in-vitro. They are able to inhibit the proliferation and the migration of endothelial cells also causing apoptosis. In-vivo studies have also established the anti-angiogenesis effect (Fournier et al., 2002; Scavelli et al., 2007; Ribatti et al., 2008).

Another important attribute of bisphosphonates is to have anti-neoplastic effects. Recent studies, both in vitro and in vivo, have described a direct effect on the cancer cell adhesion, invasion and viability, with pro-apoptotic properties on cancer cells. Important biochemical factors such as TGF-β and IGF-1 are released from bone matrix up on osteoclastic resorption. The inhibition of the osteoclastic activity, deprive also tumour cells by these essential growth factors (Santini et al., 2003; Cheng et al., 2004).

2.1.4 Denosumab

Denosumab (Dmab) is a fully human immunoglobulin G2 monoclonal antibody with high affinity and specificity for RANKL. By binding to RANKL, denosumab inhibits RANKL from activating its only receptor RANK on the surface of osteoclasts and their precursors (Tometsko et al., 2004).

Prevention of RANKL-RANK interaction inhibits osteoclast formation, function and survival thereby decreasing bone resorption and interrupting cancer-induced bone destruction. Dmab has the same physiological effects on RANKL as endogenous OPG, demonstrating rapid inhibition of bone resorption but with reversible effect (Bekker et al., 2004).

Dmab follows non-linear, dose-dependent pharmacokinetics. The bioavailability of one subcutaneous denosumab injection is 61% and serum concentrations are detected within 1 hour. Maximal serum concentrations are achieved in 5-21 days and denosumab may be detectable for 9 months or longer. Based upon monoclonal antibody pharmacokinetics, denosumab is most likely cleared by the reticuloendothelial system with minimal renal filtration and excretion. The elimination half-life of denosumab is 32 days and the terminal half-life is 5-10 days and it does not incorporate into bone (Tsourdi et al., 2011; Narayanan 2013).
Numerous clinical and pre-clinical studies have shown that RANKL antagonists prevent osteolysis with a significant reduction in skeletal tumour burden (Roodman and Dougall, 2008; Tsourdi et al., 2011). In these studies, the combination of RANKL inhibition with hormonal therapy and/or chemotherapy resulted in significantly greater inhibition of skeletal tumour growth than either single agent alone in the breast, prostate or lung cancer models examined, respectively (Tometsko et al., 2004). The indications for denosumab are similar to the ones for bisphosphonates (Moen et al., 2011).

2.1.5 Rosomazumab

After the discovery that sclerostin deficiency causes rare genetic conditions that are characterised by high bone mass and resistance to fracture, sclerostin became a therapeutic target for the treatment of osteoporosis (Balemans et al., 2001; Brunkow et al., 2001). Sclerostin, a negative regulator of bone formation that is secreted by osteocytes, inhibits WNT signaling down-regulating this stimulus for osteoblast development and function (Li et al., 2005; Poole et al., 2005). Romosozumab is a monoclonal antibody that binds and inhibits sclerostin, with a dual effect of increasing bone formation and decreasing bone resorption (McClung et al., 2014). In a recent randomised clinical trial on osteoporosis involving 7,180 postmenopausal women randomly assigned to receive subcutaneous injections of romosozumab (at a dose of 210 mg) or placebo, it was found that the drug was associated with a lower risk of vertebral fracture at 12 months. Two cases of osteonecrosis of the jaw were observed in the romosozumab group (Cosman et al., 2016).

2.2 Antiangiogenic medications

Angiogenesis is defined as the formation and growing of new blood vessels through the secretion of several mediators including vascular endothelial growth factors (VEGF), endothelial-1, platelet-derived growth factors (PDGF), basic fibroblast growth factors (FGF), angiopoietins, erythropoietin, interleukine-8 and placenta growth factors (PIGF) (Al-Husein et al., 2012).
In the 1970s Judah Folkman, an American researcher, started to observe that in the absence of neovascularisation, cancerous cells could not grow exponentially (Folkman, 1971). This observation laid the basis for antiangiogenic cancer drug therapy (Wu et al., 2008; Hwang and Heath, 2010).

A variety of angiogenesis inhibitors are now licenced for the treatment of various cancers as well as other eye illnesses. As a consequence, an increasing number of patients with metastatic solid tumours have been treated with these medicines (De Falco, 2014). Antiangiogenic drugs classified into two groups (Al-Husein et al., 2012; Falcon et al., 2016):

1. Tyrosine kinase inhibitors (TKIs).
2. Protein biological agents (PBAs).

TKIs are pharmacologic drugs that decrease receptor tyrosine kinase activity. The majority are small molecule with strong inhibitory action at the VEGFR-2 intracellular domain's catalytic binding site. Sunitinib, sorafenib, and pazopanib, among the first generation of antiangiogenic TKIs, have a broader spectrum of kinase targets, including PDGFRs, receptor tyrosine kinase (c-Kit) inhibitors, and epidermal growth factor receptor (EGFRs) (Al-Husein et al., 2012; Falcon et al., 2016). PBAs, the second medication type, contains monoclonal antibodies (MABs) like bevacizumab and ziv-aflibercept. The first operates by modifying the action of vascular endothelial growth factor A (VEGF-A; VEGF hereafter), while the latter is a recombinant fusion protein that allows it to act as a VEGF receptor decoy (VEGF-trap), binding VEGF, VEGF-B, and placental growth factor (PIGF) (Falcon et al., 2016). VEGF-trap (aflibercept) medications are recombinant decoy receptor fusion proteins produced by fusing domains 2 and 3 of vascular endothelial growth factor receptor-1 (VEGFR-1). These bind to or "tras" the various isoforms of VEGF, as well as VEGF-B and PIGF, inhibiting neo-angiogenesis. There are other type of antiangiogenic drugs called mammalian target of rapamycin (mTOR) inhibitors. mTOR inhibitors seem to influence VEGF and platelet-derived growth factors (PDGF) expression, leading to vascular antiproliferative effects (Del Bufalo et al., 2006; Moriya et al., 2014).
2.2.1 Pharmacology: Pharmacodynamics, Pharmacokinetic and 
Mechanism of Action

The discovery of medications that target angiogenesis by inhibiting VEGF and 
their receptors (VEGFR) in the past few decades has resulted in a significant 
shift in the area of cancer. Antiangiogenic medicines work in various ways to 
target the same pathway. The VEGF/VEGFR pathway is one of the most 
important in angiogenesis. There are five members of the VEGF family: VEGF-
A, B, C, D, and PIGF (Roskoski, 2017). VEGFR-1 (like tyrosine kinase FLT-1, 
gene name FLT1) and VEGFR-2 (kinase insert domain receptor KDR, gene 
name KDR) are mostly engaged in angiogenesis and neovascularization, 
respectively; VEGFR-3 (like tyrosine kinase FLT-4, gene name FLT4) is 
primarily involved in lymphovascular invasion. VEGF-A may activate VEGFR-
1, VEGFR-2, or neuropilin-1 (NRP1), with the latter receptor also being involved 
in proliferation and vascular permeability (Shibuya, 2011). VEGF-A is a 45 kDa 
glycoprotein released by a wide range of human malignancies. It is a 
proliferative peptide that is unique to endothelial cells and is activated by 
hypoxia. It also causes migration, differentiation, vascular permeability, and the 
mobilisation of endothelial progenitor cells (Hicklin and Ellis, 2005). VEGF 
receptors are transmembrane tyrosine kinase receptors: the ligand promotes 
their dimerization, which auto-phosphorylates and transduces the extracellular 
signal through either a mitogen activated protein (MAP) kinase or a 
phosphoinositide 3-kinase/mammalian target of rapamycin (PI3K/mToR) 
pathway to enhance the proliferation and survival of the endothelial cells 
(Hicklin and Ellis, 2005). Monoclonal antibodies and their derivatives are 
around 150 kDa in size and do not pass the cell membrane. This medication 
class has a wide range of targets. Bevacizumab, a recombinant humanised 
monoclonal antibody IgG1 targeting all isoforms of circulating VEGF-A, is one 
example. Aflibercept is another MAB with a larger range of activity than 
bevacizumab. This medication is a recombinant protein that is the fusion of a 
VEGFR-1 and a VEGFR-2 extracellular domain part to a crystallizable fragment 
(Fc) of immunoglobulin and is capable of "trapping" circulating VEGFR-1 and 
VEGFR-2 ligands, notably VEGF-A and VEGF-B. (Holas
Papadopoulos et al., 2012). Aflibercept was created with a larger range of activity in mind than bevacizumab. It is a recombinant protein composed of a VEGFR-1 and a VEGFR-2 extracellular region fused to an immunoglobulin crystallizable fragment (Fc). Aflibercept may thereby "capture" circulating VEGFR-1 and VEGFR-2 ligands, such as VEGF-A, VEGF-B, and PIGF, which are responsible for the bulk of angiogenesis mechanisms (Holash et al., 2002; Papadopoulos et al., 2012). PKIs specifically target the VEGFR family, namely VEGFR-1 and VEGFR-2. They also frequently suppress factors of the PDGFRs family, such as PDGFR- (Davis et al., 2011). PDGF, a PDGFR ligand, stimulates the proliferation of mesenchymal cells, notably fibroblasts and smooth muscle cells of arteries. Imatinib, a PDGFR-inhibitor, may have anti-vascular/anti-angiogenic properties by targeting vascular pericytes inside smooth muscle cells (Rocha et al., 2007; Ruan et al., 2013). Similarly, fibroblast growth factor (FGF) and its receptors (FGFRs) influence blood vessel development (Presta et al., 2005). Because of their large molecular weight, anti-cancer MABs have a different pharmacokinetic profile than PKIs. Their spread is gradual, and their distribution volume is modest. They, just like many other therapeutic IgG, have a half-life of around 20 days and are delivered intravenously. The reticuloendothelial system degrades them into amino acids and peptides by circulating phagocytic cells (Newsome and Ernstoff, 2008). PKIs' bioavailability varies according to their solubility, passive permeability, and interaction with carrier-mediated transporters. The quantity of distribution is the apparent volume in which the medicine is disseminated. A medication diffuses differently in various tissues based on its lipophilic-hydrophilic balance. PKIs are mostly metabolised by the liver through phase 1 (oxido-reduction processes primarily involving CYP3A4) and phase 2 reactions (glucosyl conjugation). The majority of PKIs have a very low renal clearance. As a result, dosage modification based on renal impairment is seldom necessary for most PKIs (Gougis et al., 2017).

Table 2.2 and Table 2.3 summarise the pharmacological characteristics of the antiangiogenic drugs.
Table 2.2 Monoclonal antibodies pharmacological characteristics

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Bevacizumab</th>
<th>Aflibercept (Ziv-Aflibercept)</th>
<th>Ramucirumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Circulating VEGF A</td>
<td>Circulating VEGF A, VEGF B and PIGF</td>
<td>Tumoral VEGFR-2</td>
</tr>
<tr>
<td>Type of MAB</td>
<td>Humanized IgG1</td>
<td>Combination of VEGFR receptor and Fc region</td>
<td>Humanized IgG1</td>
</tr>
<tr>
<td>Volume of distribution (L)</td>
<td>2.88</td>
<td>5.5</td>
<td>15</td>
</tr>
<tr>
<td>Terminal half-life (days)</td>
<td>19.6</td>
<td>15</td>
<td>5.5</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Reticuloendothelial system</td>
<td>Reticuloendothelial system</td>
<td>Reticuloendothelial system</td>
</tr>
<tr>
<td>Dose adaptation if renal or hepatic impairment</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2.3 Protein kinase inhibitors pharmacological characteristics

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Sorafenib</th>
<th>Sunitinib</th>
<th>Pazopanib</th>
<th>Vandetanib</th>
<th>Axitinib</th>
<th>Regorafenib</th>
<th>Cabozantanib</th>
<th>Nintedanib</th>
<th>Lenvatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability (%)</td>
<td>43</td>
<td>NR</td>
<td>26</td>
<td>NR</td>
<td>52</td>
<td>69</td>
<td>Unknown</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>Volume of distribution (L)</td>
<td>~200</td>
<td>~2000</td>
<td>~10</td>
<td>~4000</td>
<td>~200</td>
<td>NR</td>
<td>~300</td>
<td>~1000</td>
<td>~100</td>
</tr>
<tr>
<td>Active metabolite</td>
<td>No</td>
<td>N-desethyl-sunitinib</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>N-oxide, N-desmethyl 14-70</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Terminal half-life (hours)</td>
<td>25-48</td>
<td>41-93</td>
<td>31</td>
<td>216-247</td>
<td>2.5-6.1</td>
<td>14-70</td>
<td>55-100</td>
<td>12-14</td>
<td>28</td>
</tr>
<tr>
<td>Main Metabolism</td>
<td>Hepatic</td>
<td>Hepatic</td>
<td>Hepatic</td>
<td>Hepatic</td>
<td>Hepatic</td>
<td>Hepatic</td>
<td>Hepatic</td>
<td>Hepatic</td>
<td>Hepatic</td>
</tr>
<tr>
<td>Excretion in urine in %</td>
<td>19</td>
<td>16</td>
<td>4</td>
<td>36</td>
<td>23</td>
<td>19</td>
<td>27</td>
<td>&lt;1</td>
<td>25</td>
</tr>
</tbody>
</table>

2.3 Novel and other drugs causing osteonecrosis

Besides antiresorptive and antiangiogenic medicines, King et al. emphasised the possibility of additional medications to cause MRONJ (King et al., 2019). Furthermore, recent systematic studies performed by Sacco et al. 2020 and 2021 have emphasised the possible relationship between tumour necrosis factor-alpha (anti-TNF-α) and recreational substances with MRONJ (Sacco et al., 2020; Sacco et al., 2021a). Past medications, such as immunosuppressant drugs, radiopharmaceuticals, and selective oestrogen receptor modulators (SERMs), have also been linked to the onset of ONJ, according to King et al. (King et al., 2019). Radiopharmaceuticals are generally categorised based on the kind of radiation they emit: alpha particles, beta particles, gamma rays, or positron rays. Radiopharmaceuticals have a variety of applications, including the research and imaging of a wide range of benign and malignant disorders. They may be taken orally or intravenously, and they function by binding to reactive areas in the bone tissue (Baumann et al., 2005). They direct highly concentrated radiation (typically beta particles or gamma rays) to the
appropriate areas (Wieder et al., 2014). Radiopharmaceuticals have been widely used in the localization and management of bone metastatic tumors in conjunction with chemotherapy, when required (Baumann et al., 2005). MRONJ has been linked to radium 223, but other radiopharmaceuticals used to treat bone metastases, such as rhenium 186, strontium 89, samarium 153, and radium 222, have not been directly linked to the disease (King et al., 2019). Radium 223 in particular is a radiopharmaceutical agent that generates alpha particles. The medicine works on calcium hydroxyapatite in bone and localises in regions of osteoclast activity, allowing it to treat metastases at various body locations (Wieder et al., 2014). The proof of MRONJ development in radium 223 treatment patients is weak; nevertheless, a cross-disciplinary team leader evaluation in 2013 identified MRONJ as a possible consequence. This review's results were developed by assembling data from several trials (1 phase III, 3 phase II, 3 phase I, expanded access trials). MRONJ has been recorded in four individuals who received radium 223; however, all four patients also had bisphosphonate treatment. According to current research, radium 223 may have an additional impact when paired with other bone-modulating medications in the onset of MRONJ (U.S. National Library Clinicaltrials.gov., 2016).

SERMs are oestrogen receptor partial agonists used to preserve bone mass in postmenopausal women. They are used to preventing breast cancer as well as treat osteoporosis because they function as oestrogen receptor antagonists in breast tissues (An, 2016). SERMs influence bone turnover by inhibiting osteoclast activity, lowering bone resorption as a consequence of their mode of action as oestrogen receptor agonists (Hadji, 2012). However, there has only been one recorded occurrence of ONJ in a patient using raloxifene. However, the patient had a history of oral bisphosphonate usage in addition to other comorbidities (Baur et al., 2015). Methotrexate (MTX), an immunosuppressant used as a first-line systemic therapy for a variety of autoimmune disorders such as RA, psoriatic arthritis, and severe Crohn's disease, has been linked to the development of MRONJ. MTX has been shown to alter bone turnover by reducing osteoblast growth as well as the creation of new osteoclasts. The impact of MTX on bone turnover, in addition to its immunosuppressive effects, might explain the development of MRONJ in MTX patients (Wheeler et al., 1995; Anüsssek et al., 2012). The addition of MTX to bisphosphonate
medication has long been known to increase the risk of jaw necrosis. Recently, a case published by Aghlaoo et al. and two cases reported by Henien et al. shown that ONJ may arise spontaneously or as a consequence of tooth extraction in patients receiving MTX solo treatment (Aghaloo et al., 2017; Henien et al., 2017). Corticosteroids and other immunosuppressive medicines have also been related to ONJ. Their mechanism seems to be complex (Sacco et al., 2021d). Corticosteroids have been proven to inhibit VEGF synthesis and hence have a direct influence on angiogenesis (Takano-Murakami et al., 2009). Corticosteroids inhibit osteoclast and osteoblast precursor recruitment, resulting in early apoptosis. Researches have reported solitary incidences of non-healing mandibular premolar sites following tooth extraction in long-term steroid users who did not have any other MRONJ-associated drugs (Nisi et al., 2014; Wong et al., 2015). TNF-α inhibitors are novel drugs that attach to TNF receptors, particularly to less the strong inflammatory response caused by autoimmune disorders. Early clinical studies with infliximab, adalimumab, and etanercept found no significant risks of osteomyelitis (OSM) or osteonecrosis of the jaw (ONJ) (Weinblatt et al., 2003; Di Fede et al., 2016). However, a recent systematic analysis conducted by Sacco et al. indicated that a group of individuals exposed to only anti TNF-α medications might develop osteonecrosis in the absence of typical MRONJ risk factors (Sacco et al., 2020). Nonetheless, the author’s assertion was based on poor-quality data with a significant risk of bias and indicating overall remission of ONJ lesions in more than 80% of individuals. This research also discovered that in some situations, TNF-α inhibitor medication was deliberately stopped and then resumed after the intraoral lesion had completely resolved (Sacco et al., 2020). Furthermore, the same author conducted a systematic research highlighting the probable link between several forms of illegal substances and MRONJ (Sacco et al., 2021a). It is commonly known that various recreational drugs operate as a dopamine, serotonin, and norepinephrine reuptake inhibitor, resulting in neurotransmitter dysregulation. Indeed, serotonin has been investigated and connected to bone mass and morphological regulation (Rosen., 2009). According to Sacco et al., the frequent medication associated to MRONJ was 'Krokodil,' the major component of which is desomorphine. The review determined that the possible cause of MRONJ produced by the 'Krokodil' drug was most likely caused by the
use of certain chemicals utilised in the illegal drug's manufacture (e.g., hydriodic acid and red phosphorus) (Allen and Cantrell, 1989; Kunalan et al., 2012; Sacco et al., 2021a). As a result, phosphorus was assumed to be in the final product. As a result, it was proposed that red phosphorus might initiate and cause MRONJ (Poghosyan et al., 2014). The review was based on poor-quality data with a significant risk of bias, which might be attributed to a lack of ethics in clinical trial conduct.

2.4 Clinical use of the bone targeting drugs

Antiresorptive and antiangiogenic drugs are widely used medication. Their clinical use is mainly related to manage malignant bone disease (multiple myeloma and metastatic solid cancer), osteoporosis, giant cell tumours and Paget disease.

2.4.1 Bisphosphonates and denosumab in cancerous bone disease

Bisphosphonates are commonly used to reduce the risk of skeletal complications of multiple myeloma and metastatic solid cancer, which include pain, pathological fractures, hypercalcaemia of malignancy, and nerve compression, (Coleman, 2001).

Osteolysis in malignant bone disease is mediated by osteoclasts, which are activated by a range of cytokines including TGF-β, the PTHrP and RANKL (Chirgwin & Guise, 2000; Roodman, 2004a). A number of very robust studies have demonstrated that bisphosphonates can notably decrease the risks of SREs associated with multiple myeloma, breast cancer, prostate cancer, and a range of other solid cancers. (Cranney et al., 2002; Djulbegovic et al., 2002; Pavlakis et al., 2005). For example, Wong et al reported that monthly infusions of zoledronic acid (4 mg) in patients with breast cancer resulted in reducing the risk of developing a SREs by 41%, whereas the use of pamidronate (90 mg) and ibandronate (6 mg) led to a risk reduction of 33% and 20% respectively (Wong et al., 2012).

Two studies in patients with bone metastases secondary to breast or prostate cancer confirmed the non-inferiority of denosumab in preventing SREs with respect to zoledronic acid. In patients with metastatic breast cancer, monthly
treatment with 120 mg denosumab was superior to treatment with 4 mg zoledronic acid per month in delaying the time to the first SREs by 18% (Stopeck et al., 2010; Fizazi et al., 2011).

The most comprehensive data on denosumab for the treatment of multiple myeloma come from a randomised, double-blind study in which denosumab was compared with zoledronic acid in patients with confirmed bone metastases from solid tumours or multiple myeloma. Of the 1,776 patients enrolled in this trial, 180 suffered from multiple myeloma. With monthly injections of 120 mg denosumab, the median time to SREs was 21 months, compared with 16 months in patients receiving monthly injections of zoledronic acid, resulting in a non-inferiority of denosumab compared to zoledronic acid (Henry et al., 2011).

2.4.2 Bisphosphonates and denosumab in non-cancerous bone disease

Oral administration of bisphosphonates, such as alendronate, risedronate, etidronate and ibandronate, is often used in the management of post-menopausal osteoporosis (PMO), glucocorticoid-induced osteoporosis, Paget's disease of bone, giant cell tumours and osteogenesis imperfecta (Drake et al., 2008).

PMO is the most common bone disease in women. It is a skeletal condition characterised by reduced bone mass, and deterioration of the bone architecture causing an increased risk of fracture (Hodsman et al., 2002). The pathogenesis of osteoporosis includes: failure of optimal strength during development of the bone tissue, increase of bone resorption, and insufficient bone formation response (Raisz, 2005). The prevention of PMO fractures is an important public health intervention, as osteoporotic fractures are associated with notable morbidity and mortality (Cauley et al., 2000).

Bisphosphonates represent the main anti-resorptive agents for the treatment of osteoporosis (Hochberg et al., 2002). A large body of evidence supports the beneficial effects of bisphosphonates in increasing bone density and reducing the risk of osteoporotic fracture (Chen et al., 2015b; Zhang et al., 2015). Paget's disease is the second most common bone metabolic disorder after osteoporosis. It is associated with bone fragility, osteoarthritis, and risk of
osteosarcoma development (Lyles et al., 2001). Bisphosphonates represent the standard of care in patients with Paget (Lyles et al., 2001; Langston & Ralston, 2004).

Osteogenesis imperfecta (OI) is a genetic disease caused by mutations in genes coding type I collagen (Biggin & Munns, 2014), which result in a predisposition to bone fractures. Oral and intravenous bisphosphonates are regularly used in children with OI in order to reduce their risk of bone fractures (Castillo & Samson-Fang, 2009).

Bisphosphonate indications in less common disorders include (Agarwala et al., 2002; Gibbons et al., 2003; Goto et al., 2003; Haas et al., 2003; Berthelot, 2006; Breuil & Euller-Ziegler, 2006; Fantasia, 2009; Landesberg et al., 2009; Silverman, 2011):

- Avascular necrosis of the femoral head
- Benign osteolytic tumours of the bone
- Bone compromised related to beta-thalassemia
- Calcinosis in juvenile dermatomyositis
- Chronic recurrent multifocal osteomyelitis
- Fibrous dysplasia of the bone
- Giant cell lesions of the jaw
- Giant cell tumours of the bone
- Gaucher’s disease
- Langerhans cell histiocytosis
- Multicentric reticulohistiocytosis
- Osteolysis surrounding failing orthopaedic implants and peri-prosthetic bone loss
- Osteonecrosis of the hip
- Reflex sympathetic dystrophy syndrome
- Rheumatoid arthritis
- Transplant-induced bone loss.

In a phase 2 study on 412 postmenopausal women, denosumab, given at a dose of 60 mg every 6 months over 1 year, increased the lumbar spine bone
mineral density (BMD) by 6.7% compared with a loss of 0.6% in the placebo group (McClung et al., 2006). These effects were sustained in a 12-month extension of this trial (Lewiecki et al., 2007). Continuation of the trial for another 24 months using a number of different treatment regimens, including discontinuation and restarting therapy, showed that long-term treatment with denosumab (for up to 48 months) increased BMD at the lumbar spine and at the total hip of 11.8 and 6.1% respectively (Miller et al., 2008).

The phase 3 FREEDOM trial study on 7,868 women, who received either biannual s.c. injections of 60 mg denosumab or placebo for 3 years, established that denosumab was significantly effective in the treatment of postmenopausal osteoporosis by increasing BMD. In the treatment group, the incidence of radiomorphometric vertebral fractures, clinical hip and clinical non-hip, non-vertebral fractures decreased by 68%, 40% and 20% respectively (Cummings et al., 2009).

### 2.4.3 Antiangiogenic medications in cancerous bone disease

Pre-clinical research revealed that tumours stimulate the formation of new capillaries from the neighboring vasculature (sprouting angiogenesis), and that this mechanism is essential for tumour development beyond 2-3 mm in size (Figure 2.2). As a result, it was hypothesized that inhibiting sprouting angiogenesis may reduce tumour development in cancer patients (Folkman, 1971). Further research demonstrated that (a) VEGF-A is a crucial determinant of sprouting angiogenesis, (b) VEGF is overexpressed in most solid malignancies, and (c) VEGF inhibition may limit tumor formation in pre-clinical studies (Ellis and Hicklin, 2008; Carmeliet and Jain, 2011). Based on these findings, various treatments that target angiogenesis by inhibiting the VEGF signalling pathway have been developed. Because angiogenesis is thought to be required for metastasis growth in all areas of the body, it is expected that antiangiogenic treatment would assist patients with metastatic illness. However, findings have varied among cancer types, suggesting that although the metastases of certain malignancies are receptive to this sort of treatment, others are not. These medicines have been particularly effective in metastatic renal cell carcinoma (mRCC), with four medications currently FDA authorised in this scenario, notably sorafenib, sunitinib, pazopanib, and axitinib. In a phase
III randomised study (placebo-controlled phase) of patients who had progressed on prior treatment, sorafenib was the first TKI to show activity in mRCC (Escudier et al., 2007). When placebo-treated patients switching to sorafenib were omitted from the study, progression-free survival (PFS) increased significantly (5.5 vs. 2.8 months) and overall survival (OS) improved (Escudier et al., 2009). A second research evaluating single agent sunitinib with interferon-a in mRCC patients (who had not previously received therapy) found that the sunitinib group had a substantial improvement in PFS (11 vs. 5 months) (Motzer et al., 2007). Increase in OS was reported in the sunitinib arm (26.4 versus 21.8 months), and rise in OS was even more pronounced in a subset-analysis of patients who did not get any post-study cancer therapy (28.1 vs. 14.1 months) (Motzer et al., 2009). Pazopanib as a single drug was later demonstrated to improve PFS in mRCC in the first-line scenario (11.1 vs. 2.8 months), although considerable crossover from placebo to pazopanib confused the final OS study (Sternberg et al., 2010; Sternberg et al., 2013). A recent phase III study comparing sunitinib and pazopanib found that both medicines had comparable effectiveness, and single agent treatment with either medication is currently recommended as standard of care in mRCC (Motzer et al., 2013). Axitinib, a more recently produced TKI, has shown benefit in the second-line scenario in patients who have advanced on first-line TKI treatment and is currently indicated for mRCC in this situation (Rini et al., 2011). TKIs have also been demonstrated to be effective as a single treatment in progressed hepato-cellular carcinoma and evolved pancreatic neuroendocrine tumours (PNET). In a randomised phase III trial of hepatocellular carcinoma, sorafenib increased OS from 7.9 to 10.7 months vs placebo, resulting in FDA approval in 2007. (Llovet et al., 2008). Sunitinib was approved by the FDA for the treatment of PNET based on the findings of a randomised placebo-controlled study that demonstrated a doubling of PFS from 5.5 months in the control arm to 11.4 months in the sunitinib arm, though the OS (Raymond et al., 2011) analysis was hampered by patient cross-over from the control arm to the sunitinib arm. Bevacizumab, a humanised monoclonal antibody that binds selectively to VEGF-A alone, has shown effectiveness in numerous metastatic indications. In metastatic colorectal cancer (mCRC), the combination of chemotherapy plus bevacizumab was demonstrated to result in improved PFS
(10.6 vs. 6.2 months) and OS (23 vs. 15.3 months) compared to the chemotherapy-only group (Hurwitz et al., 2004). Based on these findings, bevacizumab was authorised for the treatment of metastatic colorectal cancer in conjunction with chemotherapy. Subsequent phase III trials have also shown that adding bevacizumab to chemotherapy in mCRC is helpful (Giantonio et al., 2007; Saltz et al., 2008; Cunningham et al., 2013). Aflibercept, a new fusion protein that binds to three VEGF family ligands: VEGF-A, VEGF-B, and placental growth factor, provides more evidence for the effectiveness of anti-angiogenic treatment in colorectal cancer (PIGF). Aflibercept's anti-angiogenic actions may extend beyond the inhibition of VEGF-A by targeting VEGF-B and PLGF, two other growth factors with roles in angiogenesis and/or the survival of newly created capillaries (Fischer et al., 2007; Fischer et al., 2008; Zhang et al., 2009). In metastatic colorectal cancer, adding aflibercept to chemotherapy increased PFS and OS compared to chemotherapy alone (Van Cutsem et al., 2012). Furthermore, there was a considerable divergence of the survival curves in this research, with 2-year survival considerably higher in the aflibercept arm compared to the control arm (28% vs. 18.7%). (Van Cutsem et al., 2012). Based on these findings, aflibercept was recently authorised for the treatment of metastatic colorectal cancer in conjunction with chemotherapy. In non-squamous non-small cell lung cancer (NSCLC), two phase III studies found that adding bevacizumab to chemotherapy improved PFS, but only one research found that it improved OS (Sandler et al., 2006; Reck et al., 2009; Reck et al., 2010). A recent meta-analysis including 2,000 patients found a minor but substantial increase in OS of 4% after merging data from these two phase III trials (plus data from two phase II investigations) (Soria et al., 2013). Two important trials (ICON7 and GOG218) exploring the addition of bevacizumab to chemotherapy in the first-line treatment of ovarian cancer have been published (Burger et al., 2011; Perren et al., 2011). Both trials found a substantial improvement in PFS, ranging from 2.4 to 3.8 months. OS statistics were not significant in the GOG218 trial (but were confounded by cross-over), while OS data for the ICON7 study are yet awaited. However, in ICON7, bevacizumab improved overall survival in the high-risk group when compared to chemotherapy alone (36.6 vs. 28.8 months). The addition of bevacizumab to chemotherapy in recurrent ovarian cancer has exhibited a considerable
increase in PFS, albeit this has not translated into an OS advantage (Aghajanian et al., 2012).

Figure 2.2. Graphical representation of sprouting angiogenesis in tumour growth.

2.4.4 Adverse effects of bisphosphonates and denosumab

Bisphosphonates have been associated with a wide range of adverse effects, including osseous and extra-osseous adverse effects (Papapetrou, 2009) (summarised in Table 2.4).

<table>
<thead>
<tr>
<th>Extra-osseous adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oesophageal erosion and cancer</td>
</tr>
<tr>
<td>Conjunctivitis, uveitis and other ocular adverse effects</td>
</tr>
<tr>
<td>Acute tubular necrosis and focal segmental glomerulosclerosis</td>
</tr>
<tr>
<td>Hypocalcaemia</td>
</tr>
<tr>
<td>Acute phase response</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Bone adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Musculoskeletal pain</td>
</tr>
<tr>
<td>Atypical fractures</td>
</tr>
<tr>
<td>Osteonecrosis of the jaws</td>
</tr>
</tbody>
</table>

Table 2.4 Adverse event categories

Upper gastrointestinal tract adverse effects, such as nausea, vomiting and dyspepsia, have been observed in several patients. Developments of oesophageal erosions have been reported by several authors in individuals
using oral bisphosphonates (De Groen et al., 1996). In 2009 a series of 23 cases of oesophageal cancer was reported in patients treated with alendronate (Wysowski, 2009). Solomon et al observed that the rate oesophageal cancer in patients treated with bisphosphonates was 0.27/1,000, compared to a rate of 0.48/1,000 in patients treated with other anti-osteoporotic medications (Solomon et al., 2009).

Reported ocular adverse effects include conjunctivitis uveitis periorbital oedema, retinal detachment, transient ocular myasthenia and optic neuritis. These associations are not well demonstrated, though (Aurich-Barrera et al., 2006; Raja et al., 2007; French & Margo, 2008; Dasanu et al., 2009; Seth et al., 2009; Procianoy & Procianoy, 2010).

Renal toxicity is another adverse event reported in patients treated with bisphosphonates. The majority of renal toxicity events have been described with the use of intravenous BPs (Balla, 2005). The most common renal disorders observed have been acute tubular necrosis and focal segmental glomerulosclerosis, which are more commonly observed in individuals with pre-existing chronic kidney disease, diabetes mellitus, and hypertension (Body, 2006; Perazella & Markowitz, 2008).

Hypocalcaemia has been mainly reported in association with intravenous bisphosphonates and is more commonly observed in patients with previous hypo-parathyroidism, vitamin D deficiency and kidney failure (Schussheim et al., 1999; Liamis et al., 2009; Zuradelli et al., 2009).

An additional extra-osseous adverse event is represented by the acute phase response (APR). APR is a reaction, which may occur in patients that start bisphosphonates treatment (amino-bisphosphonates). The clinical manifestation of the APR is represented by an acute and transient range of symptoms, such as fever and myalgia, which can last up to 7-14 days) (Adami et al., 1987). The APR is relatively common (10-30%) after the first intravenous infusion and its frequency decreases with subsequent infusions. It has never been associated with patients treated with non-amino-bisphosphonates (etidronate, clodronate and tiludronate). The APR mechanism has been partially clarified and seems to be related to the release of tumour necrosis factor alpha and IL-6 (Olson & Van Poznak, 2007).

A number of studies have reported an increased risk of atrial fibrillation (AF)
associated with bisphosphonates (Cummings et al., 2007, Sørensen et al., 2008, Heckbert et al., 2008).

Musculoskeletal pain has been associated with the use of alendronate and risedronate, with a very low incidence. However, a recent study carried at the Mayo Clinic, did not show a significant increase of musculoskeletal pain associated with the use of bisphosphonates (Caplan et al., 2010).

Since 2003 a large number of reports have demonstrated the association between the use of bisphosphonates and osteonecrosis of the jaw (Kühl et al., 2012). Poor oral hygiene, invasive dental procedures or denture use, and prolonged exposure to high doses of IV bisphosphonates appear to increase the risk of bisphosphonate related osteonecrosis of the jaw (BRONJ) development (Marx et al., 2005; Ficarra et al., 2005; Migliorati et al., 2006). On the other hand, in the FREEDOM study on osteoporotic patients denosumab has reported a higher incidence of eczema (3.0 vs 1.7%) and cellulitis (0.3 vs < 0.1%) compared with placebo (Cummings et al., 2009).

Mild and asymptomatic hypocalcaemia has been reported in the DECIDE study (Brown et al., 2009). Moreover, it has been noticed that patients with impaired renal function are particularly prone to hypocalcaemia after denosumab administration and this is generally more pronounced compared with that observed after use of intravenous bisphosphonates (Bekker et al., 2004). In the osteoporosis setting with a denosumab dosing of 60 mg every 6 months, the risk of osteonecrosis of the jaw appeared very low and was not observed during the initial evaluation of the FREEDOM and HALT study. However, in the open label extension of the FREEDOM trial, two cases of osteonecrosis of the jaw were reported in patients who were transitioned from placebo to denosumab (Cummings et al., 2009, Smith et al., 2009).

Regarding the management of malignant conditions, the recommended and approved dose of denosumab is 120 mg every 4 weeks. In a trial between denosumab and zoledronic acid in bone metastases due to breast cancer and prostate cancer, acute-phase reactions were reported significantly less frequently in the denosumab group compared to the zoledronic acid (8% vs 10%, and 18% vs 27% respectively) one. Hypocalcaemia, on the other hand, occurred almost twice as often in patients receiving denosumab than in those receiving zoledronic acid for metastatic bone disease secondary to breast and
prostate cancer (5 vs 3% and 13 vs 6% respectively) (Stopeck et al., 2010, Fizazi et al., 2011).

The prevalence of osteonecrosis of the jaw was similar between patients receiving bisphosphonates (1.4%) and denosumab (2.0%) for bone metastases (Lipton et al., 2012). These figures are 100- to 1000-fold higher compared with the osteoporosis setting owing to the higher cumulative dose employed in oncology.

2.4.5 Adverse event of antiangiogenic medications

Antiangiogenic medications have been linked to a number of side effects, including hypertension, thromboembolic events, hypophosphatemia, thrombocytopenia, cardiac dysfunctions, skin problems, and medication-related osteonecrosis of the jaw (MRONJ) (Boehm et al., 2010; Guarneri et al., 2010; Ara and Pastushenko., 2014).

Table 2.5 lists the most usual and frequent adverse effects. Antiangiogenic medicines were just recently linked to MRONJ. This medication class's principal activity is to block VEGF, hence suppressing neovascular formation (Ruggiero et al., 2014; Ruggiero et al., 2022). Despite the rising number of osteonecrosis patients connected with this medication, the pathophysiology of MRONJ related with antiangiogenic medicines remains unknown. VEGF, on the other hand, is widely established to play a critical role in the control of osteoclastic function, promotion, and differentiation (Nakagawa et al., 2000; Sivolella et al., 2013). Furthermore, angiogenesis suppression impairs bone's capacity to regenerate, remodel, and repair, and increases vulnerability to superinfections (Chang et al., 2018). More research is needed to determine the underlying mechanism that causes bone necrosis and the precise pathophysiology of anti-angiogenic medications that cause MRONJ (Sacco et al., 2022b).

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Common adverse events</th>
<th>Moderately Frequent adverse events</th>
<th>Rare adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>hypertension, proteinuria</td>
<td>bleeding, arterial and venous thromboembolic events, hypophosphatemia, thrombocytopenia stomatitis, nausea, diarrhea, fatigue, hand foot syndrome</td>
<td>gastrointestinal perforation, nephrotic syndrome, cardiac dysfunction</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>hypertension, hypothyroidism, thrombocytopenia, neutropenia, dysgeusia</td>
<td></td>
<td>proteinuria, congestive heart failure, QTc prolongation, torsades-de-point, pancreatitis, liver dysfunction</td>
</tr>
</tbody>
</table>
Sorafenib  
- hypertension, hand-foot syndrome, skin rash/desquamation, fatigue, hypothyroidism, leukocytopenia, hypophosphatemia, diarrhea  
- nausea, proteinuria  
- cardiac dysfunction, bleeding, gastrointestinal perforation

Pazopanib  
- nausea, hypertension, diarrhea, fatigue  
- anorexia, vomiting, hair depigmentation, elevated liver enzymes, rash, leukocytopenia  
- dyspnea, hyperkalemia

Cediranib  
- hypertension, fatigue, diarrhea  
- nausea, vomiting, proteinuria, hand-foot syndrome, anorexia  
- central nervous system ischemia

Vandetanib  
- skin rash, diarrhea  
- hypertension, diarrhea, QTc prolongation  
- central nervous system ischemia

Axitinib  
- hypertension, fatigue, diarrhea  
- stomatitis, nausea, vomiting  
- bleeding, thromboembolic events, proteinuria, bowel ischemia

Combretastatin  
- hypertension, hypotension, tachycardia, bradycardia, nausea, fatigue  
- visual disturbances, dyspnea  
- bowel ischemia

ASA404 (vadimezan)  
- hypertension, hypotension, nausea, fatigue  
- urinary incontinence, visual disturbances, anxiety, confusion, tremor  
- left ventricular failure, QTc prolongation

Table 2.5 Reported adverse events among the antiangiogenic drugs (Boehm et al., 2010).

2.5 Definition of medication-related osteonecrosis of the jaws (MRONJ)

Historically, jaw osteonecrosis associated with phosphorous mixture used by workers in a match factory was first described at the end of the 19th century (Marx, 2008).

Since 2001, oral and intravenous BPs therapies have been in extensive use to treat osteoporosis and SREs due to solid metastatic cancer. Consequently, the incidence of jaw osteonecrosis has notably increased (Marx, 2003; Migliorati et al., 2003; Wang et al., 2003).

In 2007 a special task force including experienced clinicians, surgeons and researchers published a position paper on BRONJ on behalf of the American Association of Oral and Maxillofacial Surgeons (AAOMS) (AAOMS, 2007). Through the years, this position paper has been updated and slightly revised. The last modification took place in 2014 with the change of the previous nomenclature of BRONJ (bisphosphonate related osteonecrosis of the jaw) to a new one, MRONJ (medication-related osteonecrosis of the jaw), due to raising number of cases of jaw osteonecrosis in patients with history of other anti-resorptive agents (e.g. denosumab) and anti-angiogenic medications (Ruggiero et al., 2014). The 2014 AAOMS’s position paper was updated in 2022 without significant changes (Ruggiero et al., 2014; Ruggiero et al., 2022).
The 2022 AAOMS’s position papers defined MRONJ as follows:

1. Current or previous treatment with antiresorptive therapy alone or in combination with immune modulators or antiangiogenic medications.
2. Exposed bone tissue or bone tissue in the maxillofacial region that has persisted for more than eight weeks, which can be probed through an oral or extra-oral fistula.
3. No history or current radiation therapy in the head and neck region, or obvious metastatic disease to the jaws.

MRONJ occurs commonly in the mandible (65%), followed by the maxilla (26%) and, infrequently, as multifocal in both of the jaws (9%) (Marx et al., 2005; Woo et al., 2006).

2.5.1 Epidemiology data

Measuring the incidence of a rare illness or condition is difficult since a very large number of subjects is required. MRONJ frequency varies based on a variety of different criteria, such as an individual’s medical history, type of medication, duration of drug therapy, and type of dental treatment (Berenson et al., 1998). Recently, it was revealed that MRONJ may be initiated by tooth extraction (61.7%), spontaneous onset (14.8%), or ill-fitting dentures (7.3%) in the same proportions independent of medication delivery mode (Yamazaki et al., 2012; Fliefel et al., 2015). According to the existing research, the frequency of MRONJ is much greater in cancer patients using intravenous bisphosphonate and high dosage denosumab compared to non-oncology patients. In osteoporosis clinical studies, research participants allocated to placebo groups had a risk of MRONJ that varied from 0% to 0.02% (Grbic et al., 2010; Papapoulos et al., 2012; Cosman et al., 2016). The risk of MRONJ among research individuals using BPs ranges from 0.02% to 0.05%. MRONJ probability was observed to be greater in osteoporotic patients exposed to Dmab than in BPs (Bone et al., 2017; Saag et al., 2017; Hallmer et al., 2018).
According to the existing research, the risk of MRONJ in romosozumab-exposed patients is 0.03% to 0.05% (Saag et al., 2017; Cosman et al., 2016). There is relatively little information available on the prevalence of MRONJ in the paediatric population for osteogenesis imperfecta and other disorders. There were no incidences of MRONJ detected in a group of 486 participants treated for 4.5 to 6.8 years in a systematic analysis assessing the risk of MRONJ among children with osteogenesis imperfecta (Hennedige et al., 2014). In contrast, the risk of MRONJ in the cancer population is much greater than in the non-oncology group of patients. The cumulative incidence of MRONJ is estimated to be 5% among cancer patients exposed to zoledronate, ranging from 0% to 18% (Peddi et al., 2013; Valachis et al., 2013; Barrett-Lee et al., 2014; Coleman et al., 2014; Wang et al., 2014; Himelstein et al., 2017; O'Carrigan et al., 2017; Raje et al, 2018) MRONJ risk ranged from 0% to 6.9% among cancer patients exposed to Dmb, with most studies finding rates of 5% (Wang et al., 2014; Gnant et al., 2015; O'Carrigan et al., 2017; Raje et al, 2018; Coleman et al., 2020; Ng et al., 2021). When compared to antiresorptive drugs, the degree of data supporting other pharmaceutical families as risk factors for MRONJ is quite low and is based on case reports or case series (Fusco et al., 2016; Nicolat-Galitis et al., 2019; King et al., 2019). To assess the risk of MRONJ related with non-antiresorptive drugs, further controlled prospective trials will be necessary. However, according to studies, the use of antiangiogenic medications in conjunction with antiresorptive therapies is known to raise the risk of MRONJ formation, with an estimated 16% of recurring rates (Christodoulou et al., 2009). Regardless of the drug's prescription, the length of antiresorptive treatment is a risk factor for developing MRONJ. Clinical studies found that among cancer patients given zoledronate or Dmb, the probability of developing MRONJ was 0.5% and 0.8% at one year, 1.0% and 1.8% at two years, and 1.3% and 1.8% at three years (Henry et al., 2014). Saad et al. combined three-blind phase 3 studies and found comparable outcomes, including a plateau after two years for individuals exposed to Dmb (Saad et al., 2012). According to a more recent systematic study by Ng et al., the probability of MRONJ among cancer patients treated with zoledronate was 1.6% to 4% after two years and 3.8% to 18% after more than two years (Ng et al., 2021). Similarly, the odds of developing MRONJ with Dmb were 1.9% with
24 months of exposure and 6.9% with >24 months of exposure (Ng et al., 2021).

2.5.2 Pathophysiology

Regarding the aetiology and pathophysiology of MRONJ, very little information is currently available (Marx, 2003; Ruggiero et al., 2004). So far, many ideas have been presented, however it is unclear that a single hypothesis can explain the disease's pathophysiology, which is likely to be complex. The available theories centre on:

a) bone remodelling suppression or inhibition,
b) angiogenesis inhibition,
c) inflammation and/or infection,
d) soft tissue toxicity, and
e) immune dysfunction.

It has been proposed that MRONJ is caused by the inhibition or suppression of bone tissue remodelling after a tooth extraction. It is also plausible that decreased bone remodelling/turnover has a deleterious impact on intra-bony re-vascularization and angiogenesis (Allen et al., 2010). Bisphosphonates have been shown to have anti-angiogenic effect via inhibiting vascular endothelial growth factor (VEGF). Bisphosphonates have been shown in vitro and in vivo to lower VEGF levels, resulting in a considerable reduction in microvessel density (Gacche & Meshram, 2014; Pabst et al., 2014). This inhibitory impact on intra-bony neo-angiogenesis may lead to ischaemic bone disease and necrosis. Inflammation and/or infection of the bone has been proposed as a typical case for MRONJ onset, and numerous studies have identified oral infection as a major contributor to risk (Marx et al., 2005; Ficarra et al., 2005; Boonyapakorn et al., 2008). One of the early ideas on MRONJ development was soft tissue toxicity. Bisphosphonates have been shown in vitro to reduce the proliferation of oral epithelial cells, leading some researchers to speculate that bone tissue necrosis may be caused by persistent exposure of the alveolar bone after dental surgery (Reid et al., 2007; Landesberg et al., 2008; Pabst et al., 2014). Immunodeficiency and the related risk of infection
might be an involved in the pathogenesis mechanism, since the vast majority of patients with MRONJ are immunocompromised as a result of concurrent chemotherapy (Kikuiri et al., 2010; Lopez-Jornet et al., 2011; Ali-Erdem et al., 2011, Kabilova et al., 2014; Kuroshima et al., 2014).

2.5.3 Risk Factors

MRONJ risk factors may be divided into several categories as the following:

- Medication-related factors
- Systemic factors
- Local factors
- Other factors

Medication-related factors include the type, potency, mode of administration, and term of use/cumulative dose of the drug administrated. Current research shows that nitrogen-containing bisphosphonates (zolendronate, ibandornate, pamindornate, residronate alendronate) are more likely to develop MRONJ than non-nitrogen-containing bisphosphonates (Yamashita et al., 2012). Similarly, it has been reported that intravenous bisphosphonates have a greater risk of osteonecrosis than oral bisphosphonates (Ruggiero, 2004; Marx et al., 2005; Thumbigere-Math et al., 2012). According to the findings of a number of studies, the length of treatment with bisphosphonates and denosumab (cumulative dose) is another factor that raises the probability of developing MRONJ (Dimopoulos et al., 2006; Hoff et al., 2008; Fehm et al., 2009; Thumbigere-Math et al., 2012; Then et al., 2012). Literature indicates that the typical period to onset of MRONJ is between 12 and 24 months for zolendronate, 19 to 30 months with pamidronate, and 13-21.5 months for ibandronate (Dimopoulos et al., 2006; Hoff et al., 2008; Boonyapakorn et al., 2008; Thumbigere-Math et al., 2012). Previous research on oral bisphosphonates have shown that the risk rises when the treatment period exceeds three years (Ruggiero et al., 2009). Recent published state surveys in Germany, on the other hand, have found that the period it required for MRONJ to manifest was 33.2 months for oral bisphosphonates and 23.6 months for intravenous bisphosphonates (Urade et al., 2011). When comparing the intravenous and oral bisphosphonates, Fleisher et al. found a median onset time of 3 and 5
years (Fleisher et al., 2013). Early research on risk factors revealed that systemic variables such as medical comorbidity, chemotherapy, corticosteroids, diabetes, and ageing may all play a part in the beginning of MRONJ (Marx et al., 2005; Jadu et al., 2007; Boonyapakorn et al., 2008; Thumbigere-Math et al., 2012; Then et al., 2012). Invasive dental treatments (dento-alveolar surgery), smoking, periodontal disease/dental infections, and the use of detachable prosthesis are examples of local hazards linked with a greater chance of MRONJ formation (Wessel et al., 2008; Tsao et al., 2013). Other risk factors cited in the research include race and genetic predisposing background. According to Sedghizadeh et al, Asians are more prone to MRONJ than Caucasians, Hispanics, and Afro-Americans. This vulnerability might be attributed to a reduced body weight and a tiny skeletal segment (Sedghizadeh et al., 2013). In terms of genetic considerations, several studies have shown a variation in a single nucleotide sequence of the cytochrome P450, subfamily 2C polypeptide 8 (CYP2C8, rs1934952) may be related with a higher risk of MRONJ onset (Lai et al., 2009; Zhong et al., 2013). Another research found a connection with the RBMS3 (rs17024608) gene (Nicoletti et al., 2012).

2.6 Clinical Presentation of the MRONJ

2.6.1 Clinical Manifestations
The clinical hallmark of MRONJ is the presence of jawbone exposure through the oral mucosa or facial skin. The extension of the exposed bone can vary from a small area to several centimetres (Otto et al., 2012). Additional common signs of MRONJ are infection and swelling. Severe symptoms can also include paraesthesia/anaesthesia of the chin and lip (due to damage of the inferior alveolar nerve), pathological jaw fracture, tooth mobility and loss, and sinusitis (Otto et al., 2009; Otto et al., 2012; Mast et al., 2012; Assaf et al., 2014; Ruggiero et al., 2014; Ruggiero et al., 2022). The preferential site of the MRONJ is the mandible (2/3 of the cases) compared to the maxilla (1/3 of the cases).
2.6.2 Staging

The AAOMS 2007 position paper introduced a type of MRONJ staging/classification based on the severity of clinical signs and symptoms. Successively, in 2009 and 2014 the AAOMS has revised its staging/classification system, mainly to include some of the possible manifestations of non-exposed variant of MRONJ, in particular the presence of sinus tract. (AAOMS, 2007; Ruggiero et al., 2009; Ruggiero et al., 2014; Ruggiero et al., 2022). Table 2.6 summarises the MRONJ staging.

<table>
<thead>
<tr>
<th>Stages</th>
<th>AAOMS 2007</th>
<th>AAOMS 2009</th>
<th>AAOMS 2014</th>
<th>AAOMS 2022</th>
</tr>
</thead>
<tbody>
<tr>
<td>At risk Category</td>
<td>Patients treated with either oral or IV bisphosphonates</td>
<td>Patients treated with either oral or IV bisphosphonates</td>
<td>Patients treated with either oral or IV bisphosphonates</td>
<td>No apparent necrotic bone in asymptomatic patients who have been treated with IV or oral antiresorptive therapy.</td>
</tr>
<tr>
<td>Stage 0</td>
<td>Exposure and necrotic bone in asymptomatic patients without evidence of infection</td>
<td>Exposed and necrotic bone in asymptomatic patients without evidence of infection</td>
<td>Exposed and necrotic bone, or fistula that probes to bone in patients who are asymptomatic and have no evidence of infection</td>
<td>Exposed and necrotic bone or fistula that probes to the bone in patients who are asymptomatic and have no evidence of infection/inflammation. These patients also may present with radiographic findings mentioned for Stage 0 that are localized to the alveolar bone region.</td>
</tr>
<tr>
<td>Stage 1</td>
<td>Exposed and necrotic bone associated with infection as evidenced by pain and erythema in the region of the exposed bone with or without purulent drainage</td>
<td>Exposed and necrotic bone associated with infection as evidenced by pain and erythema in the region of the exposed bone with or without purulent drainage</td>
<td>Exposed and necrotic bone, or fistula that probes to bone, associated with infection as evidenced by pain and erythema in the region of the exposed bone with or without purulent drainage</td>
<td>Exposed and necrotic bone or fistula that probes to the bone, with evidence of infection/inflammation. These patients are asymptomatic. These patients also may present with radiographic findings mentioned for Stage 0 that are localized to the alveolar bone region.</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Exposed and necrotic bone in patients with pain, infection, and one or more of the following: pathologic fracture, extraoral fistula, or osteolysis extending to the inferior border</td>
<td>Exposed and necrotic bone in patients with pain, infection, and one or more of the following: exposed and necrotic bone extending beyond the region of alveolar bone, (i.e., inferior border and ramus in the mandible, maxillary sinus and zygoma in the maxilla) resulting in pathologic fracture, extraoral fistula, oral antral/oral nasal communication, or osteolysis extending to the inferior border of the mandible or the sinus floor</td>
<td>Exposed and necrotic bone in patients with pain, infection, and one or more of the following: exposed and necrotic bone extending beyond the region of alveolar bone, (i.e., inferior border and ramus in the mandible, maxillary sinus and zygoma in the maxilla) resulting in pathologic fracture, extraoral fistula, oral antral/oral nasal communication, or osteolysis extending to the inferior border of the mandible or the sinus floor</td>
<td>Exposed and necrotic bone or fistula that probes to the bone, with evidence of infection, and one or more of the following:</td>
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<td></td>
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<td></td>
<td>• Exposed necrotic bone extending beyond the region of alveolar bone (i.e., inferior border and ramus in the mandible, maxillary sinus and zygoma in the maxilla).</td>
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<td></td>
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<td>• Pathologic fracture. • Extraoral fistula.</td>
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<td></td>
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<td></td>
<td>• Oral antral/oral-nasal communication.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>• Osteolysis extending to the inferior border of the mandible or sinus floor.</td>
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</table>

Table 2.6 MRONJ staging according to the AAOMS positional papers 2007, 2009, 2014 and 2022.
2.6.3 Imaging

Imaging plays an important role in the assessment and management of MRONJ, particularly to understand the extent of bone involvement, which is often notably larger than the area of bone exposure (Hutchinson et al., 2010; Rocha et al., 2012).

Various imaging modalities have been used to assess MRONJ, including panoramic radiography, such as computerised tomography (CT), cone beam computed tomography (CBCT), magnetic resonance imaging (MRI), scintigraphy, single-photon emission computed tomography (SPECT) and positron emission tomography with computed tomography (PET/CT) (Chiandussi et al., 2006; Phal et al., 2007; Bisdas et al., 2008; Arce et al., 2009; Dore et al., 2009; Popovic et al., 2010; Stockmann et al., 2010; Belcher et al., 2014).

Panoramic radiography represents a basic though commonly used imaging as it is inexpensive and allows easy identification of dental disorders, osteolysis, bone sclerosis, and sequestra (Ruggiero et al., 2009; Arce et al., 2009). Sclerosis in particular is commonly seen in all MRONJ stages, and can also manifests as thickening of the lamina dura and reduction of width of the inferior alveolar nerve canal. (Phal et al., 2007). Nevertheless, panoramic radiography is known to have a sensitivity of about 50%, namely it might not detect about half of MRONJ cases (Stockmann et al., 2010). Table 2.7 report the common radiological MRONJ findings

<table>
<thead>
<tr>
<th>Radiodensity</th>
<th>Radiolucency</th>
<th>Pathological Complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerosis</td>
<td>Persisting alveolar socket, lack of healing</td>
<td>Sequestra</td>
</tr>
<tr>
<td>Thickening of the lamina dura</td>
<td>Osteolysis of the cortical and sponges bone</td>
<td>Pathological fractures</td>
</tr>
<tr>
<td>Altered dimension of the mandibular canal</td>
<td>Focal cortical disruption</td>
<td>Sinusitis</td>
</tr>
<tr>
<td>Periostal reaction</td>
<td>Periradicular radiolucency</td>
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</table>

Table 2.7 radiological finding of MRONJ

CT and CBCT have a much higher resolution and have been reported to have a sensitivity of more than 90% (Arce et al., 2009; Hutchinson et al., 2010). In
addition, CT scan, compared to panoramic radiography, provides a much clearer assessment of the extension of MRONJ (Guggenberger et al., 2013). The MRI is also reported to have a high sensitivity of more than 90% (Bisdas et al., 2008; Morag et al., 2009; Stockmann et al., 2010), and can be helpful in assessing the bone marrow abnormalities observed in early stages of MRONJ development (Garcia-Ferrer et al., 2008; Raje et al., 2008). Functional imaging such as PET and SPECT can identify areas of MRONJ but have low specificity as differentiation from dental infection and bone metastasis remains difficult. (Chiandussi et al., 2006; Dore et al., 2009; Treister et al., 2010; Stockmann et al., 2010; Fatterpekar et al., 2011; Ruggiero et al., 2014; Ruggiero et al., 2022).

2.7 Management of MRONJ

MRONJ is notoriously difficult to manage and at the moment there remains no clear guidance for clinicians regarding the most effective therapeutic intervention. Patients with MRONJ have been managed with a wide range of interventions including antibiotics, local antiseptic irrigations, analgesics, pentoxifylline, vitamin E, teriparatide, hyperbaric oxygen, sequestrectomy, surgical debridement, bone resections, and resections followed by microvascular reconstruction (Migliorati et al., 2005; Ruggiero et al., 2006; Hoefert & Eufinger, 2011; Ruggiero et al., 2014; Ruggiero et al., 2022). In general, interventions for the management of MRONJ are classified in conservative (antibiotics, pain control), minimally invasive surgery (sequestrectomy and surgical debridement) and invasive/resective surgery (large resection with/without reconstruction).

The AAOMS has published stage-specific treatment recommendations. They advise conservative management for MRONJ stage 0 and I and surgical debridement or resection was advised for MRONJ stage II and III. However there remains no evidence-based consensus (AAOMS, 2007, Ruggiero et al., 2009, Ruggiero et al., 2014; Ruggiero et al., 2022).

2.7.1 Surgical VS Non-surgical management of BRONJ

Historically non-surgical/conservative management has been used in patients with early stages of BRONJ/MRONJ, in those who decline or are not fit for
surgical intervention. The aim of conservative management is to decrease pain and reduce infection/bacterial colonisation of the necrotic bone ((Montebugnoli et al., 2007; Sedghizadeh et al., 2008; Schipmann et al., 2013).

Conservative management can be divided into categories according to (Ruggiero, 2015):

- Antimicrobial treatment
- Pharmacological treatment other than antimicrobial treatment
- Other treatment

Antimicrobial treatments include the use of topical, oral and intravenous agents with the intent of decreasing bacterial colonisation. Despite the unclear pathogenesis of the MRONJ, there is evidence that oral flora and specific biofilms participate to the osteonecrosis process and its progression (Kumar et al., 2010; Moretti et al., 2011). Indeed, the use of antimicrobials has been associated with reduction in painful symptoms and signs of infection. Topical chlorhexidine gluconate 0.12% can be useful in as a preventative agent in order to minimise the risk of bacterial contamination of the exposed bone (Jenkins et al., 1988; Jenkins et al., 1994). Oral and intravenous antibiotics have been typically used in MRONJ patients with painful symptoms and frank signs of infection including pus discharged, swelling, and fistulas (Hansen et al., 2007; Sedghizadeh et al., 2008).

Oral amoxicillin (with and without clavulanic acid) is often used as a first line of antibiotic therapy, followed by clindamycin and metronidazole (Pushalkar et al., 2014; Hinson et al., 2014). Intravenous antibiotics can be used for MRONJ patients with sings of severe infection not responding to oral antibiotics (Wilson et al., 1996; Wu et al., 2014).

Occasional reports of MRONJ patients treated with pentoxifylline and vitamin E or teriparatide have been reported in the literature. Pentoxifylline and vitamin E have been previously used in the management of osteoradionecrosis and might theoretically be beneficial in patients with MRONJ (Epstein et al., 2010; Magremanne et al., 2014). Teriparatide is a recombinant parathyroid hormone,
and can promote bone remodelling via stimulation of osteoblast activity and proliferation (Subramanian et al., 2011).

Although the use of pentoxifylline/vitamin E or teriparatide is to be considered experimental, preliminary results seem to suggest some beneficial effect in MRONJ patients (Lau et al., 2009; Ohbayashi et al., 2013). Hyperbaric oxygen therapy has also been attempted in the management of MRONJ, though results remain preliminary and so far inconsistent (Freiberger et al., 2012; Rupel et al., 2014).

Surgical management has historically been advised in patients with advanced stage (stage II and III). The surgical interventions described in the literature include:

- Debridement/sequestrectomy
- Segmental resection.

Debridement or sequestrectomy usually consists of removing necrotic bone within the alveolus bone. The success rate can vary (15%-100%), but it is typically higher if the debridement is followed by soft tissue or multilayer soft tissue primary closure (Markose et al., 2009; Heufelder et al., 2014; Rupel et al., 2014). In order to ensure better outcomes, it is advisable to plan the surgical procedure with adequate imaging (CT, CBCT, MRI and bone scintigraphy), so to assess the extent of necrotic bone disease and relevant margins (Stockmann et al., 2010).

The use of fluorescence-guided debridement has also been suggested in order to identify the margins of bone necrosis (Fleisher et al., 2008; Otto et al., 2013). A number of preliminary studies have also suggested the potential benefit of laser therapy, ozone therapy and autologous platelet-rich of plasma (Agrillo et al., 2012, Vescovi et al., 2012a; Vescovi et al., 2012b; Del Fabbro et al., 2015) in addition to debridement/sequestrectomy.

Patients with advanced MORNJ or severe MRONJ symptoms not responding to conservative and minimally invasive treatment might benefit from segmental resection. The outcomes of segmental resection seem notably high, although there remain concerns regarding the morbidity of this approach, especially in fragile patients with incurable cancers undergoing multiple courses of
chemotherapy (Ferrari et al., 2008; Mücke et al., 2009; Marx et al., 2009; Pautkeet et al., 2011).

Recently, two systematic reviews have analysed the current evidence regarding the major surgical procedure as curative treatment option in patient with advanced MRONJ stage (Sacco et al., 2018; Sacco et al., 2021c). The results of using microvascular free flaps technique were quite promising with an overall success rate of 96.16% and with recurrence rate of 6.41% (Sacco et al., 2018). While the segmental mandibular resection without any reconstruction the recurrence rate was only 2% (Sacco et al., 2021c).

Despite the promising date of both reviews, the authors highlighted that all the studies included in these reviews had a significant degree of heterogenicity and the risk of bias was high in all of the studies included (Sacco et al., 2018; Sacco et al., 2021c). In more contemporary years, we noticed a shift in the literature in managing MRONJ. Indeed, the attention has been on the use of adjuvant treatments prior any surgery. Human amniotic membrane has lately been documented for having beneficial effects of bone healing disease. Research has described it, as a bio-compatible scaffold with suitable mechanical properties (permeability, stability, elasticity, flexibility, resorbability, and transparency) (Fénelon et al., 2018). A meta-analysis by Sacco et al reported that human amniotic membrane has a very high degree of success rate of 91.2% with a relief of symptoms (pain) in 92.5% of the cases. However, the review was based on Levels 2b and 4 publications of the Oxford Evidence-Based Medicine Hierarchies (Sacco et al., 2023).
CHAPTER 3 RISK REDUCTION STRATEGIES

3.1 Risk Reduction Strategies for Patients at Risk of Medication-related Osteonecrosis of the Jaw

MRONJ is a potentially severe condition that is often challenging to manage. A number of risk reduction strategies have been attempted over the years with the aim of reducing the development of MRONJ in individuals exposed to anti-resorptive and anti-angiogenic medications (Dimopoulos et al., 2009; Ripamonti et al., 2009). The vast majority of relevant literature includes uncontrolled and small prospective and retrospective studies and is, therefore, of limited evidence (Ruggiero et al., 2014; Ruggiero et al., 2022).

3.2 Risk assessment and modifiable risk factors

Risk reduction strategies are based on the identification and control of factors that increase the likelihood for a disease to develop. Several studies have reported that the risk of developing MORNJ is associated with a number of factors that are relevant to (i) the medication, (ii) the underlying disease and medical comorbidities, and the local (dental) triggers. These factors have been described in detail in chapter 2 (narrative review) and include: length of therapy and cumulative dosage, type of bisphosphonate, underlying disease (osteoporosis vs cancer), medical comorbidities (e.g. diabetes), dental extractions and other surgical procedures to the jawbones, dental infections, use of dentures with associated trauma to the oral mucosa (Ruggiero et al., 2014; Yarom et al., 2007; Thumbigere-Math et al., 2012; Then et al., 2012; Ruggiero et al., 2014; Ruggiero et al., 2022).

3.3 Reducing the length/cumulative dosage of anti-resorptive therapy or switching to less potent anti-resorptive agents.

It is well recognised that the length of exposure to antiresorptive medication is one of the main risk factors for MRONJ (Bamias et al., 2005; Badros et al., 2006). Changing bisphosphonates’ schedule of administration could lead to a reduction of the incidence of MRONJ, while maintaining the same therapeutic
efficacy. In a retrospective single-centre study, Corso et al have investigated the incidence of MRONJ and skeletal-related events (SREs) in a cohort of multiple myeloma patients. A total of 106 patients were treated with zoledronic acid administered either with a conventional schedule of monthly infusions (n=51 patients) or with a reduced dosage regimen of monthly infusions during the first year, followed by a 3 monthly dose (n=55 patients). The incidence of SRE was similar in both groups, while the prevalence of MRONJ was eight-fold lower in the reduced dosage group (six MRONJ cases vs one MORNJ case) (Corso et al., 2007).

In a phase 3 open-label randomised non-inferiority trial of a reduced dosage of zometa (one infusion very 4 weeks) vs traditional schedule (one infusion very 4 weeks), Amadori et al reported a lower prevalence of MRONJ in the group of patients using the reduced dosage (1.4% vs 1.9%) (Amadori et al., 2013).

It is also well established that patients receiving yearly IV zoledronic acid have a much lower risk of developing MRONJ than patients using the monthly schedule. Similarly, available literature has clearly indicated that patients using the 6-monthly schedule of denosumab have a much lower prevalence of MRONJ that patient on the monthly schedule.

No data is available in literature on the potential benefits of reducing anti-resorptive dosage in osteoporotic patients with respect to decreasing MRONJ risk.

3.4 Reducing dental infections and the need for dental extractions through a programme of dental check-ups/preventative dentistry

There is a general consensus that patients before commencing and during anti-resorptive therapy should undergo a constant dental monitoring and should maintain a good oral hygiene to avoid any potential surgical interventions. All patients should receive a programme of dental prophylaxis so to prevent dental infection and the need for dento-alveolar surgery. (Dimopoulos et al., 2009, Ripamonti et al., 2009).

De Iuliis et al reported no cases of MORNJ in a group of 200 patients who received a dental assessment and preventative dentistry before starting zoledronic acid therapy (De Iuliis et al., 2014).
Ripamonti et al compared a group of cancer patients who prospectively underwent a programme of dental prevention before starting anti-resorptive therapy with a historical cohort who did not receive prophylactic dental screening/treatment. The outcome of the study showed that there was a significant reduction of MRONJ incidence in the group who received prophylactic dental screening and treatment (Ripamonti et al., 2009). Bonacina et al published similar outcomes highlighting the importance of dental preventive measures before starting any bisphosphonate treatments (Bonacina et al., 2011).

Vandone et al reported 6 cases of MRONJ in a group of cancer patients prospectively managed with baseline dental assessment and a programme of prophylactic dentistry as opposed to 11 cases of MRONJ in the historical group of patients who did not receive any dental screenings/preventative intervention (Vandone et al., 2012).

Dimopoulos et al published a study that investigated whether the incidence of MRONJ in 90 consecutive patients decreased after the implementation of preventive measures compared with 38 patients who received the drug before dental screening. There was a statistically significant, almost 3-fold reduction in the incidence of osteonecrosis when preventive measures were put in place (Dimopoulos et al., 2009).

More recently, Bramati et al reported on the beneficial effects of a strict programme of dental prophylaxis in 212 cancer patients. The identified no cases of MRONJ as opposed to 8.6% prevalence of MORNJ in an historical cohort who did not receive dental prophylaxis (Bramati et al., 2015).

Although these preventive measures are currently applied globally, there remains no robust evidence of the risk reduction effects due to the lack of well-designed clinical trial.

3.5 Use of antibiotics in patients undergoing dental extractions

Osteonecrosis of the jaws may develop spontaneously or can be triggered by invasive dental procedures, mainly tooth extractions. A number of peri-operative measures have been proposed with the aim of reducing the risk of MRONJ. However, the vast majority of these measures are supported by weak,
if any, evidence (Edwards et al., 2008; Montefusco et al., 2008; Bagan et al., 2009; Diniz-Freitas et al., 2012; Kuhl et al., 2012; Cabras et al., 2021).

A systematic review was published on the use of risk-reduction perio-operative procedures including antibiotic prophylaxis in patients treated with anti-resorptive or antiangiogenic drugs and undergoing dental extractions. No randomized controlled trials were found, with all published studies consisting of case series or cohort studies (Diniz-Freitas and Limeres, 2016; Cabras et al., 2021). This review found no convincing evidence supporting any of the proposed MRONJ risk reduction protocols in patients treated with anti-resorptive or anti-angiogenic drugs subjected to tooth extraction.

Montefusco et al studied a group of bisphosphonates-exposed multiple myeloma patients receiving dental extractions and observed 8 cases of MRONJ in the 71 patients who did not receive prophylactic antibiotics, as opposed to no MRONJ case in 43 patients who received antibiotic prophylaxis (Montefusco et al., 2008)

Lodi et al were probably the first authors to propose a specific protocol of antibiotic therapy for tooth extraction in patients treated with intravenous BPs, based on local and systemic infection control measures. They conducted a prospective study of 23 patients who received 38 dental extractions. No cases of MRONJ over a minimum follow-up period of 12 months were detected (Lodi et al., 2010).

3.6 Modified surgical procedures in patients undergoing dental extractions.

A number of surgical procedures have been suggested to potentially reduce the risk of MRONJ in patients undergoing dental extractions.

In their prospective study, Mozzati et al recruited 176 patients using intravenous zoledronic acid who received a total of 542 extractions. Study participants were randomised into two groups of surgical protocols: dental extraction with mucoperiosteal flap so to allow first-intention closure and healing vs dental extraction with mucoperiosteal flap with the addition of plasma rich in growth factors (PRGF) placed in the alveolar socket before first intention closure. After a follow-up period of between 24-60 months, they observed 5 cases of MRONJ
(1.8%) in the first group and none in the second (Mozzati et al., 2012). Scoletta et al also proposed the use of autologous plasma rich in growth factors associated to systemic antibiotic treatment in order reduce the risk of MRONJ. Their prospective uncontrolled study involved 65 patients who received 220 extractions, with MRONJ developing in 7.6% of the cases (Scoletta et al., 2011).

Vescovi et al studied the effect of low-power laser irradiation associated with antibiotic prophylaxis in reducing the post-operative risk of MRONJ. They studies a series of 91 cancer patients on intravenous BPs who received neodymium-doped yttrium aluminum garnet (Nd:YAG) laser bio-stimulation immediately after extraction and weekly for 6 weeks or until closure of the surgical wound, and observed 5 cases of MRONJ (5.5%) (Vescovi et al., 2013). The same authors reported no cases of MRONJ in 126 osteoporosis patients using oral BPs who underwent tooth extraction following the same procedure of post-operative Nd:YAG laser bio-stimulation (Vescovi et al., 2013).

Mozzati et al conducted a prospective study of 700 patients undergoing 1480 extractions, who were randomised to two groups: in the first group, extraction surgery involved the raising of a mucoperiosteal flap to allow first-intention closure and the use of a resorbable haemostatic sponge in the extraction socket; in the second group, extraction was performed without flap and with no haemostatic sponge. After 12-72 months of follow-up, no cases of MRONJ were detected in either group. This study was conducted on osteoporosis patients using oral bisphosphonates (Mozzati et al., 2013).

In an observational longitudinal uncontrolled study, Ferlito et al performed dental extraction with the removal of the alveolar bone (alveolectomy) and antimicrobial therapy (antibiotics and mouthwash) in 43 cancer patients using zoledronate. After a follow-up of 12 months, they observed no cases of MRONJ (Ferlito et al., 2011).

Scoletta et al reported two case series of patients receiving both IV and oral bisphosphonates who underwent dental extractions as per surgical protocols using (i) autologous plasma rich in growth factors, flap surgery and piezoelectric equipment, and (ii) autologous plasma rich in growth factors only. They reported five and one case of MRONJ respectively, however there was no
comparison to concurrent or historical controls (Scoletta et al., 2011, Scoletta et al., 2013)

Lodi et al conducted a prospective case series of a surgical protocol including alveoloplasty with primary closure. Twenty-three consecutive patients using intravenous bisphosphonates received this surgical protocol and were followed up for 3-36 months. No patient developed MRONJ (Lodi et al., 2010).

In a randomised control clinical trial performed in 176 patients using IV bisphosphonates, Mozzati et al reported that no cases of MRONJ were observed in any of the 91 patients treated with PGRF following dental extraction compared with 5 cases of MRONJ (1.9%) in the 85 patients who did not receive PRGF (Mozzati et al., 2012).

Matsumoto et al have recently published a prospective study of 19 patients treated with denusumab who underwent dental extraction as per the following protocol: pre-operative antibiotic, alveolectomy and primary closure. Patients were followed up for 3 months, and 2 cases of MRONJ were observed (10.5%) (Matsumoto et al., 2017).

A recent RCT study with a sample size of 160 patients has confirmed that a primary wound closure using a full-thickness mucoperiosteal flap is superior compared to a primary wound closure using a split-thickness flap (Ristow et al., 2021). Ristow et al reported a high MRONJ incidence in the group where split-thickness flap (23.4% vs 6.3%). However, the study presented a number of limitations including the sample size and the patients heterogenicity (in terms of comorbidities and length of drug exposure) (Ristow et al., 2021).

3.7 Suspension of anti-resorptive therapy in patients undergoing dental extractions and use of bone turn-over biomarkers.

Marx et al suggested that the carboxy-terminal telopeptide cross-linked type 1 collagen (CTX) test can be used to assess the degree of bone turnover suppression and, consequently, the risk of developing MRONJ. He suggested that patients with a marked decrease in bone resorption, indicated by an abnormally low (<150 pg/mL blood) CTX I fasting serum level, have the greatest risk of developing MRONJ (Marx et al., 2007).

However there remains little evidence to support the measurement of CTX as
a predictor of the risk of MRONJ development prior to dental extraction (Hellstein et al., 2011, Ruggiero et al., 2014).

Indeed O’Connell et al conducted a prospective study on 21 patients receiving oral bisphosphonates for osteoporosis/osteopenia and requiring dentoalveolar surgery. All patients underwent a preoperative fasting blood serum analysis of their CTX level. The analysis resulted in 10 of the 21 patients (47.6%) with a CTX level less than 150 pg/mL. Regardless of this finding, all patients had the planned surgery without delay. During a follow-up period of 5 months (range 3 to 11), all patients healed normally without the development of postoperative MRONJ (O’Connell et al., 2012).

Hutcheson et al carried out a prospective study with a much bigger sample size including 950 patients requiring dental extraction and receiving treatment for osteoporosis with oral bisphosphonate. All patients underwent a preoperative fasting blood serum analysis of their CTX level. Of these 950 patients, 282 (29.7%) had a CTX level less than 150 pg/mL. These patients were offered a “drug holiday”, however, only 101 agreed to it. Of the remaining 181 who underwent extraction with low levels of CTX, four developed MRONJ. None of the patients with a CTX level greater than 150 pg/mL (either at baseline or following a “drug holiday”) developed MRONJ (Hutcheson et al., 2014).

A systematic literature review with meta-analysis conducted by Enciso et al showed no significant increased risk of developing MRONJ in patients with an serum CTX value below 150 pg/mL.. The review was based on 16 articles (9 controlled studies and 7 case series). The meta-analysis, conducted on 9 controlled studies, revealed no significant difference in mean serum CTX values between patients with MRONJ and control participants. Moreover, a notable risk of bias was highlighted for many of the reviewed studies (Enciso et al., 2016).

Similar results were reported in another systematic review by Dal Prá et al (Dal Prá et al., 2017).

The concept of a ‘drug holiday’ has been introduced by some authors who suggested that the temporarily suspension of anti-resorptive medications in individuals due to undergo dental extraction, or immediately after dental extraction, might result in a reduced risk of MRONJ development (Khan et al., 2008, Yoneda et al., 2010). The International Task Force on Osteonecrosis of
the Jaw suggested that, “in those patients at high risk for the development of ONJ, including cancer patients receiving high dose bisphosphonates or denosumab therapy, consideration should be given to withholding anti-resorptive therapy following extensive oral surgery until the surgical site heals with mature mucosal coverage.” This task force suggests a “drug holiday” of approximately 6 weeks starting from the day of the surgery so to allow complete healing of the bone (Carini et al., 2012).

Hasegawa et al compared the outcomes of 434 dental extractions in 201 patients divided into two groups: those who had discontinued oral bisphosphonates 3 months before surgery with those who continued to take oral bisphosphonates. After 120 weeks of follow-up, they observed 1 case of MRONJ in the group that had not discontinued oral anti-resorptive therapy (Hasegawa et al., 2013). However, in a much larger study of 1175 patients using oral bisphophonate, they found no evidence that temporary suspension of anti-resorptive therapy might results into a reduced risk of developing MRONJ (Hasegawa et al., 2017).

3.8 The role of genetic and pharmacogenomics in MRONJ preventive strategy

Pharmacogenetics has emerged as a viable option in recent years as a testing method to identify the possible genetic tendency and pathophysiology of MRONJ (Sarasquete et al., 2008). The purpose of these tests was to see whether variations in a patient’s genetic code might influence their responses to certain medications. A great number of genome-wide association studies (GWAS), candidate gene studies (CGs), and whole-genome studies (WGs) have been carried out in recent years. This has been done in an effort to obtain a better knowledge of the several types of genetic variations involved in MRONJ (Sarasquete et al., 2008; Hicks et al., 2017). An earlier study found a significant correlation between MRONJ and genetic variation in a number of genes, including TGF-β, RANK, OPG, and OPN, which are responsible for osteoclast differentiation and activation. Nonetheless, despite the fact that several genetic studies, including GWAS, WES, and CGS, have been published in an effort to define the appropriate pathogenic pathways, these
mechanisms have not yet been discovered (Guo et al., 2020). In addition, despite the fact that potential associations between the development of MRONJ and a number of genes have been documented in published research, none of these studies has been successful in elucidating the role of any gene as a risk factor for the development of MRONJ. The vast amount of research published in the effort to prevent and find a link between genetic predisposition and MRONJ was highlighted in a recent meta-review published by Sacco et al. (Sacco et al., 2022a). The review found a lot of inconclusive and discordant opinions that failed to determine any links between pharmacogenomics and MRONJ susceptibility (Sacco et al., 2022a).

3.9 Conclusion

There is no robust evidence supporting the efficacy of any of the above-mentioned strategies in reducing the risk of MRONJ development, with the exception of the very likely benefits of reducing the cumulative dosage of anti-resorptive therapy. There remains therefore no convincing guidance for clinicians regarding the surgical protocols to adopt in individuals using anti-resorptive medications who are due to undergo dental extractions. The literature suggests a variety of protocols and procedures that have very little, if any, supporting evidence, which translated into an unmet need of better research and well-designed clinical studies.
CHAPTER 4 KNOWLEDGE GAP

4.1 Knowledge gap and objectives of the present study
Dento-alveolar surgery is known to represent one of the major risk factors for MRONJ development (Ruggiero et al., 2014; Ruggiero et al., 2022). Due to inconsistent results of previous research, it remains unclear whether the use of dedicated peri-operative or surgical procedures might result in a lower risk of MRONJ development. This is a major unmet healthcare need as anti-resorptive medications are widely used by individuals with metastatic cancer and osteoporosis, and a notable proportion of these individuals regularly undergo dental extraction due to concomitant periodontal disease or non-restorable dental caries or fractures.

Recently an umbrella review published by Sacco et al has underlined the limited high strength evidence to support many of the current recommendations associated to medication-related osteonecrosis of the jaw (Sacco et al., 2021b). In addition, Sacco et al has also reported the low-quality systematic reviews and meta-analyses performed in the last 18 years of research with a particular emphasis on preventive strategies type of reviews (Sacco et al., 2021b).

The aim of the present research is to perform a systematic review of available literature investigating MRONJ risk reduction strategies in individuals who use anti-resorptive drugs and are undergoing dental extractions. Additionally, two retrospective service evaluation cohort studies will also be conducted to evaluate the role of conservative adjusted protocols in two different risk categories of MRONJ patients. The results of these studies will be used to inform the design of a future clinical study aimed at comparing risk reduction strategies in individuals who use anti-resorptive drugs and are due to undergo dental extractions.
Figure 4.1: Research Gantt chart
CHAPTER 5 RISK REDUCTION STRATEGY IN PATIENT AT RISK OF MEDICATION RELATED OSTEONECROSIS OF THE JAW UNDERGOING DENTAL EXTRACTION: SYSTEMATIC REVIEW AND META-ANALYSIS

5.1 Abstract

Background: Medication-related Osteonecrosis of the jaw (MRONJ) has been known to be associated with patients receiving bisphosphonate (BP) therapy. However, recently it has been recognised that other medications, such as RANK ligand inhibitors (Denosumab) and antiangiogenic drugs can also cause MRONJ. Despite the evidence base over a twenty-year period, limited progress has been made in terms of preventive measures to be undertaken to reduce the risk of ONJ for patients undergoing dental extractions. The aim of this paper was to identify oral surgery protocols that may help to prevent or reduce the incidence of MRONJ following dental extractions. Material and methods: A multi-database (PubMed/MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials- CENTRAL) systematic search was performed up to May 2023. Any type of comparative studies considering human patients with a history of or current treatment with antiresorptive or antiangiogenic drugs and were undergoing dentoalveolar surgery was considered. Results: Nine studies fulfilled the inclusion criteria for the systematic analysis, with a total of 1948 patients. Four of the studies were case-control studies (CCS) and five were randomised clinical trials (RCT). The overall MRONJ overall occurrence was 1.5% (n=29). The RCT studies reported osteonecrosis predominantly in the study groups and (n=15) while in the control groups and (n=10). On the other hand, the CCS studies reported equally distribute osteonecrosis in both groups and (study groups n=2 and control groups n=2). Conclusions: With the current limited scientific evidence, we are unable to support or refute the effectiveness of MRONJ prevention protocols in oral surgery. Indeed, additional high-quality studies are needed.
5.2 Materials and Methods

5.2.1 Search protocol
The present systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist (Liberati et al., 2009).

The protocol was registered in the international platform of registered systematic review and meta-analysis protocols (INPLASY) under the number INPLASY202160064.

An electronic search of the literature was performed using the following databases: PubMed/MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) up to May 2023. A two-stage process was carried out to increase the precision and quality of the search: an initial screening of titles and abstracts identified the studies to be subsequently assessed in depth as full text. The search was carried out independently by two authors. Any disagreement was resolved through discussion between the authors and a third author. Data screening and extraction forms were used to verify the study’s eligibility as per pre-defined inclusion/exclusion criteria, carry out the methodological quality assessment, and extract data on study characteristics and relevant outcomes. For studies considered eligible for inclusion but presented insufficient or missing information in the title, abstract or content, authors were contacted for further clarification and a period of 6 weeks was allowed for their response. Where no clarification was provided, the relevant missing data were presented as not reported. Clinical questions were broken down and formulated using the PICO framework (Schardt et al., 2007).

5.2.2 Focused question and PICO framework
The focused question for this review was: “in individuals exposed to anti-resorptive and/or anti-angiogenic medications undergoing dental extractions, can any risk-reduction strategy lessen the risk of developing MRONJ?” The population of interest included any patient previously exposed to or actively receiving treatment with MRONJ-associated medications. The interventions included any type of risk-reduction strategy. The outcome was the development of MRONJ to the site of dental extraction.
5.2.3 Studies Objectives

• Primary objective
The primary objective of this review was to assess the effects of risk-reduction interventions in lessening the risk of MRONJ development in individuals exposed to anti-resorptive and/or anti-angiogenic medications undergoing dental extractions. For this objective, we considered no restrictions on the duration of drug therapy prior to dental surgery as well as no restrictions on the stratification of groups into patients defined as “low risk” and patients defined as “high risk”, based on the type of disease (cancer or osteoporosis), the type of MRONJ-associated medication and the cumulative duration of anti-resorptive therapy prior to dental extraction.

• Secondary objectives
Further data, where available, were collected on safety of and adverse effects of the proposed interventions. Additionally, we attempted to assess the effects of risk-reduction interventions in lessening the risk of MRONJ after stratifying individuals into homogeneous risk groups, where possible. Patients were classified at high risk of MRONJ development when they had (i) a diagnosis of metastatic cancer or multiple myeloma and received intravenous BPs for more than two years or (ii) a diagnosis of osteoporosis and received oral BPs or intravenous BPs at osteoporosis dosage for more than five years. Patients were classified at low risk of MRONJ development when they had (i) a diagnosis of metastatic cancer or multiple myeloma and received intravenous BPs for less than two years or (ii) osteoporosis and received oral BP or intravenous BPs at osteoporosis dosage for less than five years. The risk stratification was based on available literature suggesting a higher incidence of MRONJ after a median of two years of zoledronic acid and five years of alendronic acid in cancer and osteoporosis patients respectively (Mercer et al., 2013; Gabbert et al., 2015; Fung et al., 2017). The stratification into risk groups was not attempted for studies recruiting patients using denosumab or antiangiogenic agents due to a lack of studies suggesting a clear-cut threshold of risk.
5.2.4 Inclusion Criteria

- **Types of studies**
  We included only comparative type of studies such as randomised control trials (RCTs) and case-control studies (CCS). Considering that the first case of MRONJ was reported in 2003, papers were searched from January 2003 to May 2023. Included studies were also restricted to those where there had been a minimum of 8-week follow-up post-operatively and no history of radiation therapy to the jaws, as per the established definition of MRONJ (Khosla et al., 2007; Ruggiero et al., 2014, Ruggiero et al., 2022). Animal studies, case series, case reports and literature reviews were excluded. No language restrictions were imposed on the search.

- **Types of interventions**
  We considered any intervention aimed at reducing the risk of MRONJ in individuals undergoing dental extractions, including but not limited to local application of antimicrobial agents such as Chlorhexidine mouthwash, perioperative antibiotics (before and/or after surgery), primary wound closure/suturing, alveolectomy/alveoplasty, use of piezosurgery, use of platelet-rich growth factor (PRGF), use of low-power laser, sonosurgery, temporary suspension of antiresorptive or antiangiogenic therapy (drug holiday), and use of hyperbaric oxygen before and/or after surgery.

5.2.5 Data extraction

All selected papers were analysed to identify the following data: author(s), year of publication, study design, population characteristics, type of drug therapy, characteristics of adjusted dental extraction protocol and MRONJ occurrence rate. The extracted data were registered in tables. When some information was not reported, the box was filled in with NR (Not Reported).

5.2.6 Statistical analysis

Descriptive analysis was used for quantitative variables and as numbers and proportions for categorical findings. A meta-analysis was performed only for the RCT studies. The analyses compared the efficacy (number of MRONJ events)
of the experimental extraction protocol (study group) versus the control group. We planned to measure the effect of risk-reduction intervention as risk ratios (RR) with 95% confidence intervals (CI) (primary objective). The statistical heterogeneity will be tested with Cochran’s Q-test and the I2 statistic. Estimates of effect will be combined with a random-effects model in case of heterogeneity (Q-test p-value < .10). The surgical extraction techniques were compared for the development of MRONJ to possibly identify a more predictable surgical protocol for avoiding MRONJ. The Z test was used to compare the proportions between groups. Results were considered significant at the 5% level (p < 0.05). Analyses were done using JAMOVI (version 2.2.5 - Computer Software, Sydney, Australia)

5.2.7 Risk of bias assessment
Three authors independently assessed the risk of bias in the included RCTs and case-control studies as per the “Cochrane Handbook for Systematic Reviews of Interventions” guidelines and “Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I)” respectively (Sterne et al., 2016; Sterne et al., 2019). The assessment of the quality of evidence and a summary of the findings was conducted using the software GRADE profiler (GRADEpro), as recommended by the Cochrane Collaboration and GRADE Working Group.

5.3 Results
The article selection process is illustrated in Figure 5.1. The literature search yielded 1585 references after removal of duplicates. After screening the titles and abstracts, 24 articles were further examined as full text. Of these, 15 articles were eliminated as the inclusion criteria were not met. This resulted in 9 studies to be included in this systematic review. As there were no comparable studies and because quantitative data could not be statistically combined for a meta-analysis, a narrative/descriptive summary was undertaken.
• **Summary of included studies**

Nine studies were included in the systematic review, of which four were CCS (Hanson et al., 2010; Hasegawa et al., 2013; Asaka et al., 2017; Kang et al., 2020), and five were RCTs (Mozzati et al., 2012, Mozzati et al., 2013; Poxleitner et al., 2020; Ristow et al., 2021; Ottesen et al. 2022). Out of the four CCS, one study compared the use of perioperative antibiotics versus perioperative Chlorhexidine 0.2% mouth rinse (Hanson et al., 2010), whilst another evaluated the use peri-operative antibiotics and bisphosphonate discontinuation with platelet-rich growth factor and wound suturing/healing by secondary intention versus peri-operative antibiotics and bisphosphonate discontinuation with wound suturing/healing by secondary intention (Asaka et al., 2017). The other two CCS evaluated perioperative antibiotics with bisphosphonate discontinuation versus perioperative antibiotics only (Hasegawa et al., 2013; Kang et al., 2020). Out of the five RCTs, one assessed the addition of platelet-rich growth factor to alveoplasty, perioperative antibiotics and wound closure by primary intention (Mozzati et al., 2012), whereas another trial compared mucoperiosteal flap, alveoplasty with wound closure by primary intention and perioperative antibiotics versus wound healing.
by secondary intention, the use of a haemostatic gelatine sponge and perioperative antibiotics (Mozzati et al., 2013).

Another RCT compared alveoplasty, the use of platelet-rich fibrin, wound healing by secondary intention, perioperative antibiotics and chlorhexidine versus alveoplasty, wound healing by primary intention, perioperative antibiotics and Chlorhexidine (Poxleitner et al., 2020). Ristow et al. compared alveoplasty, primary wound closure with a mucoperiosteal flap, perioperative antibiotics and chlorhexidine, and bisphosphonate discontinuation versus alveoplasty, primary wound closure with a mucosal/epi-periosteal flap, perioperative antibiotics and chlorhexidine, and bisphosphonate discontinuation (Ristow et al., 2021). The fifth RCT (Ottesen et al, 2022) included in this systematic review evaluated perioperative antibiotics and chlorhexidine, antiresorptive discontinuation, and mucoperiostal flap with primary closure versus perioperative antibiotics and chlorhexidine, and antiresorptive discontinuation only.

A summary of the above studies and related patient groups is provided in Tables 5.1 and 5.2. Of the nine studies reviewed, one RCT recruited only oncology participants exposed to intravenous bisphosphonates (Mozzati et al., 2012), one RCT recruited only oncology patients exposed to denosumab and bisphosphonates (Ottesen et al 2022), three CCS and two RCTs included only osteoporotic patients undertaking oral bisphosphonate therapy (Hanson et al., 2010; Hasegawa et al., 2013; Mozzati et al., 2013; Asaka et al., 2017; Poxleitner et al., 2020) and one RCT and one CCS included a mixed group of oncology and osteoporotic patients receiving either oral or intravenous bisphosphonates Table 5.1 (Kang et al., 2020; Ristow et al., 2021).
**Table 5.1** Patient stratification according the type of disease (cancer VS osteoporosis) and route of drug administration.

*The stratification was based on the median time of drug exposure.

**Cancer patients on IV ZOL:**
- Gabbert et al 2015: median 25.0, Mean 30.3 (SD 22.6) 963 cancer patients,
- Fung et al 2017: median 2.2 years (Mean 26.4 months) 237 cancer patients.

**Osteoporotic patients on PO ALE:**
- Mercer et al 2013: Median 60 months 91 osteoporotic patients,
- Fung et al 2017: median 5.3 (mean 63.6 months) 112 osteoporotic patients
<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Population in study</th>
<th>Intervention</th>
<th>Males: Females ratio</th>
<th>Patient’s number in control group</th>
<th>Patient’s number in study group</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanson et al 2010</td>
<td>CCS</td>
<td>33</td>
<td>Perioperative antibiotics vs perioperative Chlorhexidine 0.2% mouth rinse</td>
<td>unclear</td>
<td>11</td>
<td>22</td>
<td>History or currently taking bisphosphonate therapy needed dental extraction</td>
<td>Not reported</td>
</tr>
<tr>
<td>Mozzati et al 2012</td>
<td>RCT</td>
<td>176</td>
<td>Dental extraction using platelet-rich growth factor (PRGF) with primary intention closure vs dental extraction with primary intention closure</td>
<td>75:101</td>
<td>85</td>
<td>91</td>
<td>Current IV bisphosphonate therapy (Zoledronic acid) for oncological pathology Need for dental extraction</td>
<td>History of irradiation to the head and neck Dental extractions prior to study period</td>
</tr>
<tr>
<td>Mozzati et al 2013</td>
<td>RCT</td>
<td>700</td>
<td>Dental extraction performed with alveoloplasty and primary wound closure vs wound healing with secondary intention</td>
<td>23:677</td>
<td>366</td>
<td>334</td>
<td>Current oral bisphosphonate therapy Oral Bisphosphonate for more than 24 months Need for dental extraction</td>
<td>History of irradiation to the head and neck Dental extractions prior to study period</td>
</tr>
<tr>
<td>Hasegawa et al 2013</td>
<td>CCS</td>
<td>212</td>
<td>Drug holiday vs perioperative antibiotics</td>
<td>19:193</td>
<td>111</td>
<td>101</td>
<td>Current oral bisphosphonate therapy Need for dental extraction</td>
<td>Not reported</td>
</tr>
<tr>
<td>Asaka et al 2017</td>
<td>CCS</td>
<td>102</td>
<td>Dental extraction using platelet-rich growth factor (PRGF) vs simple dental extraction</td>
<td>9:93</td>
<td>73</td>
<td>29</td>
<td>Oral bisphosphonate for osteoporosis or steroid-induced osteoporosis for more than 1 year Need for dental extraction</td>
<td>History of irradiation to the head and neck High dose steroid (50mg or more per day); Active infection; Poor general conditions; Jaw cancer</td>
</tr>
<tr>
<td>Kang et al 2020</td>
<td>CCS</td>
<td>465</td>
<td>Drug holiday vs perioperative antibiotics</td>
<td>45:420</td>
<td>179</td>
<td>286</td>
<td>History bisphosphonate therapy Needed dental extraction</td>
<td>History of irradiation to the head and neck Unclear diagnosis of osteonecrosis before tooth extraction or who exhibited osteonecrosis during the procedure.</td>
</tr>
<tr>
<td>Poxelitner et al 2020</td>
<td>RCT</td>
<td>77</td>
<td>Dental extraction using platelet-rich fibrin (PRF), alveoloplasty and wound healing by secondary intention vs dental extraction with alveoloplasty</td>
<td>1:78</td>
<td>39</td>
<td>38</td>
<td>Osteoporosis History or currently taking bisphosphonate therapy Needed dental extraction</td>
<td>History of irradiation to the head and neck Neoplastic involvement/disease of the maxillofacial region</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>N</td>
<td>Procedure</td>
<td>Control Group</td>
<td>Criteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------</td>
<td>----</td>
<td>----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ristow et al 2021</td>
<td>RCT</td>
<td>160</td>
<td>Dental extraction with full thickness muco-periosteal flap with wound closure by primary intention vs extraction with split-thickness muco-periosteal mucosal flap with wound closure by primary intention</td>
<td>Controls = extraction with split-thickness muco-periosteal mucosal flap with wound closure by primary intention</td>
<td>History or currently taking bisphosphonate therapy or denosumab therapy patients with malignancies with metastasis and adjuvant antiresorptive therapy; Exposed bone or existing diagnosis of osteonecrosis of the jaw at the extraction site; History of irradiation to the head and neck; History of irradiation to the head and neck; Neoplastic involvement/disease of the maxillofacial region; Age &lt;18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ottesen et al 2022</td>
<td>RCT</td>
<td>23</td>
<td>Muco-periosteal flap elevation with primary closure with and without antiresorptive drug discontinuation</td>
<td>Controls = extraction with split-thickness muco-periosteal mucosal flap with wound closure by primary intention</td>
<td>History or currently taking antiresorptive drugs (included denosumab) therapy or radiation therapy patients with malignancies with metastasis and adjuvant antiresorptive therapy; Exposed bone or existing diagnosis of osteonecrosis of the jaw at the extraction site; History of irradiation to the head and neck; History of irradiation to the head and neck; Neoplastic involvement/disease of the maxillofacial region; Age &lt;18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.2** Summary of the included studies; Randomised control trial (RCT), Case control study (CCS)
• **Stratification of patients into risk groups**

Stratification of patients into pre-defined risk groups (secondary objective) was not possible as none of the selected studies included sufficient details on the duration of antiresorptive therapy before dental extraction. Where available, the limited information provided indicated notable intra-study and inter-study heterogeneity with respect to the duration of antiresorptive therapy before surgery, therefore suggesting that data relevant to patients with different risk profiles were merged and analysed together.

• **Case-control studies**

  a) *Dental extraction using platelet-rich growth factor (PRGF) vs simple dental extraction.*

Asaka et al. investigated the effects of platelet-rich growth factor (PRGF) together with peri-operative antibiotics and BPs discontinuation in a case-control study recruiting 29 participants prospectively and compared them to a historical group of 73 individuals (Asaka et al., 2017). All participants had a diagnosis of osteoporosis and a history of oral BP therapy. All 29 participants received PRGF, which was applied onto the surgical site, and perioperative antibiotics, namely amoxicillin 250 mg three times a day or clindamycin 150 mg four times a day for 1 week, starting from the morning of the surgery. In the historical control group, no patients received PRGF. However, only 28 out of 73 patients in the control group received peri-operative antibiotics in the form of either of the following; amoxicillin, cefcapene, or clindamycin (the precise regimens were not reported). BPs therapy was suspended peri-operatively in 25 out of 29 (86.2%) subjects in the study group and 51 out of 73 individuals (69.8%) in the control group. All subjects received oral BPs therapy for at least 12 months and the mean duration of therapy was 51 months in the control group (range: 13-120) vs 31 months in the study group (range: 12-102) (Table 5.3 and Table 5.4). It was therefore not possible to stratify patients into high and low risk groups using the threshold of 5 years of oral
<table>
<thead>
<tr>
<th>Paper</th>
<th>Number of extractions</th>
<th>Extraction site for control group</th>
<th>Extraction site for study group</th>
<th>Antibiotic regime</th>
<th>Nature of extraction</th>
<th>Monitoring period</th>
<th>Postoperative imaging</th>
<th>Follow-up study group</th>
</tr>
</thead>
</table>
| Hanson et al 2010            | 64                    | Unclear                           | Unclear                         | Only for the control group:  
- 3 g amoxicillin 1 h preoperatively  
- 3 g amoxicillin oral 1 h pre-operatively and  
250 mg three times a day for 5 days post operatively  
- 1 g amoxicillin + 400 mg metronidazole pre-operatively either at  
250/200 mg 5 days  
- 1 g amoxicillin + 400 mg metronidazole pre-operatively and amoxicillin 250 mg  
tsds for 5 days post-operatively | Not reported                                                        | 4, 12, and a 24 weeks                                                      | Nil                                                                  | 6 months |
| Mozzati et al 2012           | 542                   | Mandible - 145 Maxilla - 122      | Mandible - 142 Maxilla - 133    | Co-Amoxiclav 1g tds 6/7  
Erythromycin 600mg qds 6/7 if penicillin allergic  
Commenced evening prior to surgery | Surgical extractions using intraosseous incision and full mucoperiosteal flap;  
Careful curettage of socket | Days 3, 7, 14, 21, 30, 60, 90, 120 | DPT and CT at 6 months;  
DPT 6 monthly; CT annually | 24-60 months |
| Mozzati et al 2013           | 1480                  | Mandible - 496 Maxilla - 364      | Mandible - 368 Maxilla - 252    | Co-Amoxiclav 1g tds 6/7  
Erythromycin 600mg qds 6/7 if penicillin allergic  
Commenced evening prior to surgery | Surgical extractions for study group using intraosseous incision and full mucoperiosteal flap;  
Careful curettage of socket; Flapless extraction for control group, socket filled with haemostatic gelatin sponge. | Days 3, 7, 14, 21, 30, 60, 90 | DPT at 12 months  
DPT 6 monthly | 12-72 months |
Intravenous penicillin-based (Vicillim/Ampicillin-Sulbactam)-12 control,  
32 study;  
Intravenous cephem-based (Cefmetazole) – 0 control, 12 study;  
Intravenous Lincomycin-based (Clindamycin) – 20 control, 45 study;  
Omal Penicillin-based (Amoxicillin), 1 control, 1 study;  
Omal cephem-based (Cefditoren pivoxil/Cefcapene pivoxil) - 35 control, 75  
study;  
Omal macrolide-based (Azithromycin/Clarithromycin/Roxithromycin) – 1 control, 13 study. | Simple extraction or incision/bone removal/ root sectioning/ suture | Up to 1 week  
From 1 – 2 weeks  
From 2 – 3 weeks  
From 3 – 4 weeks  
From 4 – 8 weeks  
More than 8 weeks | Nil                                                                  | Nil |
| Asaka et al 2017             | 218                   | Mandible – 89 Maxilla - 77        | Mandible – 24 Maxilla – 28      | Amoxicillin 250mg tds 7/7 or Clindamycin 150mg qds 7/7 – study group (n=29);  
Extraction +/- full mucoperiosteal flap | Extraction +/- full mucoperiosteal flap | Week 1, 2, 4, 8, 12 | 1 month – study group | 3 months |
<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Incisors</th>
<th>Canines</th>
<th>Premolars</th>
<th>Molars</th>
<th>Intravenous Antibiotics</th>
<th>Oral Antibiotics</th>
<th>Follow-Up</th>
<th>Wound Closure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kang et al 2020</td>
<td>1323</td>
<td>(Incisor – 70, Molar – 96)</td>
<td>(Incisor – 14, Molar – 38)</td>
<td></td>
<td></td>
<td>Amoxicillin/Cefcapene/Clindamycin – duration specified as “several days” – control group (n=28); Commenced morning of surgery</td>
<td>All patients received oral antibiotics prior surgery (not specified)</td>
<td>3 months</td>
<td>3 months – study group</td>
</tr>
<tr>
<td>Poxleitner et al 2020</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td></td>
<td></td>
<td>All patients received perioperative intravenous antibiotic therapy (penicillin 10,000 IU once daily or clindamycin 600 mg three times daily in case of penicillin allergy), initiated 1 day before surgery and continued until 1 day after surgery</td>
<td>Extraction +/- full mucoperiosteal flap</td>
<td>10-14 days and 90 days</td>
<td>Not reported</td>
</tr>
<tr>
<td>Ristow et al 2021</td>
<td>475</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oral antibiotics (Sultamicillin PD 375mg 1-0-1) starting a week before surgery and lasting for a week after surgery. In case of hypersensitivity to penicillin or a penicillin allergy, clindamycin (600mg) was used instead.</td>
<td>All teeth were extracted following a rigid protocol: -adjunctive antibiotic therapy -atraumatic extraction -alveoplasty -primary wound closure</td>
<td>Nil</td>
<td>6 months</td>
</tr>
<tr>
<td>Ottesen et al 2022</td>
<td>31</td>
<td>Unclear</td>
<td>Unclear</td>
<td></td>
<td></td>
<td>Oral antibiotics (amoxicillin 1000 mg and clavulanic acid 250) one hour before the surgical intervention to continue for ten days postoperatively. In case of hypersensitivity to penicillin or a penicillin allergy, clindamycin (600mg) was used instead.</td>
<td>All teeth were extracted following a rigid protocol</td>
<td>1, 3 and 6 months of follow-up</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

**Table 5.3 Extraction protocol**
<table>
<thead>
<tr>
<th>Paper</th>
<th>Complications in study group</th>
<th>Incidence of MRONJ in control group</th>
<th>Incidence of MRONJ in study group</th>
<th>Nature of MRONJ in control group</th>
<th>Nature of MRONJ in study group</th>
<th>Mean time for MRONJ occurrence</th>
<th>Treatment of MRONJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanson et al 2010 Mozzati et al 2012</td>
<td>18 patients delayed healing &gt; 8 weeks None</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>91.6 days</td>
<td>Not reported</td>
</tr>
<tr>
<td>Mozzati et al 2013</td>
<td>None</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hasegawa et al 2013</td>
<td>3 patients delayed healing &gt; 8 weeks</td>
<td>0.9% (1/111)</td>
<td>2% (2/101)</td>
<td>Intraoral fistula</td>
<td>Delayed healing beyond 8 weeks</td>
<td>12 weeks for study group</td>
<td>Antibiotics + irrigation</td>
</tr>
<tr>
<td>Asaka et al 2017</td>
<td>None</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kang et al 2020 Poxleitner et al 2020</td>
<td>Postoperative complications were observed in 7 patients: N=6 in Control group N=1 in the study group (the nature of complication was not specified)</td>
<td>0.6% (1/179)</td>
<td>0 (0%)</td>
<td>Unclear</td>
<td>Unclear</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ristow et al 2021</td>
<td>Unclear</td>
<td>5 (6.3%)</td>
<td>11 (14.3%)</td>
<td>Not reported</td>
<td>Not reported</td>
<td>8 weeks (15 patients had non vital bone at the time of the dental extraction as histopathology confirmed)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Ottesen et al 2022</td>
<td>Three patients died during the trial period</td>
<td>0 (0%)</td>
<td>4 (30.8%)</td>
<td>-</td>
<td>Three patient with exposed bone stage I and one patient with stage 0</td>
<td>3 months later</td>
<td>Two of the four patients had minor surgical block resections the other two were not reported</td>
</tr>
</tbody>
</table>

Table 5.4 MRONJ outcome
BPs therapy as predefined in our protocol. There were no recorded adverse effects associated with the study interventions. No cases of MRONJ were recorded in either of the groups, therefore suggesting no notable difference between the two interventions in reducing the risk of MRONJ. This case-control study was considered to have a serious risk of bias in confounding, selection, measurement of the outcomes and reported results (Figure 5.2).

Figure 5.2 Risk of bias summary for CCS

b) Perioperative antibiotics vs perioperative Chlorhexidine 0.2% mouth rinse

The aim of this research was to evaluate and compare the effect of systemic anti-microbial therapy Vs the local anti-microbial therapy. This study was conducted a case-control study including 33 osteoporosis patients undergoing dental extractions divided into two intervention groups: perioperative antibiotics (n=11 patients) and perioperative chlorhexidine (n=22 patients) (Hanson et al., 2010).

The authors admitted that due to staff turnover, the study guidelines were not rigidly adhered to, which resulted in seven different prophylactic protocols. Most patients were taking alendronic acid (71.0%), followed by risedronic acid (19%) and ibandronic acid (9.5%).

The duration of oral BP therapy was not reported and therefore unable to stratify the study participants as low or high risk. There were no cases of MRONJ in either the peri-operative antibiotic or chlorhexidine group (Table 5.3 and Table
with no suggested difference between the two interventions in MRONJ risk reduction. However, a total of 18 patients reported incomplete soft tissue healing at 8 weeks, therefore potentially classifiable as MRONJ non-exposed cases; unfortunately, their allocation group was not specified. This case-control study was considered to have a serious risk of bias in confounding, selection, measurement of the outcomes and reported result (Figure 5.2).

c) Drug holiday vs perioperative antibiotics

The aim of Hasegawa et al study was to evaluate and compare two different risk reduction strategy: drug holiday vs the use of perioperative systemic anti-microbial drug therapy. In this study, the authors recruited 201 osteoporosis patients due to have dental extractions. A group of patients received a peri-operative antibiotic and a 3-month period of discontinuation of oral BPs therapy prior to dental extraction (group A, n=101) and the other group received only per-operative antibiotics prior the tooth avulsion (group B, n=111) (Hasegawa et al. 2013).

Most patients were taking alendronic acid (61.2%), followed by risedronic acid (38.3%), combined therapy, (3.5%) and minodronic acid (0.5%). Two percent of cases were stated as “unknown oral bisphosphonate”. The duration of BP therapy was stated for all patients except for 40 individuals, with the mean duration being 23.6 (range 1-180 months) and 32.6 months (range 1-120 months) in the two groups, Hence, we were unable to stratify the included patients as low and high risk as per the protocol. Of note, no information was provided regarding the duration of antibiotic use or the type of surgical procedure performed. Furthermore, there was a discrepancy between the number of patients stated as recruited (n=201) versus the total number of patients reported in the study Tables (n=212). The authors reported two cases (1.9%) of incomplete soft tissue healing at 8 weeks (which is in keeping with non-exposed MRONJ) in group A and one case of MRONJ (0.9%) in group B (Table 5.3 and Table 5.4). These results suggest possible reduction in the risk of MRONJ development when extractions were performed with peri-operative antibiotics and a prior 3-month discontinuation of oral BPs therapy in osteoporosis patients. Adverse effects of the study interventions were not
report. This study was considered to have a serious risk of bias in confounding, selection, measurement of the outcomes and reported result (Figure 5.2).

On the other hand, another study of Kang et al similarly was aiming to evaluate and compare two group of osteoporosis patients undergoing dental extractions. Similarly to Hasegawa et al, Kang et al investigated two different risk reduction strategies: pre-operative antibiotics administered 1 hour before extraction (group A of patients, n=179) vs peri-operative antibiotics with discontinuation of BPs therapy prior to dental extraction (group B of patients, n=286) (Kang et al., 2020). No details of the type of antibiotic or the time of BP discontinuation were provided. The authors reported the mean duration of BPs therapy (40 ± 35.6 SD months in group A vs 14 ± 16.8 SD months in group B). The majority of patients received oral bisphosphonates (88%), reported as being alendronic acid in 56% of the discontinuation group. 3% of patients in both groups received oral alendronic acid and intravenous injections of ibandronate. The presence of comorbidities and risk factors for MRONJ was not reported in this study. Kang et al did not carry out a separate analysis of patients on BP treatment for more than three years and therefore risk stratification could not be undertaken (Table 5.3 and Table 5.4). One patient in group A developed MRONJ (0.5%) compared to no cases in group B. There were no other adverse effects reported. This case-control study was considered to have a serious risk of bias in confounding, selection, measurement of the outcomes and reported result (Figure 5.2).

- Randomised clinical trials

  d) Dental extraction using platelet-rich growth factor (PRGF) with primary intention closure vs dental extraction with primary intention closure.

The aim of the Mozzati et al study was to evaluate and compare two different risk reduction strategies: dental extraction using PRGF and alveoloplasty with primary closure and extraction with only primary closure. Mozzati et al. recruited only cancer patients on bisphosphonate IV. A total of 176 cancer patients requiring dental extractions were randomly assigned to two groups: a group treated with PRGF, perioperative antibiotics, alveoplasty, and primary wound
closure (group A, n = 91) vs. a group treated only with perioperative antibiotics and primary wound closure (group B, n = 85) (Mozzati et al., 2012). Antibiotics were given peri-operatively on the evening prior to the dental extraction and continued post-operatively for 6 days. In the case of penicillin allergy, Erythromycin 600mg four times a day for 6 days was provided. A total of 542 extractions were performed with orthopantomography and CT imaging being undertaken preoperatively and at six months postoperatively. Subjects were followed up clinically at days 3, 7, and 14 and up to 120 days postoperatively. No cases of MRONJ were reported in group A, whereas MRONJ developed in five patients (5.8%) of group B (Table 5.3 and Table 5.4).

These results suggest that the addition of PRGF, perioperative antibiotics, alveoloplasty, and primary wound closure may reduce the risk of MRONJ development in high risk oncology patients. There were no reported adverse effects. This study was classified as having a high risk of bias of random sequence generation, allocation concealment, blinding of participants and outcome assessment. There was a low risk of attrition bias and selective reporting (Figure 5.3).

![Figure 5.3 Risk of bias summary for RCTs studies included in the research](image.png)
e) Dental extraction performed with alveoloplasty and primary wound closure vs wound healing with secondary intention

The aim of the Mozzati et al study was to evaluate and compare two different risk reduction strategies: dental extraction performed with alveoloplasty and primary wound closure vs extraction with wound healing with secondary. Mozzati et al recruited in an RCT 700 patients on oral BP therapy. All the participants were randomly allocated to dental extraction performed with alveoloplasty and primary wound closure (group A, n=334) and extraction with wound healing with secondary (group B, n=366) (Mozzati et al., 2013). The researcher reported no study blinding. The duration of oral BP therapy was recorded as being greater than 24 months for all patients, with no further details on the cumulative length of therapy. We were therefore unable to stratify the included patients into low and high-risk groups. No participant in either group developed MRONJ, therefore suggesting no difference between the two interventions in reducing the risk of MRONJ (Table 5.3 and Table 5.4). Although the randomization was computer-generated, no information on allocation concealment was reported in the study, therefore the risk of selection bias was high. We considered the study to be at risk of performance and detection to be high, as the outcome assessors were not blinded to the nature of the interventions. There was a low risk of attrition bias or incomplete reporting (Figure 5.3).

f) Dental extraction using platelet-rich fibrin (PRF), alveoloplasty and wound healing by secondary intention vs dental extraction with alveoloplasty and wound healing by primary intention.

The aim of the Poxleitner et al study was to evaluate and compare two different risk reduction strategies: dental extraction with PRF, alveoloplasty and secondary intention healing vs extraction with alveoloplasty with healing by primary intention. Poxleitner et al randomly assigned via block randomization 77 osteoporotic patients, with both groups also receiving perioperative intravenous antibiotics (one day prior to surgery and continued until 1 day after surgery) and perioperative chlorhexidine mouthwash (Poxleitner et al., 2020). All patients received perioperative intravenous antibiotic therapy (penicillin 10, 000, 000 IU
once daily or clindamycin 600 mg three times daily in case of penicillin allergy), which was initiated one day prior to surgery and continued until 1 day after surgery. Most patients were taking alendronic acid (36%), followed by denosumab (31.2%), ibandronic acid (12%), risendronic acid (10.4%) and zolendronic acid (9.1%). All the study participants had a BPs history, with a mean duration of therapy of 3 years (group A, range of 1-10 years) and 4 years (group B, range of 1-7 years). We were unable to stratify the included patients. None of the patients in either group developed MRONJ (Table 5.3 and Table 5.4). This study was classified as having a low risk of bias of random sequence generation, unclear risk of bias in allocation concealment, high risk of bias in blinding of participants and outcome assessment. There was a low risk of attrition bias and selective reporting (Figure 5.3).

**g) Dental extraction with full thickness muco-periosteal flap with wound closure by primary intention vs extraction with split-thickness muco-periosteal mucosal flap with wound closure by primary intention**

The aim of the Ristow et al study was to evaluate and compare two different surgical procedure strategies: dental extraction with full thickness muco-periosteal flap with primary wound closure vs split-thickness muco-periosteal and primary wound closure. Ristow et al conducted an RCT of 160 participants undergoing antiresorptive medications because of cancer or osteoporosis (Ristow et al, 2021). Participants were randomized into two intervention groups: full thickness muco-periosteal flap with wound closure by primary intention (group A, n= 82) vs split-thickness muco-periosteal flap with wound closure by primary intention (protocol B, n= 78). The authors did not report the length of drug therapy across the groups, hence we were unable to stratify the included patients into low and high-risk groups. However, the authors performed a sub-group analysis by stratifying the participants into two groups: cancer patients undergoing intravenous bisphosphonates or subcutaneous denosumab (high-risk) and osteoporosis patients having weekly oral bisphosphonates or six monthly subcutaneous denosumab (low risk). All patients received peri-operative antibiotics (Sultamicillin 375mg) prior the surgical intervention and for one week postoperatively. Patients with a
hypersensitivity or allergy to penicillin were prescribed clindamycin 600mg. Additionally, 0.2% chlorhexidine mouthwash was used by all the patients 2 days before surgery and for at least 5 days postoperatively. The study assessors were all blinded for the treatment allocation. After 8 weeks from the beginning of the study 2 patients dies from the group A and 1 patient died in the group B. Five participants (5/80, 6.3%) of group A developed MRONJ compared to eleven individuals in group B (11/77, 14.3%). Sub-group analysis showed that 3 (3/44, 6.8%) of the high-risk cancer participants allocated to group A developed MRONJ compared to 13 (13/40, 32.5%) in group B, and 2 (2/36, 5.6%) of the low-risk osteoporosis patients of group A developed MRONJ compared to 5 (5/37, 13.5%) in group B.

These results suggest that performing a full thickness muco-periosteal flap with wound closure by primary intention is associated with a reduced risk of developing MRONJ compare to the split- thickness muco-periosteal flap with wound closure by primary intention. Although the difference in outcome between the patients allocated to the two protocols was reported to be statistically significant, the actual number of patients in each sub-groups who actually developed MRONJ post-operatively remains unclear as the authors included patients who were switched intra-operatively from protocol B to protocol A due to evidence of necrotic bone intraoperatively (pre-existing MRONJ prior to surgery) (Table 5.3 and Table 5.4). This study was considered as low risk of bias for random sequence generation, allocation concealment, blinding of participants and outcome assessment, attrition bias and selective reporting (Figure 5.3).

h) Dental extraction with antiresorptive discontinuation and muco-periosteal flap elevation with primary closure vs extraction with muco-periosteal flap elevation with primary closure

The aim of the Ottesen et al study was to evaluate and compare two different surgical procedure strategies: muco-periosteal flap elevation with primary closure with and without antiresorptive drug discontinuation. Ottesen et al conducted a single blinded randomized feasibility study including a total of 23 cancer participants on high-dose of antiresorptive drugs (Ottesen et al, 2022). All the patients included were randomized into two groups:
respectively: group A (n= 13) was allocated to perioperative antibiotics, chlorhexidine, muco-periosteal flap elevation with primary closure and antiresorptive discontinuation (for one month prior to dental extraction and three months after extraction), and group B (n= 10) was allocated to perioperative antibiotics, chlorhexidine, and muco-periosteal flap elevation with primary closure only.

In the group A n=8 patients were treated with denosumab while, n=5 patients were treated with bisphosphonate. While, in the group B, n=5 patients were treated with denosumab and, n=5 patients treated with bisphosphonate.

Three patients died during the trial period. All 3 patients were receiving denosumab therapy.

The median duration of denosumab intake was 9 months (range from 2 to 30), and for bisphosphonate intake 17.5 months (range from 4 to 96) in group B.

One patient in denosumab treatment had history of IV bisphosphonate (zometa). Therefore, we were unable to stratify the included patients into low and high-risk groups as per our protocol.

Perioperative antibiotics were provided to all the patients one hour before the surgical intervention and continued for ten days postoperatively. All patients used 0.12% chlorhexidine mouthwash two times a day until suture removal (10 days postoperatively).

During the follow-up, four patients developed MRONJ (30.8%) in the group A (drug holiday group). All four patients were treated with denosumab (Table 5.3 and Table 5.4).

This study was considered at low risk of bias for random sequence generation, allocation concealment and outcome assessment, but high risk for attrition bias, selective reporting and blinding of participants (Figure 5.3).

5.3.1 Meta-analysis results

The analysis was carried out using the log odds ratio as the outcome measure. A random-effects model was fitted to the data. The amount of heterogeneity (i.e., tau²), was estimated using the restricted maximum-likelihood estimator (Viechtbauer, 2005). In addition to the estimate of tau², the Q-test for heterogeneity (Cochran, 1954) and the I² statistic are reported. In case any
amount of heterogeneity is detected (i.e., \( \text{tau}^2 > 0 \), regardless of the results of the Q-test), a prediction interval for the true outcomes is also provided. Studentized residuals and Cook's distances are used to examine whether studies may be outliers and/or influential in the context of the model. Studies with a studentized residual larger than the \( 100 \times (1 - 0.05/(2 \times k)) \) the percentile of a standard normal distribution are considered potential outliers (i.e., using a Bonferroni correction with two-sided alpha = 0.05 for \( k \) studies included in the meta-analysis). Studies with a Cook's distance larger than the median plus six times the interquartile range of the Cook's distances are considered to be influential. The rank correlation test and the regression test, using the standard error of the observed outcomes as predictor, are used to check for funnel plot asymmetry.

A total of \( k = 5 \) studies were included in the analysis. The observed log odds ratios ranged from \(-2.5260\) to \(2.2973\), with the majority of estimates being positive (80%). The estimated average log odds ratio based on the random-effects model was \( \hat{\mu} = 0.3389 \) (95% CI: \(-1.1727\) to \(1.8504\)). Therefore, the average outcome did not differ significantly from zero (\( z = 0.4394, p = 0.6604 \)) Table 5.5. According to the Q-test, there was no significant amount of heterogeneity in the true outcomes (\( Q(4) = 6.0951, p = 0.1922, \text{tau}^2 = 1.0997, I^2 = 37.7069\% \)).

<table>
<thead>
<tr>
<th>Tau</th>
<th>Tau²</th>
<th>I²</th>
<th>H²</th>
<th>R²</th>
<th>df</th>
<th>Q</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td>1.049</td>
<td>1.0997 (SE= 2.1152)</td>
<td>37.71%</td>
<td>1.605</td>
<td>.</td>
<td>4.000</td>
<td>6.095</td>
<td>0.192</td>
</tr>
</tbody>
</table>

Table 5.5 Heterogeneity statistics analysis.

A 95% prediction interval for the true outcomes is given by \(-2.2125\) to \(2.8902\). Hence, although the average outcome is estimated to be positive, in some studies the true outcome may in fact be negative. An examination of the studentized residuals revealed that none of the studies had a value larger than \(\pm 2.5758\) and hence there was no indication of outliers in the context of this model. According to the Cook's distances, none of the studies could be considered to be overly influential. Neither the rank correlation nor the
A regression test indicated any funnel plot asymmetry ($p = 0.8167$ and $p = 0.7039$, respectively) Figure 5.4 and 5.5.

Figure 5.4 Funnel Plot

<table>
<thead>
<tr>
<th>Study</th>
<th>Effect Size</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mozzoli 2012</td>
<td>-2.53</td>
<td>[-5.34, 0.38]</td>
</tr>
<tr>
<td>Mozzoli 2013</td>
<td>0.09</td>
<td>[-3.83, 4.01]</td>
</tr>
<tr>
<td>Postetner 2020</td>
<td>0.05</td>
<td>[-3.89, 4.00]</td>
</tr>
<tr>
<td>Ristow 2020</td>
<td>0.93</td>
<td>[-0.18, 2.03]</td>
</tr>
<tr>
<td>Ollason 2021</td>
<td>2.30</td>
<td>[-0.75, 5.36]</td>
</tr>
<tr>
<td>RE Model</td>
<td>0.34</td>
<td>[-1.17, 1.85]</td>
</tr>
</tbody>
</table>

Figure 5.5 Forrest Plot

5.3.2 Assessment of quality of evidence

The 5 GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) were used to assess the quality of evidence. A “Summary of findings” table was produced to summarise the results and quality of evidence for each outcome (Table 5.6) using the GRADEpro software (GRADEpro GDT, 2015). In assessing the overall quality of evidence presented by the selected studies, eight out of nine were assessed to have low quality due to a high risk of bias as each study had at least one domain rated at high risk. One out of nine was assessed high quality due to a
low risk of bias in its domains. Therefore, the estimates of the effect of the results are uncertain and the presented results have to be interpreted cautiously as the rates of osteonecrosis may have been underestimated or over-estimated.
<table>
<thead>
<tr>
<th>Study/author</th>
<th>Type of adjusted protocol</th>
<th>Type of study</th>
<th>Quality assessment</th>
<th>No. of patients</th>
<th>Grade of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies</strong></td>
<td><strong>Designed Protocol</strong></td>
<td><strong>Study design</strong></td>
<td><strong>Risk of bias</strong></td>
<td><strong>Inconsistency</strong></td>
<td><strong>Indirectness</strong></td>
</tr>
<tr>
<td>Hanson et., al 2010</td>
<td>Perioperative antibiotics vs perioperative Chlorhexidine 0.2% mouth rinse</td>
<td>Case controlled trial</td>
<td>Serious</td>
<td>Serious</td>
<td>Serious</td>
</tr>
<tr>
<td>Mozzati et., al 2012</td>
<td>Dental extraction using platelet-rich growth factor (PRGF) with primary intention closure vs dental extraction with primary intention closure</td>
<td>Randomised controlled trial</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
</tr>
<tr>
<td>Mozzati et., al 2013</td>
<td>Dental extraction performed with alveoplasty and primary wound closure vs wound healing with secondary intention</td>
<td>Randomised controlled trial</td>
<td>Not serious</td>
<td>Serious</td>
<td>Not serious</td>
</tr>
<tr>
<td>Hasegawa et., al 2013</td>
<td>Drug holiday vs perioperative antibiotics</td>
<td>Case controlled trials</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Serious</td>
</tr>
<tr>
<td>Asaka et., al 2017</td>
<td>Dental extraction using platelet-rich growth factor (PRGF) vs simple dental extraction</td>
<td>Case controlled trial</td>
<td>Not serious</td>
<td>Serious</td>
<td>Serious</td>
</tr>
<tr>
<td>Kang et., al 2020</td>
<td>Drug holiday vs perioperative antibiotics</td>
<td>Case controlled trials</td>
<td>Serious</td>
<td>Serious</td>
<td>Serious</td>
</tr>
<tr>
<td>Posletiner et., al 2020</td>
<td>Dental extraction using platelet-rich fibrin (PRF), alveoplasty and wound healing by secondary intention vs dental extraction with alveoplasty and wound healing by primary intention.</td>
<td>Randomised controlled trial</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
</tr>
<tr>
<td>Ristow et., al 2020</td>
<td>Dental extraction with full thickness mucoperiosteal flap with wound closure by primary intention vs extraction with split-thickness mucoperiosteal mucosal flap with wound closure by primary intention</td>
<td>Randomised controlled trial</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
</tr>
<tr>
<td>Ottesen et al 2021</td>
<td>Muco-periosteal flap elevation with primary closure with and without antiresorptive drug discontinuation</td>
<td>Randomised controlled trial</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
</tr>
</tbody>
</table>

**Table 5.6 Summary of findings**

*GRADE Working Group grades of evidence.* High: We are very confident that the true effect lies close to that of the estimate of the effect; Moderate: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; Low: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect; Very low: We have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect. *Quality of evidence downgraded one level on the basis of unclear risk of selection bias. †Quality of evidence downgraded one level on the basis of heterogeneity. ‡Quality of evidence downgraded one level on the basis of indirectness. ‰Quality of evidence downgraded one level on the basis of imprecision.
5.4 Discussion
Dental extraction is one of the most commonly reported risk factors for the development of MRONJ (Lodi et al., 2010), with a 2017 systematic review of 4,106 MRONJ individuals reporting a history of prior dental extraction in 45% of cases (McGowan et al., 2017). The pathogenesis of MRONJ associated with dental extraction remains unclear, with current hypotheses suggesting that bone necrosis may be triggered by post-operative infection of the alveolar bone, insufficient mucosal healing or bone remodelling suppression (Otto et al., 2015).

Accordingly, a number of clinical studies have attempted reducing the risk of MRONJ development associated with dental extractions through investigating interventions to control infection, support mucosal wound closure and facilitate bone healing. The systematic review by Gaudin et al assessed thirteen studies and found that the risk of developing MRONJ after dental extraction may be minimised when extraction protocols include alveolectomy (Gaudin et al., 2015). However there remain concerns regarding their conclusions as the article did not follow carefully the PRISMA guidelines. In addition to that, they performed a proportional type of meta-analysis without considering significant differences among the study design. Equally, Beth-Tasdogan et al. found insufficient evidence to support any specific surgical protocol as being superior in reducing the development of MRONJ (Beth-Tasdogan et al., 2017). However, this review did not include important comparative studies such as CCS as well as did not perform any assessment of the quality of evidence (Hanson et al., 2010; Hasegawa et al., 2013; Asaka et al., 2017; Kang et al., 2020).

The present systematic review was rigorously designed in order to overcome the limitations of previous reviews and to provide clinicians with a clear and updated assessment of the level of evidence behind the suggested risk-reduction strategies. In particular, for the first time, we planned risk stratification of participants with a view to assess the effect of the proposed intervention upon homogeneous groups of individuals with similar MRONJ risk profile.

We identified nine studies including five RCTs and four CCS that met our search criteria. Of note, all but one study assessed a range of synchronous interventions with potential impact upon one or more suggested pathogenetic pathways of MRONJ, so that a critical assessment of the role of each intervention remains difficult. For example, one study assessed the effects of perioperative antibiotics, mucoperiosteal
flap, alveoplasty, and wound suturing/healing by primary intention versus perioperative antibiotics, gelatine sponge haemostatic and wound suturing/healing by secondary intention (Mozzati et al., 2013). Some studies arranged all interventions but one to be the same in the study groups. For example, the study by Ristow et al was designed so to provide all participants with perioperative bisphosphonate discontinuation, antibiotics and chlorhexidine, alveoplasty, wound closure by primary intention, but only group A with full thickness muco-periosteal flap and only group B with epi-periosteal mucosal flap. As the relative role of each intervention in reducing the risk of MRONJ is difficult to gauge and multiple synchronous interventions may have a cumulative effect upon MRONJ pathogenesis, one should refrain from extrapolating that a single intervention may be per se superior (e.g. full thickness muco-periosteal flap vs to epi-periosteal mucosal flap) in reducing the risk of MRONJ development when patients received multiple interventions (Ristow et al, 2021).

With respect to the risk of bias and quality of evidence, all but one study was found to be of poor quality and carry a high risk of bias (Table 5.3 and Table 5.4), suggesting low confidence that the reported outcomes may reliably reflect a genuine effect of the tested interventions (Hanson et al., 2010; Mozzati at al., 2012; Hasegawa et al., 2013; Mozzati et al., 2013; Asaka et al., 2017; Poxleitner et al., 2020; Kang et al., 2020; Ristow et al., 2021; Ottesen et al, 2022).

Furthermore, stratification into the risk groups pre-defined in our methods was not possible due to missing data and the large range drug therapy duration. Overall our systemic review show that the results of clinical studies are often inconsistent, as similar risk-reduction interventions were reported to be ineffective in some studies and potentially beneficial in others. This causes notable uncertainty and further reduces confidence in the effects of the proposed interventions, in addition to the above-mentioned high risk of bias and the inclusion of non-homogeneous patients’ groups with different risk profile.

Two of the four CCS included in the present systematic review showed no difference in the development of MRONJ among the study groups, therefore suggesting no notable risk reduction associated with the proposed procedures in osteoporosis individuals taking oral BPs. These include the use of perio-operative antibiotics vs peri-operative chlorhexidine, and peri-operative antibiotics with BP discontinuation and wound suturing healing by secondary intention vs peri-operative antibiotics with BP discontinuation and wound suturing/healing by secondary intention with platelet-rich
growth factor (Hanson et al., 2010; Asaka et al., 2017). The other two CCS reported a possible reduction in the risk of MRONJ development in osteoporosis individuals taking oral with perioperative antibiotics and BPs discontinuation vs perioperative antibiotics only (Hasegawa et al., 2013; Kang et al., 2020). Nonetheless, the remains uncertainty regarding the most effective antibiotic regimen and timing of BPs discontinuation.

Two of the five RCTs also showed no evidence of reducing the risk of MRONJ development with the proposed interventions in osteoporosis and cancer patients using oral or intravenous BP, including (i) the use of perioperative antibiotics and wound suturing/healing by primary intention via mucoperiostal flap and alveoplasty vs perioperative antibiotics and wound suturing/healing by secondary intention with haemostatic gelatine sponge (Mozzati et al., 2013), and (ii) the use of perioperative antibiotics, chlorhexidine mouthwash, platelet-rich fibrin, alveoplasty, and wound suturing/healing by secondary intention vs perioperative antibiotics, chlorhexidine mouthwash, alveoplasty and wound suturing/healing by primary intention (Poxleitner et al., 2020). The other three RCTs, although testing interventions that are broadly similar to the two RCTs above, suggested possible protective effects in osteoporosis and cancer patients using oral or intravenous BPs, as well as denosumab. One study reported a reduced risk of MRONJ development with the use of use of platelet-rich growth factor and alveoplasty in addition to perioperative antibiotics and wound suturing/healing by primary intention vs perioperative antibiotics and wound suturing/healing by primary intention (Mozzati et al., 2013). Ristow et al. reported a reduced risk of MRONJ with full thickness mucoperiosteal flap in addition to perioperative antiresorptive discontinuation, antibiotics and chlorhexidine, and alveoplasty with wound closure by primary intention vs epi-periosteal mucosal flap with antiresorptive discontinuation, antibiotics and chlorhexidine, and alveoplasty with wound closure by primary intention (Ristow et al., 2020). Of note this was the only study at low risk of bias and where the protective effects were also confirmed after stratifying participants into risk groups by underlying diagnosis and antiresorptive medications including denosumab (but not the length of anti-resorptive therapy). Nonetheless, confidence in their results is reduced by the fact that, although a risk reduction study should recruit individuals who have not yet developed the disease of interest, some participants who were found intraoperatively to have necrotic bone pre-existing to the dental extraction were not excluded from the study. Additionally, a
discrepancy of the number of MRONJ before and after subgrouping on the article was found, which was not clarified by the authors despite the request. Ottesen et al. also reported a reduced risk of MRONJ development with antiresorptive discontinuation (including denosumab) in addition to peri-operative antibiotics and chlorhexidine, and mucoperiosteal flap with primary closure vs peri-operative antibiotics and chlorhexidine, and mucoperiosteal flap with primary closure (Ottesen et al., 2022).

5.5 Conclusion
Overall, looking at the results and quality of studies included in the present systematic review, no reliable guidance can be provided to clinicians who manage patients at risk of MRONJ undergoing dental extractions. Further well-designed clinical trials are definitely needed in order to test the hypothesis that a particular intervention, or a combination of more interventions, may effectively reduce the risk of MRONJ development in this patient group. The results of the present systematic review may be helpful in identifying a potential preliminary suggestion of efficacy of some risk-reduction interventions, with a view to inform and guide the design of future trials. It is also imperative that a homogeneous population with an enriched phenotype (patients with a notable high risk of MRONJ development due to underlying disease, type of antiresorptive agent and cumulative length of therapy) is identified as the target group for future clinical trials.
Chapter 6 - Dental extraction adjusted protocol in patients considered at high risk of developing osteonecrosis: a retrospective service evaluation cohort study

6.1 Abstract

**Purpose:** The aim of this single-centre retrospective hospital-based service evaluation study was to determine the incidence of medication-related osteonecrosis of the jaw in oncologic patients who were prescribed antiresorptive or antiangiogenic medication following dental exodontia adjusted surgical protocol.

**Materials and methods:** This retrospectively analysis looked over a period of eight years and included data that were collected from the dental records of individuals who had dental extractions with a specific adjusted protocol. Patients who had previously been treatment with a combination of drugs (antiresorptive + antiangiogenic) or patient exposed to head and neck radiotherapy were not included in this evaluation.

**Results:** A total of 43 patients were included in the analysis. 107 tooth extractions were carried out over the period of this research. A significantly increased risk of developing medication-related osteonecrosis of the jaw was evident in the cohort of patients analysed (n=19; 44.2%)

**Conclusion:** According to the findings of this research, oncologic patients are at high risk of developing medication-related osteonecrosis of the jaw following dental extraction. This risk does still remaining high despite the minimal traumatic dental extractions' protocol implemented.

6.2 Introduction

Antiresorptive drugs are effective treatments that are often used to successfully treat common and uncommon bone targeted benign diseases such as postmenopausal osteoporosis, osteogenesis imperfecta, and hypercalcaemia. Additionally, antiresorptive medications are also used to treat conditions like Paget's disease. They have also been used as a preventative measure and therapy for skeletal bone metastasis illnesses associated with solid malignant tumours and multiple myeloma in recent years. This is done by administering the medicine via a different method of drug administration or in a different dose (Brown et al., 2009; Cummings et al., 2009; Hellstein et al., 2011; Henry et al., 2011). However, one of the significant adverse
effects that these medications have, is represented by the osteonecrosis of the jaw (Marx, 2003; Ruggiero et al., 2014; Ruggiero et al., 2022). Numerous studies have referred to MRONJ as an area of necrotic bone in the oral and maxillofacial region. This necrotic bone might appear with or without exposed bone, depending on the circumstances. In individuals who had never been exposed to any kind of ionising radiation in the head and neck area before, this region's effects should have lasted for longer than eight weeks (Ruggiero et al., 2014; Ruggiero et al., 2022). MRONJ is only seen very rarely in people who have osteometabolic illnesses (the incidence of this condition varies from 0% to 0.4%), but it appears much more often in people who have metastatic cancer (the frequency of this condition ranges from 0.2–6.7%). (Ruggiero et al., 2014). Extreme suppression of bone turnover, in addition to other local and general variables, seems to be a risk factor for the development of MRONJ. However, the exact aetiopathogenesis of MRONJ is not fully understood. The most important potential risk factors related to the development of MRONJ include a history of recent trauma or evacuation of dentoalveolar teeth, the length of time the patient was exposed to the medication, and the patient's underlying medical state (oncologic or non-oncologic patients) (Saia et al., 2010; Otto et al., 2015; Ruggiero et al., 2014; Ruggiero et al., 2022). The regular use of simple and surgical excision of non-restorable teeth in patients with osteometabolic illness after BPs therapy has been based on an individual's risk classification in recent years. This has been the case in the majority of cases (Di Fede et al., 2018; Nicolatou-Galitis et al., 2019). In recent years, alveoloplasty and primary wound closure have been shown to be beneficial in decreasing the risk of MRONJ formation (Schiodt et al., 2019). Nevertheless, a number of studies on alveoloplasty and primary wound closure ignored the patient's degree of risk for developing osteonecrosis (Otto et al., 2015). The evaluation of an individual's risk profile, broken down into high risk and low risk categories, is very necessary in order to choose the most suitable dental treatment. The rate of bone turnover suppression is largely determined by the type of patient being treated (oncologic or non-oncologic), as well as the dosage regimen and the length of treatment. As a result, the cumulative risk of MRONJ in patients receiving antiresorptive drugs for any skeletal disorder increases over time. This reflects the fact that the risk is proportional to the rate of bone turnover suppression. On the other hand, this link with the antiangiogenic medication treatment has received far more criticism (Ruggiero et al., 2022). At this time, there are no data available from clinical
trials that compare monotherapy with an antiangiogenic drug alone to any kind of preventative dental extraction technique. The goal of this retrospective hospital-based service evaluation study was to find and validate a dental extraction surgical protocol in high-risk oncologic patients who had previously received antiresorptive and/or antiangiogenic drug therapy at one of London’s largest service providers. The study was carried out with the intention of identifying and validating the protocol.

6.3 Materials and Methods
This is a single-centre retrospective hospital-based service evaluation study that included only oncologic patients taking antiresorptive or antiangiogenic drug therapies who underwent dental extraction in a specific clinic and with a specific protocol in the Oral Surgery department of King’s College Hospital between January 2012 and December 2019. All the patients were clinically and radiographically assessed prior to any surgery. Teeth that were considered non-restorable, and could potentially present a risk to the patient’s health were extracted by dedicated oral surgeons. After surgery, patients were followed up for a period of 6 months, respectively at 8 and 24 weeks. Consent was obtained from all the patients included in this study. Indeed, patients were informed about the possible sequelae of dental extraction. The inclusion criteria for this service evaluation were:

1) Adult patients over the age of 18 who were treated with antiresorptive or antiangiogenic drugs for oncologic or bone metastasis purposes.
2) Patients require dental extractions due to non-restorable teeth considered in the initial consultation.
3) Minimal invasive dental extraction protocol followed

The exclusion criteria of this service evaluation were

1) clinical signs of MRONJ during the first visit,
2) pregnant or breast-feeding women,
3) radiation to the head and neck region

All the patients’ extractions were carried out under local anaesthesia or local anaesthesia supplemented with intravenous sedation.
6.3.1 Dental extraction protocol
All the patients included in the study were treated using a standard atraumatic technique in order to maintain alveolar bone and preserve socket anatomy. Postoperative antibiotics such as amoxicillin (500 mg, three times daily) for five days or metronidazole (400 mg, three times daily) being prescribed for five days if the use of amoxicillin was contraindicated were prescribed for all the patients. Additionally, chlorhexidine 0.2% mouthwash was dispensed post-operatively to all patients for use 3 times daily for at least 2–3 weeks following the surgery. Patients were given standard postoperative instructions following the treatment. A cold, soft diet for the first day was suggested. Normal oral hygiene procedures were resumed two days after the surgery.

6.3.2 Study variables and outcome measured
The primary outcome of this service evaluation was to evaluate the incidence of MRONJ in oncologic patients prescribed antiresorptive and/or antiangiogenic drug therapy following dental extraction with a specific protocol. This was determined by a clinical examination of mucosal wound closure and new dental x-rays.

Secondary outcomes evaluated were:
1) Patients’ demographics (age and gender)
2) Type of primary cancer
3) The region of the MRONJ presented (maxilla or mandible)

6.3.3 Data analysis
The data generated by the retrospective analysis of patients’ dental records were recorded in a Microsoft Excel file. Descriptive statistics were computed using JAMOVI version 2.3.18. Results are expressed as a percentage or as mean values, including the standard deviation of the mean and range. In addition, analyses were performed using Fischer’s exact test for categorical variables. The statistical significance level was set at 0.05 (or 5%). Moreover, a Pearson correlation coefficient (r) test was used to measure correlation and the strength of the relationship among some of the variables.
6.4 Results

6.4.1 Baseline characteristics

A total of 43 patients (n=20 females (46.5%) and 23 males (53.5%) with a mean age of 67.1 ± SD 9.47 ranging from 47 to 88 years of age were included in the study. N=25 of the patients (58.1%) suffered from multiple myeloma (MM), n=8 (18.6%) suffered of prostate cancer (PC), n=6 (14%) suffered of breast cancer (BC) followed by respectively non-Hodgkins lymphoma (n= 2; 4.7%), melanoma (n= 1; 2.3%) and clear cell kidney cancer (CKC) (n= 1; 2.3%). All 43 patients had single or multiple antiresorptive drug therapies. No patients with history or current antiangiogenic drug therapy met the inclusion criteria for this study. The overall length of antiresorptive drug duration was 26 ± SD 27.6 months, ranging from 1 to 120 months. There was no significant difference in the duration of intake with regard to the route of administration. A total of 107 dental extractions were performed, and all the patients were followed-up for 6 months. A summary of the descriptive data can be found in table 6.1.

<table>
<thead>
<tr>
<th>Summary</th>
<th>Data – (N and/or %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients n°</td>
<td>Female n° = 20 (46.5%)</td>
</tr>
<tr>
<td></td>
<td>Male n°=23 (53.5%)</td>
</tr>
<tr>
<td>Cancer Type</td>
<td>MM n°= 25 (58.1%)</td>
</tr>
<tr>
<td></td>
<td>PC n°= 8 (18.6%)</td>
</tr>
<tr>
<td></td>
<td>BC n°= 6 (14%)</td>
</tr>
<tr>
<td></td>
<td>NHL n°= 2 (4.7%)</td>
</tr>
<tr>
<td></td>
<td>Melanoma n°= 1 (2.3%)</td>
</tr>
<tr>
<td></td>
<td>CKC n°= 1 (2.3%)</td>
</tr>
<tr>
<td>Type of Antiresorptive Drugs</td>
<td>ZOL n°= 25 (58.1%)</td>
</tr>
<tr>
<td></td>
<td>CLO n°= 10 (23.3%)</td>
</tr>
<tr>
<td></td>
<td>PAM n°= 2 (4.7%)</td>
</tr>
<tr>
<td></td>
<td>Dmab n°= 2 (4.7%)</td>
</tr>
<tr>
<td></td>
<td>PAM + ZOL n°= 1 (2.3%)</td>
</tr>
<tr>
<td></td>
<td>ZOL +ALD n°= 1 (2.3%)</td>
</tr>
<tr>
<td></td>
<td>ZOL + CLO n°= 1 (2.3%)</td>
</tr>
<tr>
<td></td>
<td>ZOL + Dmab n°= 1 (2.3%)</td>
</tr>
<tr>
<td>Length of Drug Administration</td>
<td>ZOL (15.1 months)</td>
</tr>
<tr>
<td>(months- express in mean)</td>
<td>CLO (49.4 months)</td>
</tr>
<tr>
<td></td>
<td>PAM (18.5 months)</td>
</tr>
<tr>
<td></td>
<td>DMZ (18 months)</td>
</tr>
<tr>
<td></td>
<td>PAM + ZOL (72 months)</td>
</tr>
<tr>
<td></td>
<td>ZOL +ALD (8 months)</td>
</tr>
<tr>
<td></td>
<td>ZOL + CLO (54 months)</td>
</tr>
<tr>
<td></td>
<td>ZOL + DMZ (41 months)</td>
</tr>
<tr>
<td>Route of Administration</td>
<td>IV n°= 28 (65.1%)</td>
</tr>
<tr>
<td></td>
<td>Oral n°= 10 (23.3%)</td>
</tr>
<tr>
<td></td>
<td>IV + Oral n°=2 (4.7%)</td>
</tr>
<tr>
<td></td>
<td>SC n°= 2 (4.7%)</td>
</tr>
<tr>
<td></td>
<td>IV+SC n°= 1 (2.3%)</td>
</tr>
<tr>
<td>Total number of MRONJ</td>
<td>MRONJ n°= 19 (44.2%)</td>
</tr>
<tr>
<td>MRONJ Type</td>
<td>Exposed n°= 17 (39.5%)</td>
</tr>
<tr>
<td></td>
<td>Non-Exposed n°= 2 (4.7%)</td>
</tr>
<tr>
<td></td>
<td>No MRONJ n°= 24 (55.8%)</td>
</tr>
<tr>
<td>MRONJ location</td>
<td>Upper Jaw n°= 8 (42.1%)</td>
</tr>
<tr>
<td></td>
<td>Lower Jaw n°= 11 (57.9%)</td>
</tr>
</tbody>
</table>
A total of \( n=19 \) (44.2%) patients developed MRONJ after dental extraction. Respectively \( n= 8 \) (40%) female and \( n= 11 \) (47.8%) male. The incidence was predominantly in multiple myeloma \( (n= 9) \) followed by prostate cancer \( (n= 5) \), breast cancer \( (n=3) \) and clear cell kidney cancer \( (n=1) \). According to the region, the incidence of MRONJ was predominantly in the mandible \( (n=11; 57.9\%) \) compare to the maxilla \( (n=8; 42.1\%) \). Additionally, in the male cohort, the incidence of MRONJ was respectively in the mandible \( (n=6; 31.6\%) \) and in the maxilla \( (n=5; 26.3\%) \), while in the female cohort, the incidence of MRONJ was \( n=5 \) (26.3%) in the mandible and \( n=3 \) (15.8%) in the maxilla.

According to Fischer’s exact statistical test, there was statistically significant difference with regards to gender and MRONJ location (mandible Vs maxilla) at time of surgery \( (p < 0.001) \). However, there was no statistically significant difference between gender and MRONJ development and MRONJ development and antibiotic family used (Penicillin vs. Macrolides), respectively, \( p < 0.760 \) and \( p < 0.217; \) Table 6.2, Figure 6.1 and Figure 6.2.

<table>
<thead>
<tr>
<th>Category</th>
<th>Odds ratio Value</th>
<th>95% Confidence of Interval</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>MRONJ (Female vs Male)</td>
<td>0.727</td>
<td>0.216</td>
<td>2.44</td>
</tr>
<tr>
<td>MRONJ (Penicillin vs Macrolides)</td>
<td>2.56</td>
<td>0.728</td>
<td>9.00</td>
</tr>
</tbody>
</table>

Table 6.2 Evaluation of MRONJ risk factors
6.4.3 Analysis of correlation factors

According to the Pearson correlation coefficient \((r)\) test, there was a weak negative statistical correlation among many of the variables. However, few positive and strong correlations were found between MORNJ type and MRONJ development \((r=0.977)\), MRONJ site and MRONJ development \((r=0.907)\), and MRONJ site and MRONJ type \((r=0.881)\). Table 6.3 summarises the correlation findings.

![Figure 6.1 Gender distribution of MRONJ development.](image1)

![Figure 6.2 Perioperative antibiotics distribution in MRONJ cases.](image2)

<table>
<thead>
<tr>
<th>MRONJ Development</th>
<th>Gender</th>
<th>MRONJ Development</th>
<th>Type of MRONJ</th>
<th>MRONJ site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson's r</td>
<td>-0.079</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>p-value</td>
<td>0.616</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>95% CI Upper</td>
<td>0.227</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>95% CI Lower</td>
<td>-0.370</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Type of MRONJ</td>
<td>Pearson's r</td>
<td>0.977</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Table 6.3 Correlation matrix

<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>MRONJ Development</th>
<th>Type of MRONJ</th>
<th>MRONJ site</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>0.819</td>
<td>&lt; .001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>95% CI Upper</td>
<td>0.267</td>
<td>0.988</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>95% CI Lower</td>
<td>-0.333</td>
<td>0.958</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MRONJ site</td>
<td>Pearson's r</td>
<td>-0.093</td>
<td>0.907</td>
<td>0.881</td>
</tr>
<tr>
<td>p-value</td>
<td>0.552</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>—</td>
</tr>
<tr>
<td>95% CI Upper</td>
<td>0.213</td>
<td>0.949</td>
<td>0.934</td>
<td>—</td>
</tr>
<tr>
<td>95% CI Lower</td>
<td>-0.383</td>
<td>0.833</td>
<td>0.790</td>
<td>—</td>
</tr>
</tbody>
</table>

6.5 Discussion

This study examined the incidence of MRONJ following dental extractions in oncologic patients prescribed antiresorptive or antiangiogenic therapy. Additionally, we aimed to evaluate the significance of minimally invasive dental extraction in managing the risk of developing MRONJ. Epidemiological data on patients treated with BPs are scarce, owing to the difficulty of tracking information (due to the multiplicity of bisphosphonates, a lack of patient records, pathologies, and poor compliance with regular drug intake) (Sparabombe et al., 2014). Therefore, the number of new cases of MRONJ in these patients could be partly underestimated due to several factors (difficult access to secondary care, asymptomatic or missed diagnosis). Hence the importance of this analysis. In this study, dental extractions were performed in both the maxilla and mandible. A sample of 43 patients was selected of which all of them had antiresorptive drug therapy. No patients with history or undergoing antiangiogenic drug therapy met the inclusion criteria for this study.

Mucosal healing in the surgical site was evaluated as the primary outcome, since it is the most immediate clinical parameter that can indicate an eventual MRONJ onset. All the surgical procedures and the follow-up care were done by an experienced oral surgeon. The incidence of MRONJ in oncologic patients who underwent dental extraction in this study was n= 19 (44.2%). This data is towards the higher end of the range previously published (Ruggiero et al., 2014; Khan et al., 2015; Ruggiero et al., 2022). Although MRONJ may develop spontaneously, several studies have reported that dental extraction or other surgical interventions involving intraoral bone exposure (Assael, 2006; Migliorati et al., 2006). Hence, dental extractions and/or any dentoalveolar surgical procedures in patients receiving antiresorptive and other
antiangiogenic drugs are significantly important in dentistry as well as oral and maxillofacial surgery. Additionally, the data showed that males were statistically more likely to develop MRONJ than females. However, this data might be represented by the cohort of patients included in this study (a larger group of male patients). Moreover, this study suggested that the use of antibiotics as well as the type of antibiotics given to patients at risk of MRONJ are not reducing the chance of developing osteonecrosis significantly. However, the study’s results are in line with current literature trends. Based on epidemiological observational studies, tooth extraction often precedes the manifestation of MRONJ. Therefore, it is defined as a precipitating or triggering event (Abu-Id et al., 2008, Otto et al., 2012). As a result, some of the guidelines even recommend avoiding tooth extractions and dentoalveolar surgery under bisphosphonate intake whenever possible (Ruggiero et al., 2009, Yoneda et al., 2010). To date, few protocols have been suggested to reduce the incidence of MRONJ in oncologic patients, who are considered to be at higher risk of osteonecrosis (Regev et al., 2008; Lodi et al, 2010). Studies have suggested and proposed that dental extraction and all types of surgical interventions involving bone exposure should be avoided. However, there is no relevant research analysing this type of data in oncologic patients. In addition, several guidelines dealing with risk assessment and management of patients receiving bisphosphonates have been published (Ruggiero et al., 2009; Ruggiero et al., 2014; Ruggiero et al., 2022). However, the recommendations are partly contradictory. In the present study, the authors presented a minimally invasive surgical protocol with the purpose of preventing the development of MRONJ. Additionally, all patients in this research were provided with chlorhexidine 0.2% mouthwash to use 2–3 times daily after the extraction, as well as oral antibiotics. Although the evidence for the use of chlorhexidine and antibiotics following dental extraction in patients taking medication associated with osteonecrosis of the jaw is weak, some authors continue to advocate the use of these adjunct therapies (Tanna et al., 2017; Nicolatou-Galitis et al., 2019). A 2017 systematic review reported that antibiotic prophylaxis can be beneficial in preventing the development of MRONJ (Bermúdez-Bejarano et al., 2017). As with all prescribing decisions, benefits should be balanced with risk, particularly with reports of adverse reactions to antimicrobials and chlorhexidine. The results of this study did not, however, support the findings of others in relation to risk reduction. Several positive correlation variables were found among the type of MRONJ, MORNJ development, and MRONJ locations. This result
probably reflects the non-homogeneity of the sample population. This could perhaps be explained by the small number of patients treated within our cohort and the limitations of further statistical analysis of this group of patients. This lack of significance was an unexpected finding, which we feel would be interesting to investigate further with future studies in this area. Indeed, MRONJ was observed more frequently in the mandible than the maxilla, and the results observed in this study did show a similar pattern to other reported research (Khosla et al., 2007; Ruggiero et al., 2014; Ulmner et al., 2014). On consideration, there were some limitations to this study. Risk factors such as patients with multiple comorbidities were not evaluated, and the dental pathology necessitating extraction in each patient was not recorded. Furthermore, the small number of patients analysed in this study precluded a multivariate analysis.

6.6 Conclusion

The results of this study confirm that the risk of developing MRONJ in oncologic patients taking antiresorptive medication is significantly high after dental extractions, despite the minimally invasive approach. Indeed, this data showed that minimal trauma during dental extraction might not be the adequate adjusted protocol to follow to minimise the risk of MRONJ.
A randomised controlled trial, on the other hand, would be useful in determining the true utility of antibiotic and/or topical antiseptic regimes in such patients undergoing tooth extraction.
Chapter 7 - Dental extraction adjusted protocol in patients considered at low risk of developing osteonecrosis: a retrospective service evaluation cohort study

7.1 Abstract

Purpose: The aim of this multi-centre retrospective primary care-based service evaluation study was to determine the incidence of medication-related osteonecrosis of the jaw in patients affected by benign osteometabolic disease who were undergoing antiresorptive drug therapy following dental exodontia with an adjusted surgical protocol.

Materials and methods: For this retrospective analysis, data were taken from the dental records of people who had extractions over a five-year period. Patients with exposure to radiotherapy to the head and neck, antiangiogenic therapy, or a combination of antiresorptive and antiangiogenic drug therapy were excluded from this study.

Results: A total of 152 patients were included in the study's analysis. All the patients included in this study required dental extractions due to failed dentition and were undergoing antiresorptive drug therapy. The incidence of MRONJ was n=1 (0.7%). A delay in healing time was found in n=46 (30.3%) of the patients undergoing a minimally invasive dental extraction protocol.

Conclusion: This study showed that, following dental extraction, patients affected by benign osteometabolic disease are minimally exposed to the risk of developing medication-related osteonecrosis of the jaw. However, delayed socket re-epithelization was seen in patients with a longer duration and cumulative dosage of antiresorptive therapy.

7.2 Introduction

Bone-modifying agents (BMAs) such as bisphosphonates (BPs) and denosumab (Dmab) inhibit bone resorption and are widely used to manage numerous bone diseases and bone related conditions. Antiresorptive drugs are often prescribed to treat osteoporosis, Paget’s disease, metastatic osteolytic lesions associated with breast cancer, multiple myeloma, severe forms of osteogenesis imperfecta, and moderate to severe hypercalcemia associated with malignancies (Hillner et al., 2000;
In recent times, intravenous bisphosphonates have also been promoted for the control of osteoporosis (Black et al., 2007; Delmas et al., 2009). Post-menopausal osteoporosis is a very common disorder (Sambrook and Cooper, 2006). Less potent bisphosphonates such as alendronate and risedronate are first-line therapies for the treatment of post-menopausal osteoporosis, especially following a minimal-trauma fracture (Cranney et al., 2003; Wells et al., 2008). Paget’s disease is another fairly common condition for which antiresorptive drugs like bisphosphonates represent the first-line treatment (Schweitzer et al., 1995). By 2006, over 19 million prescriptions for bisphosphonates were dispensed worldwide, suggesting a good safety profile and benefit in osteoporosis management (Chapurlat and Delmas, 2006). Adverse events related to bisphosphonate usage have largely focused on gastrointestinal and renal safety, bone, joint, or muscle pain, and the development of acute phase reactions (Pazianas et al., 2010). Several recent studies have reported the possible association between bisphosphonate use, especially intravenous (IV) zoledronate or pamidronate, and jaw osteonecrosis (Marx, 2003; Carter et al., 2005; Purcell and Boyd, 2005). Since Marx first reported bisphosphonate-related osteonecrosis of the jaw (BRONJ), the disorder has been documented as a complication associated with invasive dental procedures performed on subjects during BPs therapy (Marx, 2003). However, ever since, ONJ has been documented and associated with different other medications (King et al., 2019; Sacco et al., 2020). Therefore, in 2014, the ONJ nomenclature was changed to medication-related osteonecrosis of the jaw (MRONJ), which is now widely known (Ruggiero et al., 2014). The frequency of MRONJ is reported to be higher with the use of intravenous BPs than with oral BPs (Cartsos et al., 2008). Previously, intravenous BPs were only used to treat patients with bone metastastic disease. However, in recent times, other bone diseases such as osteoporosis have also been managed with intravenous BPs (Doggrell et al., 2002; Ruggiero et al., 2022). A study assessing once yearly zoledronate for osteoporosis management in 8000 individuals reported, following separate case adjudication, a single episode of ONJ in each of the placebo and zoledronic acid groups (Black et al., 2007). The length of this study was only three years, whereas most cases of ONJ have been reported in patients taking bisphosphonates for longer periods of time. In a recent survey of over 8000 respondents, ONJ had a prevalence of 0.10% (95% confidence interval 0.05% to 2.0%) (Lo et al., 2010). A factor that is usually considered to increase the risk of
MRONJ development is dental extraction (Vahtsevanos et al., 2009; Saad et al., 2015; Ruggiero et al., 2022). The relative risk of BRONJ after tooth extraction in patients with cancer who are receiving BPs is reportedly 16–50 times higher than in patients receiving BPs who have not undergone tooth extraction (Kyrgidis et al., 2008; Vahtsevanos et al, 2009; Saad et al., 2015). Although the incidence of BRONJ in patients receiving oral BP following tooth extraction was reportedly only 0.5% in 2009 (Kunchur et al., 2009), this incidence has increased during the past 5 years; recent studies have reported higher incidences, including 2.27% in 2013 (Hansen et al., 2013), 2.5% in 2013 (Taylor et al., 2013), and 3.44% in 2017 (Jeong et al., 2017). In Japan, a retrospective study published in 2017 reported that the incidence of MRONJ after tooth extraction was 3.0% (35 of 1175 cases, 41 of 2458 teeth) (Hasegawa et al., 2017). Epidemiological data of non-cancer patients treated with oral and intravenous BPs and/or Dmab are scarce, owing to the difficulty of tracking; thus, the number of new cases of MRONJ in these patients may be undercounted (Khosla et al., 2007; Walter et al., 2009). Hence, one of the main issues related to MRONJ is the impossibility, at present, of defining guidelines for prevention based on scientific evidence, as well as establishing the role of risk factors related, due to the recent identification of the disease and the difficulty in carrying out appropriate studies. Thus, the aim of this study was to evaluate the 12-month incidence of MRONJ after tooth extractions in patients affected by osteometabolic disease associated with benign conditions treated with antiresorptive medications (any route of administration) and possible related risk factors.

7.3 Materials and Methods
This is a multi-centre retrospective primary care-based service evaluation study that included only patients affected by osteometabolic disease associated with benign conditions taking only antiresorptive drug therapies who underwent dental extraction with a specific protocol between January 2012 and December 2018. The study’ centres were three. All the patients were clinically and radiographically assessed prior to any surgery. Teeth that were considered non-restorable were extracted by an oral surgeon and an oral and maxillofacial surgeon. After surgery, patients were followed up for a period of a year, respectively at 1, 2, 6, 9, and 12 months. A consent form was obtained for all the patients included in this study. Indeed, patients were informed about the possible sequelae of dental extraction. All the data were collected
retrospectively from the clinical notes by using a specific case report form matching the objective of this research.

The inclusion criteria for this service evaluation were:

1) Adult patients over the age of 18 who were treated with antiresorptive drugs due to benign osteometabolic disease
2) Patients require dental extractions due to non-restorable teeth considered in the initial consultation.

The exclusion criteria of this service evaluation were:

1) Clinical signs of MRONJ during the first visit
2) Cancer patients with or without metastatic bone disease
3) Pregnant or breast-feeding women
4) Radiation to the head and neck region.

All the patients’ extractions were carried out under local anaesthesia or local anaesthesia supplemented with intravenous sedation.

7.3.1 Dental extraction protocol
All the patients included in the study were treated using an atraumatic technique in order to maintain alveolar bone and preserve socket anatomy.

The atraumatic dental extraction technique was initiated by using a periotome. The periotome was kept in a pen-holding positioned on the long tooth axis into the gingival sulcus. This was used to first detach the cervical gingival fibres. Consecutively, the periotome with minimal movement was advanced to periodontal ligament space several millimetres tangentially to the root surface to break down the periodontal ligament fibres up to 2/3rd of the length of the root. After which, forceps were used with gentle movement until the tooth was extracted, avoiding bone and soft tissue disruption. Post-operative antibiotics such as co-amoxiclav (875 mg + 125 mg twice daily) for five days or clarithromycin (500 mg twice daily) for five days if the use of amoxicillin was contraindicated were prescribed for all the patients. Additionally, chlorhexidine 0.2% mouthwash was dispensed post-operatively to all patients for use 3 times daily for at least 2-3 weeks following the surgery. Patients were given standard
postoperative instructions following the treatment. A cold, soft diet for the first day was suggested. Normal oral hygiene procedures were resumed 2 days after the surgery.

7.3.2 Study variables and outcome measured
The primary outcome of this service evaluation was to evaluate the incidence of MRONJ in patients affected by benign metabolic disease undergoing antiresorptive drug therapy following dental extraction with a specific protocol. This was determined by a clinical examination of mucosal wound closure and new dental x-rays.

Secondary outcomes evaluated were:
4) Patients demographic (age and gender)
5) Type of bone disease
6) Type of antiresorptive medication
7) The region of the MRONJ presented (maxilla or mandible or both)
8) Delayed healing

Delayed dental socket was defined as a persistent breach in the oral mucosa and/or bone exposure in the mandible or maxilla that does not heal within four weeks, as documented in the previous study (Amler, 1969).

7.3.3 Data analysis
The data generated by the retrospective analysis of patients’ dental records were recorded in a Microsoft Excel file. Descriptive statistics were computed using JAMOVI version 2.3.18. Results are expressed as a percentage or as mean values, including the standard deviation of the mean and range. In addition, analyses were performed using Fisher’s exact test for categorical variables. The statistical significance level was set at 0.05. Moreover, a Pearson correlation coefficient ($r$) test was used to measure correlation and the strength of the relationship among some of the variables.

7.4 Results
7.4.1 Baseline characteristics
A total of 152 patients, $n=127$ (83.6%) female and $n=25$ (16.4%) male, with a mean age of $69.7 \pm SD 6.49$ ranging from 59 to 82 years of age, were included in the study.
N=123 of the patients (80.9%) suffered from osteoporosis (OP), n= 20 (13.2%) suffered from steroid induced osteoporosis (SIOP), n= 8 (5.3%) suffered from Paget’s disease, and n= 1 (0.7%) suffered from osteomalacia (Table 7.1). All 152 patients had antiresorptive drug therapy. N=95 (62.5%) patients had bisphosphonate therapy, while n= 57 (37.5%) had denosumab. The average duration of bisphosphonate consumption was 47.4 SD 17.2 months, with a range of 12 to 78 months (Table 7.1). A total of 307 teeth (123 in the maxilla and 181 in the mandible) were extracted. All the patients were followed-up for 6 months.

<table>
<thead>
<tr>
<th>Summary</th>
<th>Data – (N and/or %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients n</td>
<td>Female n = 127 (83.8%)</td>
</tr>
<tr>
<td></td>
<td>Male n = 25 (16.4%)</td>
</tr>
<tr>
<td>Type of Metabolic Disease</td>
<td>OP n = 123 (80.9%)</td>
</tr>
<tr>
<td></td>
<td>SIOP n = 20 (13.2%)</td>
</tr>
<tr>
<td></td>
<td>Paget n = 8 (5.3%)</td>
</tr>
<tr>
<td></td>
<td>Osteomalacia n = 1 (0.7%)</td>
</tr>
<tr>
<td>Type of Antiresorptive Drugs</td>
<td>BP n = 95 (62.5%)</td>
</tr>
<tr>
<td></td>
<td>Dmab n = 57 (37.5%)</td>
</tr>
<tr>
<td>Dental Extraction Site</td>
<td>Maxilla n = 53 (34.9%)</td>
</tr>
<tr>
<td></td>
<td>Mandible n = 99 (65.1%)</td>
</tr>
<tr>
<td></td>
<td>Both n = 0 (0%)</td>
</tr>
<tr>
<td>Length of Drug Administration</td>
<td>BP (47.4 months SD 17.2)</td>
</tr>
<tr>
<td>(months- express in mean)</td>
<td>Dmab (28 months SD 7.73)</td>
</tr>
<tr>
<td>Route of Administration</td>
<td>Oral n° = 62 (40.8%)</td>
</tr>
<tr>
<td></td>
<td>SC n = 57 (21.7%)</td>
</tr>
<tr>
<td></td>
<td>IV n = 33 (37.5%)</td>
</tr>
<tr>
<td>Total number of MRONJ</td>
<td>MRONJ n = 1 (0.7%)</td>
</tr>
<tr>
<td>Delayed epithelialization</td>
<td>Normal n = 106 (69.7%)</td>
</tr>
<tr>
<td></td>
<td>Delayed n = 46 (30.3%)</td>
</tr>
</tbody>
</table>

Table 7.1 Data Summary

### 7.4.2 Risk factors evaluation and MRONJ incidence

Only 1 (0.7%) patient developed MRONJ after dental extraction. The patient suffered from osteoporosis and had Dmab administered for a period of 40 months. The dental extraction surgical site was predominantly in the mandible, with n = 99 (65.1%), followed by n = 53 (34.9%) in the maxilla. The only MRONJ case affected the lower jaw, which eventually did not need any treatment. There was no statistically significant difference between the single-rooted and multirooted sites of dental extraction. Additionally, normal epithelialisation was only seen in 69.7% (n =106) of the cases, which was highly represented in the Dmab cohort of patients (20 patients out of 57). According to Fischer’s exact statistical test, MRONJ development and type of
antiresorptive drug therapy were statistically not significant with a $p < 0.375$. Additionally, it was also found that type of antiresorptive drugs and delayed healing, as well as delayed healing and gender groups were statistically not significant different (respectively $p < 0.203$ and $p < 0.634$); Table 7.2 and Figure 7.1.

The results quite interestingly have shown almost 50% of the female cases a delayed in healing time compare to the male. These results could be associated to the higher length time of drug administration compare to the male cohort.

<table>
<thead>
<tr>
<th>Category</th>
<th>Odds ratio Value</th>
<th>95% Confidence of Interval</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>MRONJ vs Type of drugs</td>
<td>5.07*</td>
<td>0.203</td>
<td>127</td>
</tr>
<tr>
<td>Antiresorptive drug vs Delayed healing</td>
<td>1.63</td>
<td>0.806</td>
<td>3.31</td>
</tr>
<tr>
<td>Delayed healing vs Gender</td>
<td>0.687</td>
<td>0.225</td>
<td>1.85</td>
</tr>
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</table>

* Haldane-Anscombe correction applied

Table 7.2 Evaluation of MRONJ and delayed healing surgical site risk factors

7.4.3 Analysis of correlation factors

According to the Pearson correlation coefficient ($r$) test, there was a weak negative statistical correlation among many of the variables except for the dental extraction site and the gender ($r = -0.159$). Additionally, several weak positive correlations were found among some variables except for delay in healing and route of administration ($r = -0.186$). Table 7.3 summarises the correlation findings.
<table>
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<tr>
<th></th>
<th>Gender</th>
<th>Route of Administration</th>
<th>Delayed healing</th>
<th>Dental Extraction Site</th>
<th>Root Morphology</th>
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</thead>
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<td><strong>Route of Administration</strong></td>
<td>Pearson’s r</td>
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<tr>
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<td>p-value</td>
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<td>—</td>
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<td>—</td>
</tr>
<tr>
<td></td>
<td>95% CI Upper</td>
<td>0.175</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>95% CI Lower</td>
<td>-0.143</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td><strong>Delayed healing</strong></td>
<td>Pearson’s r</td>
<td>-0.060</td>
<td>0.186</td>
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<tr>
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<td>p-value</td>
<td>0.459</td>
<td>0.021</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>95% CI Upper</td>
<td>0.100</td>
<td>0.336</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>95% CI Lower</td>
<td>-0.218</td>
<td>0.028</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Dental Extraction Site</strong></td>
<td>Pearson’s r</td>
<td>-0.159</td>
<td>0.035</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.050</td>
<td>0.667</td>
<td>0.988</td>
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</tr>
<tr>
<td></td>
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<td>-0.000</td>
<td>0.193</td>
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<tr>
<td></td>
<td>95% CI Lower</td>
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<td>-0.158</td>
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<tr>
<td><strong>Root Morphology</strong></td>
<td>Pearson’s r</td>
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<td>95% CI Upper</td>
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<td>0.133</td>
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</tr>
<tr>
<td></td>
<td>95% CI Lower</td>
<td>-0.218</td>
<td>-0.185</td>
<td>-0.234</td>
<td>-0.153</td>
</tr>
</tbody>
</table>

Table 7.3 Correlation matrix

7.5 Discussion
A large review by Filleul et al., concluded that tooth extraction was the main triggering factor in 67% of ONJ cases (Filleul et al., 2010). Tooth extraction is believed to be a major risk factor for the development of MRONJ (Vahtsevanos et al., 2009; Ruggiero et al., 2014). Therefore, a previous BRONJ position paper from the Allied Task Force Committee of the Japanese Society for Bone and Mineral Research suggested that nonsurgical treatments should be performed rather than surgical treatments such as tooth extraction if dental treatments are desperately required (Yoneda et al., 2010). The AAOMS position paper also recommended that procedures that involve direct osseous injury should be avoided in cancer patients receiving BMA because of the high risk of MRONJ (Ruggiero et al., 2014). According to these guidelines, surgeons might treat even severely infected teeth using a nonsurgical approach whenever
possible. Epidemiological data on patients treated with oral BPs are extremely limited due to a variety of reasons (lack of patient records, compliance with regular drug intake, etc.) (Ruggiero et al., 2022). Therefore, the number of new cases of MRONJ in these patients could be partly underestimated (missed or difficult recognition of cases of MRONJ, often less severe and asymptomatic; reduced access to specialist reference centres; studies that do not specify either the type or duration of NBP treatments, underlying diseases and risk factors). Some initial incidence assessments indicated frequencies of the order of 1 case per 10,000-100,000 people per year of exposure (Khosla et al., 2007; Walter et al., 2009). The analysis carried out by the SICMF/SIPMO commission instead estimated the prevalence of MRONJ in osteoporotic patients overall to be between 0.02% and 1% (Campisi et al., 2020). Only patients with osteoporosis, the pathology that most frequently requires the assumption of bisphosphonates (Hellstein et al., 2011), were selected in this study. Cancer patients, as well as those receiving anti-angiogenic drugs alone or in combination with bisphosphonates, were excluded because they were at high risk of developing MRONJ, even spontaneously (Campisi et al., 2020). Furthermore, it was decided to investigate of the oral and sub-cutaneous route of administration, relatively safer and with less probability of the onset of osteonecrosis (Ruggiero et al., 2014). Several studies indeed show how the intramuscular and intravenous administration have a higher risk of developing MRONJ (Ruggiero et al., 2014; Fusco et al., 2016; Ruggiero et al., 2022). The aim of this study was to look at the risk factors for MRONJ one year after tooth extraction in osteoporotic patients who had taken oral bisphosphonates and sub-cutaneous Dmab. Furthermore, the current study seeks to determine the extent of delayed dental healing following dental procedures such as tooth extraction and how this relates to bisphosphonate use. The data showed that female patients have an increased risk of delayed healing compared to male in accordance also with previous data published (Ruggiero et al., 2014; Ruggiero et al., 2022). Delayed dental healing may be an earlier or less advanced lesion compared to ONJ, but with a similar pathogenesis. By observing delayed dental healing as well as ONJ, we may therefore more broadly describe bisphosphonate associated dental disorders and increase our power to find an association between bisphosphonate use and associated dental disorders. Additionally, according to the statistical test used for this study we could not find any significance among the category/groups analysed.
(MRONJ vs type of drugs; antiresorptive drug vs delayed healing; delayed healing vs gender), with a \( p \) value above the 0.05.

Furthermore, the study highlighted a that large cohort of patients presented with delayed healing time (n= 46; 30.3%). This was more evident in the group of patients with a longer period of antiresorptive drug exposure.

In this study, the incidence of MRONJ after tooth extraction was 0.7% (n=1), which is higher than rates in previous studies. Our MRONJ cases might have included patients who had already developed MRONJ before tooth extraction, because it is difficult to distinguish early, stage 0 MRONJ. Theoretically, long-term use of antiresorptive drugs carries a high risk of MRONJ (Kajizono et al., 2015; Otto et al., 2015; Ruggiero et al., 2022). Nevertheless, non-restorable teeth and those with a poor prognosis should be extracted before the development of inflammation or infection, which could constitute a trigger in and of itself. There were no statistically significant correlations found among the variables studied. The results of this study suggest that tooth extraction with an adjusted protocol and use of peri-operative antibiotics and antiseptics should not necessarily be postponed, even in patients receiving long-term BMA.

7.6 Conclusion

Within the limits of the present study, the results showed an incidence of osteonecrosis that was relatively low in these patients. Therefore, by following a risk-based therapy, it will be possible to perform oral surgery safely. However, further studies are needed, mainly on a larger sample and considering others’ multiple administrations of different antiresorptive drugs, with particular attention to the new drugs associated with MRONJ.
Chapter 8 – General Discussion

The section that follows summarises and presents the important results of this research with respect to the proposed hypotheses. The first hypothesis that was presented for consideration in this doctoral research was to establish a reliable approach to MRONJ risk reduction methods. This was evaluated through the use of a hypothetical question in Chapter 5: "In individuals exposed to anti-resorptive and/or anti-angiogenic medications undergoing dental extractions, can any risk-reduction strategies minimise the development of MRONJ?" This question asked whether or not any risk-reduction strategy could lower the risk of developing MRONJ and was important to establish the second hypothesis of this research: "minimal invasive dento-alveolar surgery used in conjunction with local and systemic antibacterial and antiseptic therapy could reduce the frequency of MRONJ." The investigations that were carried out in order to address the latter hypothesis are reported in chapters 6 and 7. This chapter serves as a reflection on the outcomes found as well as an overall summary of the thesis. Additionally, this chapter aims to discuss the implications of this research in the management of patients who are at risk for MRONJ and are undergoing dentoalveolar surgeries and to establish a hypothetical research study (Chapter 9) based on the current observations.

8.1 Key findings

- Chapter 5
An extensive multi-database screening of the literature was performed to analyse any type of comparative study considering any exodontia-adjusted protocols. Only nine studies fulfilled the inclusion criteria for the systematic review and meta-analysis. Four of these studies were case-control studies, while the other five were randomised clinical trials. The overall MRONJ occurrence was 1.5% (n = 29) in 1948 patients. The estimated average log odds ratio based on the random-effects model was \( \hat{\mu} = 0.3389 \) (95% CI: -1.1727 to 1.8504). Additionally, according to the Q-test analysis, there was no significant amount of heterogeneity. The overall quality of the evidence presented by eight studies was found to be low, with a high risk of bias. While
only one study was found to be of high quality. In conclusion, the study reported very limited scientific evidence to support or refute the effectiveness of any MRONJ prevention study protocols included in the research.

- Chapter 6
This study was based on a single-centre retrospective hospital-based service evaluation analysis. The purpose of it was to assess the frequency of MRONJ in oncology patients undergoing a specific dental extraction protocol. The data were gathered from the clinical records over a period of eight years. Only 43 patients fulfilled the inclusion criteria for this research. A total of 107 dental extractions were performed according to the minimally invasive dental extraction approach. The study reported a significant increased risk of developing medication-related osteonecrosis following minimally invasive dental extraction with antimicrobial antibiotic adjuvant therapy (n=19; 44.2%). This study highlighted that the minimally invasive dental extraction approach in high risk MRONJ oncologic patients might not reduce the risk of osteonecrosis, with strong positive correlations found among the type of MORNJ and MRONJ development (r= 0.977), MRONJ site and MRONJ development (r= 0.907) and MRONJ site and type of MRONJ (r= 0.881).

- Chapter 7
This study was based on a multi-centre retrospective primary care-based service evaluation analysis. The aim of this study chapter was to determine the incidence of MRONJ and the delayed healing time of low risk patients affected by benign osteometabolic disease. The data were collected from the dental records of patients who had extractions following a specific protocol. The incidence of MRONJ was n=1 (0.7%). However, n = 46 (30.3%) patients undergoing a minimally invasive dental extraction protocol experienced a delay in healing time. This study highlighted that patients affected by benign osteometabolic disease are minimally exposed to the risk of developing medication-related osteonecrosis of the jaw, and minimally invasive dental extraction with antimicrobial antibiotic adjuvant therapy has no impact on reducing MRONJ compared to the frequency of osteonecrosis in other studies. Additionally, a high incidence of delayed socket re-epithelization was also reported in patients exposed to a higher cumulative dosage of antiresorptive therapy.
8.2 Strength and limitations of this PhD

The key strength of this clinical PhD is that the research was conducted not only in secondary care patients but also in multi-centre primary care. Additionally, clearly defined outcome measures were applied and used throughout the research. Furthermore, the research was not only assessing the same surgical protocol but also the efficacy of local and systemic antimicrobials in high- and low-risk MRONJ patients. Although the proposed protocol had a negative outcome in terms of reducing MRONJ in both low- and high-risk patients, we can conclude that local and systemic antimicrobials have no effect on reducing disease incidence and should be used with caution. On the other hand, there were many aspects of this research that could not be explored due to several constraints imposed by the COVID-19 pandemic and by having only one researcher analyse a rare condition. Indeed, due to their retrospective nature, chapters 6 and 7 have several biases to consider (reporting bias, selection bias, lack of calibration, and so on), but their findings add to the literature value. Hence, further studies are needed to identify a potential effective surgical protocol. As a result, in Chapter 9, we propose and design a randomised pilot study to potentially corroborate the hypothesis associated with PhD research.
Chapter – 9 Future research (alveolectomy/alveoloplasty surgical adjusted protocol in patients at risk of developing MORNJ: a randomised pilot study)

According to the findings of our research (systematic review and service evaluations in primary and secondary care), there is weak evidence to claim or refute the benefit of any of the tested risk reduction strategies for the development of MRONJ. Hence, further research is needed.

9.1 Aims
The future research study aims to investigate whether intra-operative alveolectomy/alveoloplasty and suturing (primary closure) performed at the time of dental extraction reduce the risk of MRONJ development when compared to standard of care (simple) dental extraction (with no alveolectomy/alveoloplasty or suturing).

9.2 Materials and methods

9.2.1 Trial design
The randomised pilot study will be conducted prospectively and in parallel groups. The research will be carried out at a single centre and conducted with the understanding and written consent of each patient according to the principles, which have been conducted in full accordance with ethical principles and the Declaration of Helsinki (World Medical Association, 2013). The proposed research will follow the Health Research Authority's (HRA) recommendation and will be registered on an international registry such as ClinicalTrials.gov. The research protocol will require approval by a local Research Ethics Committee (REC). After informed consent is obtained, the patients will be randomly assigned to one of two study groups:

- Test group (T-group): alveolectomy/alveoloplasty and wound primary closure
- Control group (C-group): minimally invasive dental extraction

9.2.2 Patient selection criteria
The patients were selected based on the following inclusion criteria:

- Any type of gender;
- Non-smoking patients;
- Patients who were taking or had taken bisphosphonates for cancer treatment;
- Dental extraction in any jaws;
- No restrictions on the reason and type of tooth to be extracted (e.g. periodontal disease, retained root, dental carious);
- 6 months post-intervention follow-up

The exclusion criteria were:
- Patients under the age of 18;
- Patients taking antiangiogenic medications or a combination of antiresorptive and antiangiogenic medications;
- Patients who have undergone radiotherapy in the head-neck region;

9.2.3 Sample size calculation
According to the available literature, simple dental extraction (without alveolar bone removal and without suturing) is associated with the development of MRONJ in 26% of cancer patients on high-dose or high-potency antiresorptive medication, whereas performing intra-operative alveolar bone removal (alveolectomy or alveoloplasty) and wound suturing may reduce the development of MRONJ to 6% of cases (Lazarovici et al., 2010; Saia et al., 2010; Ferlito et al., 2011; Yamazaki et al., 2012; Taylor et al., 2013). Because of the study type and the "proof-of-concept" character of this research, no formal sample size calculation will be performed. In order to estimate the effect size for this explorative pilot study, approximately 25 patients for each group (n = 50 overall) will be recruited over a period of 12 months and followed up for at least 6 months. All the included patients will need to be cancer patients with 24 months of minimum exposure to antiresorptive drugs and therefore stratified as high-risk patients (Feng et al., 2017).

9.2.4 Statistical analysis
The Shapiro–Wilk test will be used to evaluate the normality of the data distribution. Descriptive statistics will be computed using a statistical software. Results will be expressed in percentages, mean values, standard deviations, and ranges. Student's t-test will be used to analyse continuous variables and the Fischer exact test for categorical variables. The statistical significance level will be assumed when p <0.05.
9.2.5 Surgical Protocol

All dental extractions will be performed following a standardised protocol. One week before the dental extraction, every patient will have a session of oral hygiene instruction. Extractions will be performed by the same oral surgeon involved in the study. An appropriate local anaesthesia technique will be used according to the tooth to be extracted (Lidocaine 2% with 1:80,000 of adrenalin). Tooth luxation and elevation will be gently performed using hand instruments. Following the dental extraction, the patients allocated to the T-group will receive the alveolectomy/alveoloplasty piezo-surgical device. Additionally, sulcular incisions are performed buccally and lingually/palatally. If needed, a full-thickness reliving incision will be performed mesially and distally to the region of interest to achieve tissue coverage during the suturing phase.

While patients in the C-group will have an atraumatic dental extraction with no soft or hard tissue surgery. No sutures will be used in this group. All patients will receive systemic antibiotic therapy after the extraction. Amoxicillin (500 mg every 8 hours for 7 days) will be used in patients who are not allergic to penicillin; alternatively, metronidazola (400 mg every 8 hours for 7 days) will be used in cases of allergy. Figures 8.1 and 8.2 show the surgical procedures in both groups.

![Figure 8.1 Alveolectomy/alveoloplasty procedure.](image)

![Figure 8.2 Simple dental extraction](image)

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9.2.6 Randomization and blinding procedure

A block type of randomization will be performed to equalise the sample sizes for the two groups. An independent statistician will computer-generate the randomization sequence prior to the start of the research. No one involved in the study will have access to the allocation codes. An independent oral surgeon (not involved in the surgical procedure or the study) will enrol patients. Consequently, the allocation of the patients to the T-group will be performed by the operating surgeon by unsealing the sealed envelopes at the time of surgery. Additionally, to reduce the detection bias, patients will not be informed about their allocation groups. The postoperative follow-ups will be performed by a blind independent clinician to assess the outcome of the study.

To minimise any performance bias, all surgical procedures will be performed with the same protocol and by the same oral surgeon.

9.3 Outcome measure

- Primary Objective

The primary objective of the study is to investigate whether intra-operative alveolectomy/alveloplasty and suturing can reduce the risk of MRONJ development in cancer patients exposed to antiresorptive drug therapy who undergo dental extraction, compared to standard-of-care operative procedures (extraction without intra-operative alveolectomy/alveloplasty and suturing).

All surgical sites will be clinically inspected and radiographically investigated during the follow-up. According to Ruggiero et al, visual assessment will be focused on mucosa healing/bone exposure (Ruggiero et al, 2022).
Reference


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retrospective study evaluating frequency and risk factors of osteonecrosis of the jaw in 576 cancer patients receiving intravenous bisphosphonates. American Journal of Clinical Oncology, 35(4), 386–392.


Appendix A. Prisma check list

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<td>Describe the rationale for the review in the context of existing knowledge.</td>
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<td>Rationale</td>
<td>4</td>
<td>Provide an explicit statement of the objective(s) or question(s) the review addresses.</td>
<td></td>
</tr>
<tr>
<td><strong>METHODS</strong></td>
<td>5</td>
<td>Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.</td>
<td></td>
</tr>
<tr>
<td>Eligibility criteria</td>
<td>6</td>
<td>Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the data when each source was last searched or consulted.</td>
<td></td>
</tr>
<tr>
<td>Search strategy</td>
<td>7</td>
<td>Present the full search strategies for all databases, registers and websites, including any filters and limits used.</td>
<td></td>
</tr>
<tr>
<td>Selection process</td>
<td>8</td>
<td>Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.</td>
<td></td>
</tr>
<tr>
<td>Data collection process</td>
<td>9</td>
<td>Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.</td>
<td></td>
</tr>
<tr>
<td>Data items</td>
<td>10a</td>
<td>List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10b</td>
<td>List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unknown information.</td>
<td></td>
</tr>
<tr>
<td>Study risk of bias assessment</td>
<td>11</td>
<td>Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.</td>
<td></td>
</tr>
<tr>
<td>Effect measures</td>
<td>12</td>
<td>Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.</td>
<td></td>
</tr>
<tr>
<td>Synthesis methods</td>
<td>13a</td>
<td>Describe the processes used to decide which studies were eligible for each synthesis (e.g. labelling the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13b</td>
<td>Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13c</td>
<td>Describe any methods used to tabulate or visually display results of individual studies and syntheses.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13d</td>
<td>Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13e</td>
<td>Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analyses, meta-regression).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13f</td>
<td>Describe any sensitivity analyses conducted to assess robustness of the synthesized results.</td>
<td></td>
</tr>
<tr>
<td>Reporting bias assessment</td>
<td>14</td>
<td>Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).</td>
<td></td>
</tr>
<tr>
<td>Certainty assessment</td>
<td>15</td>
<td>Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.</td>
<td></td>
</tr>
</tbody>
</table>
# PRISMA 2020 Checklist

<table>
<thead>
<tr>
<th>Section and Topic</th>
<th>Item #</th>
<th>Checklist Item</th>
<th>Location where item is reported</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RESULTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study selection</td>
<td>16a</td>
<td>Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16b</td>
<td>Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.</td>
<td></td>
</tr>
<tr>
<td>Study characteristics</td>
<td>17</td>
<td>Cite each included study and present its characteristics.</td>
<td></td>
</tr>
<tr>
<td>Risk of bias in studies</td>
<td>18</td>
<td>Present assessments of risk of bias for each included study.</td>
<td></td>
</tr>
<tr>
<td>Results of individual studies</td>
<td>19</td>
<td>For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.</td>
<td></td>
</tr>
<tr>
<td>Results of synthesis</td>
<td>20a</td>
<td>For each synthesis, briefly summarize the characteristics and risk of bias among contributing studies.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20b</td>
<td>Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20c</td>
<td>Present results of all investigations of possible causes of heterogeneity among study results.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20d</td>
<td>Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.</td>
<td></td>
</tr>
<tr>
<td>Reporting biases</td>
<td>21</td>
<td>Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.</td>
<td></td>
</tr>
<tr>
<td>Certainty of evidence</td>
<td>22</td>
<td>Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.</td>
<td></td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discussion</td>
<td>23a</td>
<td>Provide a general interpretation of the results in the context of other evidence.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23b</td>
<td>Discuss any limitations of the evidence included in the review.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23c</td>
<td>Discuss any limitations of the review processes used.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23d</td>
<td>Discuss implications of the results for practice, policy, and future research.</td>
<td></td>
</tr>
<tr>
<td><strong>OTHER INFORMATION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Registration and protocol</td>
<td>24a</td>
<td>Provide registration information for the review, including register name and registration number, or state that the review was not registered.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24b</td>
<td>Indicate where the review protocol can be accessed, or state that a protocol was not prepared.</td>
<td></td>
</tr>
<tr>
<td>Support</td>
<td>24c</td>
<td>Describe and explain any amendments to information provided at registration or in the protocol.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24d</td>
<td>Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.</td>
<td></td>
</tr>
<tr>
<td>Competing interests</td>
<td>25</td>
<td>Declare any competing interests of review authors.</td>
<td></td>
</tr>
<tr>
<td>Availability of data, code and other materials</td>
<td>26</td>
<td>Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.</td>
<td></td>
</tr>
</tbody>
</table>


For more information, visit: http://www.prisma-statement.org/
Appendix B. Systematic review data collection form

Preventive Strategy in Patients at Risk of MRONJ: Systematic Review Data Extraction Form

Full Text Article

General Information

<table>
<thead>
<tr>
<th>Reviewer name:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Article Title</td>
<td></td>
</tr>
<tr>
<td>First Author</td>
<td></td>
</tr>
<tr>
<td>Journal</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td></td>
</tr>
</tbody>
</table>

Study Eligibility

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(To eliminate animal studies, retrospective studies, case series letters and reviews)

Type of intervention

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
</table>

Study Design

<table>
<thead>
<tr>
<th>RCT</th>
<th>CCS</th>
<th>Split mouth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(RCT: randomised control study; CCS: case control study)

For RCT studies: what kind of randomization method was performed

Number of centres in trial

Country where trial was conducted

Source of funding

Was ethical approval reported?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Population Characteristics / Sampling Frame

<table>
<thead>
<tr>
<th>Dates of recruitment period</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients in the study</td>
<td></td>
</tr>
<tr>
<td>Number of patients evaluated</td>
<td></td>
</tr>
<tr>
<td>Age range of recruited population</td>
<td>______ yrs</td>
</tr>
<tr>
<td>Number of males</td>
<td></td>
</tr>
<tr>
<td>Number of females</td>
<td></td>
</tr>
<tr>
<td>Type of antiresorptive or antiangiogenic medications’ patient were exposed to</td>
<td></td>
</tr>
<tr>
<td>Length of antiresorptive or antiangiogenic medications’ exposure (in months)</td>
<td></td>
</tr>
<tr>
<td>Length of the follow-up</td>
<td></td>
</tr>
<tr>
<td>How many drop-outs or losses to follow-up were reported?</td>
<td></td>
</tr>
<tr>
<td>Were inclusion/exclusion criteria specified for the study?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Preventive Strategy in Patients at Risk of MRONJ: Systematic Review Data Extraction Form**

### Intervention Characteristics Within the Groups

<table>
<thead>
<tr>
<th>What type of intervention was carried out in the Case Group (treatment group)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>What type of intervention was carried out in the Control Group?</td>
</tr>
<tr>
<td>Was any prophylactic agent used in the Case Group? specified what type and when (eg. Type of ABX, pre-op/post-op/both)</td>
</tr>
<tr>
<td>Was any prophylactic agent used in the Control Group? specified what type and when (eg. Type of ABX, pre-op/post-op/both)</td>
</tr>
</tbody>
</table>

### Outcome Measured

<table>
<thead>
<tr>
<th>How many patients in the Case Group (treatment group) did develop MRONJ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many patients in the Control Group did develop MRONJ?</td>
</tr>
<tr>
<td>How many patients in the Case Group (treatment group) did develop MRONJ in the mandible?</td>
</tr>
<tr>
<td>How many patients in the Control Group did develop MRONJ in the mandible?</td>
</tr>
<tr>
<td>How many patients in the Case Group (treatment group) did develop MRONJ in the maxilla?</td>
</tr>
<tr>
<td>How many patients in the Control Group did develop MRONJ in the maxilla?</td>
</tr>
<tr>
<td>How many patients in the Case Group (treatment group) did develop MRONJ in both jaws?</td>
</tr>
<tr>
<td>How many patients in the Control Group did develop MRONJ in both jaws?</td>
</tr>
<tr>
<td>Any incidence or side-effects were reported</td>
</tr>
</tbody>
</table>
Preventive Strategy in Patients at Risk of MRONJ: Systematic Review Data Extraction Form

Quality of studies

| Risk of bias arising from the randomization process | Low / High / Some concerns |
| Risk of bias due to deviations from the intended interventions (effect of assignment to intervention) | Low / High / Some concerns |
| Risk of bias due to deviations from the intended interventions (effect of adhering to intervention) | Low / High / Some concerns |
| Missing outcome data | Low / High / Some concerns |
| Risk of bias in measurement of the outcome | Low / High / Some concerns |
| Risk of bias in selection of the reported result | Low / High / Some concerns |
| Overall risk of bias | Low / High / Some concerns |

ROBINS-I (For CCS studies)

| Bias due to confounding | Low / Moderate / Serious / Critical / NI |
| Bias in selection of participants into the study | Low / Moderate / Serious / Critical / NI |
| Bias in classification of interventions | Low / Moderate / Serious / Critical / NI |
| Bias due to deviations from intended interventions | Low / Moderate / Serious / Critical / NI |
| Bias due to missing data | Low / Moderate / Serious / Critical / NI |
| Bias in measurement of outcomes | Low / Moderate / Serious / Critical / NI |
| Bias in selection of the reported result | Low / Moderate / Serious / Critical / NI |
| Overall bias | Low / Moderate / Serious / Critical / NI |

GRADE assessment quality

| Summary of findings | High/Moderate/Low/Very low |
Appendix C. Risk of Bias in non-randomised studies - of interventions tool

The Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) assessment tool
(version for cohort-type studies)
Version 1 August 2016

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ROBINS-I tool (Stage I): At protocol stage
Specify the review question
Participants
Experimental intervention
Comparator
Outcomes

List the confounding domains relevant to all or most studies

List co-interventions that could be different between intervention groups and that could impact on outcomes
ROBINS-I tool (Stage II): For each study

Specify a target randomized trial specific to the study

Design
Individually randomized / Cluster randomized / Matched (e.g. cross-over)

Participants

Experimental intervention

Comparator

Is your aim for this study...?
- [ ] to assess the effect of assignment to intervention
- [ ] to assess the effect of starting and adhering to intervention

Specify the outcome

Specify which outcome is being assessed for risk of bias (typically from among those earmarked for the Summary of Findings table). Specify whether this is a proposed benefit or harm of intervention.

Specify the numerical result being assessed

In case of multiple alternative analyses being presented, specify the numeric result (e.g. RR = 1.52 (95% CI 0.83 to 2.77) and/or a reference (e.g. to a table, figure or paragraph) that uniquely defines the result being assessed.
## Risk of bias assessment (cohort-type studies)

Responses, unadjusted to group, are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to signs to other questions, no formatting is used.

<table>
<thead>
<tr>
<th>Bias domain</th>
<th>Signalling questions</th>
<th>Elaboration</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias due to confounding</td>
<td>1.1 Is there potential for confounding of the effect of intervention in this study?</td>
<td>In rare situations, such as when studying harms that are very unlikely to be related to factors that influence treatment decisions, no confounding is expected and the study can be considered to be at low risk of bias due to confounding, equivalent to a fully randomized trial. There is no N (no information) option for this signalling question.</td>
<td>Y / PY / FN / FN</td>
</tr>
<tr>
<td></td>
<td>If Y / PY to 1.1: determine whether there is a need to assess time-varying confounding.</td>
<td>If participants could switch between intervention groups then associations between intervention and outcome may be biased by time-varying confounding. This occurs when prognostic factors influence switches between intended interventions.</td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td></td>
<td>If N / PN, proceed to question 1.3.</td>
<td>If intervention switches are unrelated to the outcome, for example when the outcome is an unexpected harm, then time-varying confounding will not be present and only control for baseline confounding is required.</td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>Questions relating to baseline confounding only</td>
<td>1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?</td>
<td>Appropriate methods to control for measured confounders include stratification, regression, matching, standardization, and inverse probability weighting. They may control for individual variables or for the estimated propensity score. Inverse probability weighting is based on a function of the propensity score. Each method depends on the assumption that there is no unmeasured or residual confounding.</td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td></td>
<td>1.5. If Y / PY to 1.4: Were confounding domains that were controlled for measured validity and reliability by the variables available in this study?</td>
<td>Appropriate control of confounding requires that the variables adjusted for are valid and reliable measures of the confounding domains. For some topics, a list of valid and reliable measures of confounding domains will be specified in the review protocol but for others such a list may not be available. Study authors may cite references to support the use of a particular measure. If authors control for confounding variables with no indication of their validity or reliability pay attention to the subjectivity of the measure. Subjective measures (e.g. based on self-report) may have lower validity and reliability than objective measures such as lab findings.</td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td></td>
<td>1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?</td>
<td>Controlling for post-intervention variables that are affected by intervention is not appropriate. Controlling for mediating variables estimates the direct effect of intervention and may introduce bias. Controlling for common effects of intervention and outcome introduces bias.</td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>Questions relating to baseline and time-varying confounding</td>
<td>1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?</td>
<td>Adjustment for time-varying confounding is necessary to estimate the effect of starting and adhering to intervention, in both randomized trials and RNS. Appropriate methods include those based on inverse probability weighting. Standard regression models that include time-updated confounders may be problematic if time-varying confounding is present.</td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td></td>
<td>1.8. If Y / PY to 1.7: Were confounding domains that were adjusted for measured validity and reliability by the variables available in this study?</td>
<td>See Table 1.</td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>Risk of bias judgement</td>
<td>Optional: What is the predicted direction of bias due to confounding?</td>
<td>Can the true effect estimate be predicted to be greater or less than the estimated effect in the study because one or more of the important confounding domains was not controlled for? Answering this question will be based on expert knowledge and results in other studies and therefore can only be completed after all of the studies in the body of evidence have been reviewed. Consider the potential effect of each of the unmeasured domains and whether all important confounding domains not controlled for in the analysis would be likely to change the estimate in the same direction, or if one important confounding domain that was not controlled for in the analysis is likely to have a dominant impact.</td>
<td>Low / Moderate / Serious / Critical / NI</td>
</tr>
<tr>
<td>Bias in selection of participants into the study</td>
<td>2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If Y/PN to 2.1: go to 2.4</td>
<td>2.2. If Y/PY to 2.1: Were the post-intervention variables that influenced selection likely to be associated with the intervention?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.3 If Y/PY to 2.2: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 2.4. Do start of follow-up and start of intervention coincide for most participants? |
|----|---------------------------------------------------------------------------------|
| If Y/PY to 2.2 and 2.3, or N/PN to 2.4 | 2.5. Were adjustment techniques used that are likely to correct for the presence of selection biases? |

<table>
<thead>
<tr>
<th>Risk of bias judgement</th>
<th>Optional: What is the predicted direction of bias due to selection of participants into the study?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y/PY / PN / NI</td>
<td>If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bias in classification of intervention</th>
<th>3.1 Were intervention groups clearly defined?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A pre-requisite for an appropriate comparison of interventions is that the interventions are well defined. Ambiguity in the definition may lead to bias in the classification of participants. For individual-level interventions, criteria for considering individuals to have received each intervention should be clear and explicit, covering issues such as type, setting, dose, frequency, intensity and/or timing of intervention. For population-level interventions (e.g. measures to control air pollution), the question relates to whether the population is clearly defined, and the answer is likely to be 'Yes'.</td>
</tr>
</tbody>
</table>

| 3.2 Was the information used to define intervention groups recorded at the start of the intervention? |
|----|--------------------------------------------------------------------------------|
| In general, if information about interventions received is available from sources that could not have been affected by subsequent outcomes, then differential misclassification of intervention status is unlikely. Collection of the information at the time of the intervention makes it easier to avoid such misclassification. For population-level interventions (e.g. measures to control air pollution), the question relates to whether the information may not be sufficient to avoid bias. The way in which the data are collected for the purposes of the NRSI should also avoid misclassification. |

| 3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome? |
|----|-------------------------------------------------------------------|
| Risk of bias judgement | Optional: What is the predicted direction of bias due to measurement of outcomes or interventions? |
| Y/PY / PN / NI | If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions. |

<p>| | See Table 1 |
| Low / Moderate / Serious / Critical / NI | Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable |</p>
<table>
<thead>
<tr>
<th>Bias due to deviations from intended interventions</th>
<th>If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?</td>
<td>Deviations that happen in usual practice following the intervention (for example, cessation of a drug intervention because of acute toxicity) are part of the intended intervention and therefore do not lead to bias in the effect of assignment to intervention. Deviations may arise due to expectations of a difference between intervention and comparator (for example because participants feel unlucky to have been assigned to the comparator group and therefore seek the active intervention, or components of it, or other interventions). Such deviations are not part of usual practice, so may lead to biased effect estimates. However these are not expected in observational studies of individuals in routine care.</td>
</tr>
<tr>
<td>4.2. If Y to 4.1: Were these deviations from intended intervention unbalanced between groups and likely to have affected the outcome?</td>
<td>Deviations from intended interventions that do not reflect usual practice will be important if they affect the outcome, but not otherwise. Furthermore, bias will arise only if there is imbalance in the deviations across the two groups.</td>
</tr>
<tr>
<td>If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6</td>
<td></td>
</tr>
<tr>
<td>4.3. Were important co-interventions balanced across intervention groups?</td>
<td>Risk of bias will be higher if unplanned co-interventions were implemented in a way that would bias the estimated effect of intervention. Co-interventions will be important if they affect the outcome, but not otherwise. Bias will arise only if there is imbalance in such co-interventions between the intervention groups. Consider the co-interventions, including any pre-specified co-interventions, that are likely to affect the outcome and to have been administered in this study. Consider whether these co-interventions are balanced between intervention groups.</td>
</tr>
<tr>
<td>4.4. Was the intervention implemented successfully for most participants?</td>
<td>Risk of bias will be higher if the intervention was not implemented as intended by, for example, the health care professionals delivering care during the trial. Consider whether implementation of the intervention was successful for most participants.</td>
</tr>
<tr>
<td>4.5. Did study participants adhere to the assigned intervention regimen?</td>
<td>Risk of bias will be higher if participants did not adhere to the intervention as intended. Lack of adherence includes imperfect compliance, cessation of intervention, crossovers to the comparator intervention and switches to another active intervention. Consider available information on the proportion of study participants who continued with their assigned intervention regimen.</td>
</tr>
</tbody>
</table>
### Bias due to missing data

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Were outcome data available for all, or nearly all, participants?</td>
<td>&quot;Nearly all&quot; should be interpreted as &quot;enough to be confident of the findings&quot;, and a suitable proportion depends on the context. In some situations, availability of data from 95% (or possibly 90%) of the participants may be sufficient, providing that events of interest are reasonably common in both intervention groups. One aspect of this is that review authors would ideally try and locate an analysis plan for the study. If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.</td>
</tr>
<tr>
<td>5.2 Were participants excluded due to missing data on intervention status?</td>
<td>Missing intervention status may be a problem. This requires that the intended study sample is clear, which it may not be in practice.</td>
</tr>
<tr>
<td>5.3 Were participants excluded due to missing data on other variables needed for the analysis?</td>
<td>This aims to elicit whether either (i) differential proportion of missing observations or (ii) reasons for missing observations could substantially impact on our ability to answer the question being addressed. &quot;Similar&quot; includes some minor degree of discrepancy across intervention groups as expected by chance.</td>
</tr>
<tr>
<td>5.4 If P/N to 4.3, 4.4 or 4.5: Are the proportion of participants and reasons for missing data similar across interventions?</td>
<td>NA / Y / P / N / Ni</td>
</tr>
<tr>
<td>5.5 If P/N to 5.1, or Y/P to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?</td>
<td>Evidence for robustness may come from how missing data were handled in the analysis and whether sensitivity analyses were performed by the investigators, or occasionally from additional analyses performed by the systematic reviewers. It is important to assess whether assumptions employed in analyses are clear and plausible. Both content knowledge and statistical expertise will often be required for this. For instance, use of a statistical method such as multiple imputation does not guarantee an appropriate answer. Review authors should seek naive (complete-case) analyses for comparison, and clear differences between complete-case and multiple imputation-based findings should lead to careful assessment of the validity of the methods used.</td>
</tr>
</tbody>
</table>

### Risk of bias judgement

<table>
<thead>
<tr>
<th>Bias due to missing data</th>
<th>See Table 2</th>
</tr>
</thead>
</table>

Optional: What is the predicted direction of bias due to missing data?

If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.
### Bias in measurement of outcomes

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 Could the outcome measure have been influenced by knowledge of the intervention received?</td>
<td>Some outcome measures involve negligible assessor judgment, e.g. all-cause mortality or non-repeatable laboratory assessments. Risk of bias due to measurement of these outcomes would be expected to be low.</td>
</tr>
<tr>
<td>6.2 Were outcome assessors aware of the intervention received by study participants?</td>
<td>If outcome assessors were blinded to intervention status, the answer to this question would be ‘No’. In other situations, outcome assessors may be unaware of the interventions being received by participants despite there being no active blinding by the study investigators; the answer to this question would then also be ‘No’. In studies where participants report their outcomes themselves, for example in a questionnaire, the outcome assessor is the study participant: in an observational study, the answer to this question will usually be ‘yes’ when the participants report their outcomes themselves.</td>
</tr>
<tr>
<td>6.3 Were the methods of outcome assessment comparable across intervention groups?</td>
<td>Comparable assessment methods (e.g. data collection) would involve the same outcome detection methods and thresholds, same time point, same definition, and same measurements.</td>
</tr>
<tr>
<td>6.4 Were any systematic errors in measurement of the outcome related to intervention received?</td>
<td>This question refers to differential misclassification of outcomes. Systematic errors in measuring the outcome, if present, could cause bias if they are related to intervention or to a confounder of the intervention-outcome relationship. This will usually be due either to outcome assessors being aware of the intervention received or to non-comparability of outcome assessment methods, but there are examples of differential misclassification arising despite these controls being in place.</td>
</tr>
</tbody>
</table>

### Risk of bias judgement

<table>
<thead>
<tr>
<th>Bias in selection of the reported result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the reported effect estimate likely to be selected, on the basis of the results, from...</td>
</tr>
<tr>
<td>7.1 ... multiple outcome measurements within the outcome domain?</td>
</tr>
<tr>
<td>For a specified outcome domain, it is possible to generate multiple effect estimates for different measurements. If multiple measurements were made, but only one or a subset is reported, there is a risk of selective reporting on the basis of results.</td>
</tr>
<tr>
<td>Y / N / NI</td>
</tr>
<tr>
<td>7.2 ... multiple analyses of the intervention-outcome relationship?</td>
</tr>
<tr>
<td>Because of the limitations of using data from non-randomised studies for analyses of effectiveness (need to control confounding, substantial missing data, etc), analysts may implement different analytic methods to address these limitations. Examples include unadjusted and adjusted models; use of final value vs change from baseline vs analysis of covariance; different transformations of variables; a continuously scaled outcome converted to categorical data with different cut-points; different sets of covariates used for adjustment; and different analytic strategies for dealing with missing data. Application of such methods generates multiple estimates of the effect of the intervention versus the comparator on the outcome. If the analyst does not pre-specify the methods to be applied, and multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results.</td>
</tr>
<tr>
<td>Y / N / NI</td>
</tr>
<tr>
<td>7.3 ... different subgroups?</td>
</tr>
<tr>
<td>Particularly with large cohorts often available from routine data sources, it is possible to generate multiple effect estimates for different subgroups or simply to omit varying proportions of the original cohort. If multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results.</td>
</tr>
<tr>
<td>Y / N / NI</td>
</tr>
</tbody>
</table>

### Risk of bias judgement

<table>
<thead>
<tr>
<th>Optional: What is the predicted direction of bias due to selection of the reported result?</th>
</tr>
</thead>
<tbody>
<tr>
<td>If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.</td>
</tr>
<tr>
<td>Y / N / NI</td>
</tr>
</tbody>
</table>

### Low / Moderate / Serious / Critical / NI

<table>
<thead>
<tr>
<th>Favour's experimental / Favour's comparator / Towards null / Away from null / Unpredictable</th>
</tr>
</thead>
</table>
Overall bias | Risk of bias judgement | See Table 3. | Low / Moderate / Serious / Critical / NI Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable
---|---|---|---
Optional: What is the overall predicted direction of bias for this outcome?
Appendix D. Cochrane tool for assessing risk of bias in randomised trials (RoB2)

Revised Cochrane risk-of-bias tool for randomized trials (RoB 2)
TEMPLATE FOR COMPLETION

Edited by Julian PT Higgins, Jelena Savović, Matthew J Page, Jonathan AC Sterne
on behalf of the RoB2 Development Group

Version of 22 August 2019

The development of the RoB 2 tool was supported by the MRC Network of Hubs for Trials Methodology Research (MR/L004933/2 - NS61), with the support of the host MRC CTC-2 Hub (Collaboration and Innovation for Difficult and Complex randomised controlled Trials in Invasive procedures - MR/K025648/1), by MRC research grant MR/K02520/1, and by a grant from The Cochrane Collaboration.

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Risk of bias assessment
Responses underlined in green are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to sign posts to other questions, no formatting is used.

Domain 1: Risk of bias arising from the randomization process

<table>
<thead>
<tr>
<th>Signalling questions</th>
<th>Comments</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Was the allocation sequence random?</td>
<td></td>
<td>✔️/ ☑️/ ✗/ ✗/ ✗/ ✗</td>
</tr>
<tr>
<td>1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?</td>
<td></td>
<td>✔️/ ☑️/ ✗/ ✗/ ✗/ ✗</td>
</tr>
<tr>
<td>1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?</td>
<td></td>
<td>✔️/ ☑️/ ✗/ ✗/ ✗/ ✗</td>
</tr>
</tbody>
</table>

Risk-of-bias judgement
Low / High / Some concerns

Optional: What is the predicted direction of bias arising from the randomization process?
NA / Favour experimental / Favour comparator / Towards null / Away from null / Unpredictable

235
### Domain 2: Risk of bias due to deviations from the intended interventions (effect of assignment to intervention)

<table>
<thead>
<tr>
<th>Signalling questions</th>
<th>Comments</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Were participants aware of their assigned intervention during the trial?</td>
<td>Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.2. Were carers and people delivering the interventions aware of participants’ assigned intervention during the trial?</td>
<td>Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.3. [If Y/PP/NI to 2.1 or 2.2:] Were there deviations from the intended intervention that arose because of the trial context?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.4. [If Y/PP to 2.3:] Were these deviations likely to have affected the outcome?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.5. [If Y/PP/NI to 2.4:] Were these deviations from intended intervention balanced between groups?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.6. Was an appropriate analysis used to estimate the effect of assignment to intervention?</td>
<td>Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.7. [If N/PP/NI to 2.6:] Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
</tbody>
</table>

**Risk-of-bias judgement**

Low / High / Some concerns

Optional: What is the predicted direction of bias due to deviations from intended interventions?

NA / Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable

---

### Domain 2: Risk of bias due to deviations from the intended interventions (effect of adhering to intervention)

<table>
<thead>
<tr>
<th>Signalling questions</th>
<th>Comments</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Were participants aware of their assigned intervention during the trial?</td>
<td>Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.2. Were carers and people delivering the interventions aware of participants’ assigned intervention during the trial?</td>
<td>Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.3. [If applicable:] If Y/PP/NI to 2.1 or 2.2:] Were important non-protocol interventions balanced across intervention groups?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants’ outcomes?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
<tr>
<td>2.6. If N/PP/NI to 2.3, or Y/PP/NI to 2.4 or 2.5:] Was an appropriate analysis used to estimate the effect of adhering to the intervention?</td>
<td>NA / Y / PP / PN / NI</td>
<td></td>
</tr>
</tbody>
</table>

**Risk-of-bias judgement**

Low / High / Some concerns

Optional: What is the predicted direction of bias due to deviations from intended interventions?

NA / Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable
## Domain 3: Missing outcome data

<table>
<thead>
<tr>
<th>Signalling questions</th>
<th>Comments</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Were data for this outcome available for all, or nearly all, participants randomized?</td>
<td></td>
<td>Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>3.2 If <em>NP/N</em> to 3.1: Is there evidence that the result was not biased by missing outcome data?</td>
<td></td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>3.3 If <em>NP</em> to 3.2: Could missingness in the outcome depend on its true value?</td>
<td></td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>3.4 If <em>PY/N</em> to 3.3: Is it likely that missingness in the outcome depended on its true value?</td>
<td></td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>Risk-of-bias judgement</td>
<td></td>
<td>Low / High / Some concerns</td>
</tr>
<tr>
<td>Optional: What is the predicted direction of bias due to missing outcome data?</td>
<td></td>
<td>NA / Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable</td>
</tr>
</tbody>
</table>

## Domain 4: Risk of bias in measurement of the outcome

<table>
<thead>
<tr>
<th>Signalling questions</th>
<th>Comments</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Was the method of measuring the outcome inappropriate?</td>
<td></td>
<td>Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?</td>
<td></td>
<td>Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>4.3 If <em>NP/N</em> to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?</td>
<td></td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>4.4 If <em>PY/N</em> to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?</td>
<td></td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>4.5 If <em>PY/N</em> to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?</td>
<td></td>
<td>NA / Y / PY / PN / N / NI</td>
</tr>
<tr>
<td>Risk-of-bias judgement</td>
<td></td>
<td>Low / High / Some concerns</td>
</tr>
<tr>
<td>Optional: What is the predicted direction of bias in measurement of the outcome?</td>
<td></td>
<td>NA / Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable</td>
</tr>
</tbody>
</table>
## Domain 5: Risk of bias in selection of the reported result

<table>
<thead>
<tr>
<th>Signalling questions</th>
<th>Comments</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?</td>
<td></td>
<td>Y / P / PN / N / NI</td>
</tr>
<tr>
<td>Is the numerical result being assessed likely to have been selected, on the basis of the results, from...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?</td>
<td></td>
<td>Y / P / PN / N / NI</td>
</tr>
<tr>
<td>5.3 ... multiple eligible analyses of the data?</td>
<td></td>
<td>Y / P / PN / N / NI</td>
</tr>
<tr>
<td>Risk-of-bias judgement</td>
<td></td>
<td>Low / High / Some concerns</td>
</tr>
</tbody>
</table>

Optional: What is the predicted direction of bias due to selection of the reported result?

<table>
<thead>
<tr>
<th>Overall risk of bias</th>
<th>Risk-of-bias judgement</th>
<th>Low / High / Some concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optional: What is the overall predicted direction of bias for this outcome?</td>
<td></td>
<td>NA / Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable</td>
</tr>
</tbody>
</table>
### jamovi Desktop

#### Download for macOS

#### 2.3.21 solid & current

Recommended For All Users

---

#### All Releases

<table>
<thead>
<tr>
<th>OS</th>
<th>Release</th>
<th>Format</th>
<th>Version</th>
</tr>
</thead>
<tbody>
<tr>
<td>Windows</td>
<td>solid</td>
<td>.exe</td>
<td>2.3.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.zip</td>
<td>2.3.21</td>
</tr>
<tr>
<td></td>
<td>legacy</td>
<td>.exe</td>
<td>2.2.5</td>
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<tr>
<td></td>
<td></td>
<td>.zip</td>
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<tr>
<td>macOS</td>
<td>solid</td>
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<td>2.3.21</td>
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<tr>
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<td>.dmg</td>
<td>2.2.5</td>
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<td></td>
<td>legacy</td>
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<td>ChromeOS</td>
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<td>flathub</td>
<td>2.3.21</td>
</tr>
</tbody>
</table>
Appendix F. Research Ethics Committee (REC) – ethical approval decision tool

Welcome

Not all research conducted within the UK requires review by an NHS Research Ethics Committee (REC).

This decision tool:

- **will** help you to determine if your study requires a review by an NHS REC
- **will not** tell you whether you need any other regulatory approvals and/or types of ethics review

You should check what other reviews or approvals are needed for your research irrespective of the result from this tool.

At each stage of the decision tool you will be asked a series of questions. Read each question carefully and answer by selecting the ‘YES’ or ‘NO’ buttons.

To help you with terminology, a GLOSSARY button is available at the bottom every page. All links to individual glossary items or other websites appear in blue text and open in a new window.

Select the ALGORITHM button, which is at the bottom of every page, for more detailed information about both the policy and legislation requirements for NHS REC review that apply to the UK or to particular countries of the UK.

**Post Market Surveillance** is NOT usually considered research. However, there are some circumstances where an NHS REC review may be required. Select YES below to determine if your post market surveillance requires NHS REC review.

Firstly, is your study research?

- **YES**
- **NO**
- **NOT SURE**
Data Extraction Form: Service Evaluation of Exodontia Protocol in High Risk MRONJ Patients

### Patients Characteristics

| ID number |  |
| Gender |  |
| Age |  |
| MH |  |
| Type of Cancer |  |
| Drug therapy |  |
| Type of antiresorptive therapy |  |
| Route of administration |  |
| Length of antiresorptive drug therapy (months) |  |
| Location of the performing procedure | Primary care / Secondary care |

### Type of intervention

| Number of teeth extracted |  |
| Any bone removal performed (alveoloplasty) |  |
| Location of the surgical site (mandible or maxilla or both) |  |
| Any post-op antibiotic prescribed | Yes [ ] No [ ] |
| Type of antibiotics |  |
| Duration of the antibiotics therapy |  |
| Any topical antimicrobial prescribed (mouthwash) | Yes [ ] No [ ] |
| Any pre-op topical antimicrobial given (mouthwash) | Yes [ ] No [ ] |
| Any post-op topical antimicrobial given (mouthwash) | Yes [ ] No [ ] |
| Type of mouthwash |  |
| Duration of the topical antimicrobial given (days) |  |
### Data Extraction Form: Service Evaluation of Exodontia Protocol in High Risk MRONJ Patients

#### Outcome measured

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-ups (weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any developed MRONJ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of MRONJ (exposed, not exposed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRONJ Onset (how many weeks from the exodontia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site of MRONJ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRONJ treatment modality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any MRONJ improvement or healing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix H. MRONJ low risk patients – data collection form

Data Extraction Form: Service Evaluation of Exodontia Protocol in Low Risk MRONJ Patients

<table>
<thead>
<tr>
<th>Patients Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID number</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>MH</td>
</tr>
<tr>
<td>Type of disease which required antiresorptive therapy</td>
</tr>
<tr>
<td>Drug therapy other than antiresorptive therapy</td>
</tr>
<tr>
<td>Type of antiresorptive therapy</td>
</tr>
<tr>
<td>Route of antiresorptive drug therapy</td>
</tr>
<tr>
<td>Length of antiresorptive drug therapy (months)</td>
</tr>
<tr>
<td>Location of the performing procedure</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of teeth extracted</td>
</tr>
<tr>
<td>Any bone removal performed (alveoloplasty)</td>
</tr>
<tr>
<td>Location of the surgical site (mandible or maxilla or both)</td>
</tr>
<tr>
<td>Any post-op antibiotic prescribed</td>
</tr>
<tr>
<td>Type of antibiotics</td>
</tr>
<tr>
<td>Duration of the antibiotics therapy</td>
</tr>
<tr>
<td>Any topical antimicrobial prescribed (mouthwash)</td>
</tr>
<tr>
<td>Any pre-op topical antimicrobial given (mouthwash)</td>
</tr>
<tr>
<td>Any post-op topical antimicrobial given (mouthwash)</td>
</tr>
<tr>
<td>Type of mouthwash</td>
</tr>
<tr>
<td>Data Extraction Form: Service Evaluation of Exodontia Protocol in Low Risk MRONJ Patients</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Duration of the topical antimicrobial given (days)</strong></td>
</tr>
<tr>
<td><strong>Outcome measured</strong></td>
</tr>
<tr>
<td>Follow-ups (weeks)</td>
</tr>
<tr>
<td>Any delayed healing (no full epithelialization beyond 4 weeks)</td>
</tr>
<tr>
<td>How long for full epithelialization (weeks)</td>
</tr>
<tr>
<td>Any developed MRONJ</td>
</tr>
<tr>
<td>Type of MRONJ (exposed, not exposed)</td>
</tr>
<tr>
<td>MRONJ Onset (how many weeks from the exodontia)</td>
</tr>
<tr>
<td>Site of MRONJ</td>
</tr>
<tr>
<td>MRONJ treatment modality</td>
</tr>
<tr>
<td>Any MRONJ improvement or healing</td>
</tr>
</tbody>
</table>
Appendix I. List of publications

Review

The Use of Human Amniotic Membrane (hAM) as a Treatment Strategy of Medication-Related Osteonecrosis of the Jaw (MRONJ): A Systematic Review and Meta-Analysis of the Literature

Roberto Sacco, Oladapo Akintola, Nicola Sacco, Alessandro Acocella, Monica Diuana Calasans-Maia, Massimo Maranzano, and Sergio Olate

Citation: Sacco, R.; Akintola, O.; Sacco, N.; Acocella, A.; Calasans-Maia, M.D.; Maranzano, M.; Olate, S. The Use of Human Amniotic Membrane (hAM) as a Treatment Strategy of Medication-Related Osteonecrosis of the Jaw (MRONJ): A Systematic Review and Meta-Analysis of the Literature. Medicina 2023, 59, 968. https://doi.org/10.3390/medicina59050968

Abstract: Background and objectives: Although it is very uncommon, medication-induced osteonecrosis of the jaw (also known as MRONJ) can have serious consequences. Traditionally, this adverse event has been recognised in patients who were treated with bisphosphonate (BP) drugs. Nevertheless, in recent years, it has been established that individuals having treatment with various types of medications, such as a receptor activator of nuclear factor kappa B ligand inhibitor (denosumab) and antiangiogenic agents, have had the same issue. The purpose of this research is to determine if the application of human amniotic membrane (hAM) may be used as a therapy for MRONJ.

Material and Methods: A multi-source database (MEDLINE, EMBASE, AMED, and CENTRAL) systematic search was performed. The major objective of this study is to obtain an understanding of the efficacy of hAM when it is employed as a treatment modality for MRONJ. The protocol of this review was registered in the INPLASY register under the number NPLASY202330010.

Results: The authors were able to include a total of five studies for the quality analysis, whereas for the quantity evaluation, only four studies were eligible. A total of 91 patients were considered for the investigation. After treatment with human amniotic membrane (hAM), a recurrence of osteonecrosis was observed in n=6 cases (8.8%). The combined efficacy of surgical therapy and the use of hAM resulted in an overall success rate of 91.2%. Intraoperative complications were only documented in one article, and they were mostly caused by the positioning of the hAM, which led to wound breakdown at the surgical site.

Conclusions: Based on the small amount of data and low-quality research included in this study, using human amniotic membranes to treat MRONJ might represent a feasible option. Nevertheless, further studies with a wider patient population are required to understand the long-term impacts.

Keywords: medication-related osteonecrosis of the jaw; skeletal-related events; human amniotic membrane; cancer; antiresorptive drugs; antiangiogenic drugs

1. Introduction

Since 2003, medication-related osteonecrosis of the jaw (MRONJ) has been a major concern as a severe side effect, mainly caused by bisphosphonate (BP) therapy for both...
A Comprehensive Quality Meta-Review of Genetic and Pharmacogenomic Aspects of Medication-Related Osteonecrosis of The Jaw (MRONJ)

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Abstract: Background: Antiresorptive and antiangiogenic medications can cause a serious adverse effect known as medication-related osteonecrosis of the jaw (MRONJ). In recent years, a new trend of research has emerged emphasizing the potential relation of MRONJ and genetic predisposition. Current evidence-based science of this adverse reaction is associated with poorly performed studies. Additionally, MRONJ research has recently observed a new trend of studies orientated towards the misuse of reviews. This quality meta-review intends to summarize the results of all systematic reviews and meta-analyses that have been published on MRONJ in relation to genetic and pharmacogenomics risk factors. Methods: The research study protocol was registered into the database of the International Network for the Registration of Systematic Reviews and Meta-Analyses (INPLASY) INPLASY202230002. A comprehensive search across several databases (PubMed, EMBASE, MEDLINE, and CINAHL) was conducted to locate multi-language papers published between January 2003 and November 2022. Data were collected from relevant research studies and appraised in accordance with the precise outcomes described in this evaluation. Results: Only five systematic reviews and meta-analyses were analysed in this meta-review. All the reviews included in this research presented qualities mistakes and shortcomings. Two quality assessment tools (Confidence in Evidence from Reviews of Qualitative research (CERQual) and Assessment of Multiple Systematic Reviews 2 (AMSTAR-2)) were used to evaluate each study included in this research. Conclusions: The data evaluated by this meta-review confirmed the poor-quality secondary research underpinning the genetic/pharmacogenomics aspect of MRONJ. Moreover, this study highlighted the many flaws of the current published systematic and meta-analysis studies published so far.

Keywords: MRONJ; genetic; osteonecrosis; pharmacogenomics; evidence-based medicine; meta-review

1. Introduction

One of the most serious side effects associated with the use of particular drugs is a condition known as medication-related osteonecrosis of the jaw (MRONJ). This condition is induced primarily by antiresorptive drugs and antiangiogenesis inhibitors [1,2]. Commonly, these drugs are used to treat the skeletal signs and symptoms of primary or secondary bone
Review

Systematic review of medication related osteonecrosis of the jaw (MRONJ) in patients undergoing only antiangiogenic drug therapy: surgery or conservative therapy?

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Accepted 16 March 2021
Available online 24 March 2021
Review

Evaluation of segmental mandibular resection without microvascular reconstruction in patients affected by medication-related osteonecrosis of the jaw: a systematic review

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Accepted 18 December 2020
Available online 24 December 2020
Review article

The risk of osteonecrosis of the jaw and adverse outcomes in patients using antiresorptive drugs undergoing orthodontic treatment: A systematic review

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ARTICLE INFO

Keywords:
Orthodontic treatment
Osteonecrosis
MRONJ
Tooth movement
Antiresorptive drugs

ABSTRACT

Objectives: Medication-related osteonecrosis of the jaw (MRONJ) is rare. It is a serious adverse effect of certain drugs, of which bisphosphonates (BPs) are the most widely known. The aim of this systematic review was to analyze all published evidence for the reported adverse outcomes as a result of orthodontic treatment in patients undergoing antiresorptive therapy.

Data: All types of studies involving patients undergoing orthodontic treatment and treated with antiresorptive drugs were considered. A meta-analysis was not conducted due to the high amount of variability and heterogeneity in the reporting and presentation of data among the studies meeting the inclusion criteria.

Sources: A systematic search was performed using 4 databases (PubMed, MEDLINE, EMBASE and CINAHL).

Study selection: Seven studies matched the inclusion criteria for this review, reporting a total of 29 patients. MRONJ was only reported in 1 patient. The adverse outcomes following orthodontic treatment included difficulty achieving root parallelism (n = 4), difficulty achieving complete space closure (n = 3), exaggerated tooth mobility post-debond (n = 2), increased duration of orthodontic treatment beyond expected completion (n = 1), sclerotic alveolar bone changes seen on post-op radiographic images (n = 2), and an increased amount of root resorption (n = 1).

Conclusions: The high amount of heterogeneity and limited evidence precluded a valid interpretation and analysis of the results through pooling of data. Additional data with sufficient quality, a reduction of bias, and a greater prospective cohort of patients is crucial to assess adverse effects, mechanisms of action, and associated risk factors in at-risk patients.

Clinical significance: Based on the limited evidence available in the literature, it is unclear whether orthodontic treatment alone can precipitate MRONJ. However, antiresorptive drug therapy may be associated with a suboptimal treatment outcome.

1. Introduction

Bisphosphonates (BPs) are antiresorptive drugs used to manage numerous conditions such as osteoporosis, fibrous dysplasia and metastatic bone diseases. Bisphosphonate therapy is generally well tolerated by patients [1, 2]. The purpose of antiresorptive drug therapies is to restore the bone density by decreasing remodelling of bone. BPs have direct effects on osteoclasts by significantly attenuating bone remodelling and decreasing skeletal-related complications in patients with malignant diseases or osteoporosis [1]. The first reported cases of patients developing a non-healing necrosis in the maxillofacial region was reported in 2003 by Marx et al. The disease was associated with patients taking bisphosphonates and therefore aptly named bisphosphonate-related osteonecrosis of the jaw (BRONJ) [3].

The work should be attributed to University of Manchester Division of Dentistry, School of Medical Sciences, Oral Surgery Department, Manchester, UK - Coupland 3 Building, Oxford Rd, Manchester, M13 9PL, UK.

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Review

The role of illicit drugs in developing medication-related osteonecrosis (MRONJ): a systematic review

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Accepted 10 August 2020
Available online 20 August 2020
The role of antiresorptive drugs and medication-related osteonecrosis of the jaw in nononcologic immunosuppressed patients: A systematic review

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Review

Osteonecrosis and osteomyelitis of the jaw associated with tumour necrosis factor-alpha (TNF-α) inhibitors: a systematic review

R. Sacco\textsuperscript{a,b,c,e}, S. Shah\textsuperscript{e}, R. Leeson\textsuperscript{d}, V. Moraschini\textsuperscript{c,f}, C.F. de Almeida Barros Mourão\textsuperscript{g}, O. Akintola\textsuperscript{c}, A. Lalli\textsuperscript{h}

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Available online 20 October 2019
A Systematic Review of Oxygen Therapy for the Management of Medication-Related Osteonecrosis of the Jaw (MRONJ)

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Abstract: Background: Medication-related osteonecrosis of the jaw (MRONJ) can be a life changing iatrogenic complication of antiresorptive and antiangiogenic drug therapy. It is most often associated with high doses of these medications that are used to prevent skeletal-related events in patients with cancer and bone pathologies. Unfortunately, managing MRONJ lesions has proven difficult and remains a major challenge for clinicians. Due to the lack of efficacy in treating MRONJ by surgical modalities (local debridement and free flap reconstruction), the nonsurgical management of MRONJ is still advocated to aid healing or avoid disease progression. The aim of this systematic review is to identify, analyse and understand the published evidence related to the success of oxygen therapies such as ozone (OT) and hyperbaric oxygen (HBO) in treating MRONJ. Material and methods: A multi-database (PubMed, MEDLINE, EMBASE, CINAHL and Cochrane CENTRAL) systematic search was performed by three authors. The identified articles were independently assessed for their risk of bias. Any type of study evaluating humans treated with antiresorptive and antiangiogenic drugs were considered. The aim is primarily to evaluate the success of OT and HBO in resolving MRONJ and secondarily to identify any improvements in quality of life (QoL), rate of complications, time-to-event and severity of side effects related to these treatments. Results: In total, just 13 studies were eligible for analysis. A pooled total of 313 patients (HBO group n = 82; OT group n = 231) described in these studies have shown good tolerance for oxygen therapies. Complete resolution of MRONJ was reported in 44.58% of OT patients but only 5.17% of the HBO group. Progression of MRONJ was reported only in the HBO studies in 10.34% of cases (6 patients). The quality of evidence was low or very low in all studies. This was due to limitations in how the studies were designed, run and reported. Conclusions: Based on the limited data available, it is difficult to suggest OT is better or worse than HBO or whether it is better than a placebo. As the level of evidence available is low, this necessitates larger well-designed trials to justify these interventions for patients affected by MRONJ.

Review Article

Microsurgical Reconstruction of the Jaws Using Vascularised Free Flap Technique in Patients with Medication-Related Osteonecrosis: A Systematic Review

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Background. Osteonecrosis of the jaw (ONJ) has been reported to be associated with patients receiving primarily bisphosphonate (BP) therapies. However, lately it has been documented that other medications, such as RANK ligand inhibitor (denosumab) and antiangiogenic drug, can cause ONJ. Micro-osseous-vascular reconstruction of the jaws in patients affected by medication-related osteonecrosis of the jaw represents a viable option of treatment for patients affected by stage III of the disease. However, there are still considerable doubts about the success of this procedure in the short, medium, and long term. Material and Methods. A multidatabase (PubMed/EMBASE, MEDLINE, CENTRAL) systematic search was performed. Any type of studies considering human patients treated with antiresorptive and antiangiogenic drugs was considered. The aim of the research is to primarily understand the success rate of micro-osseous-vascular reconstruction in the short, medium, and long period of time. This review has also the goal of better understanding any perioperative and postoperative complications resulting from the use of the reconstruction techniques. Results. Eighteen studies resulted eligible for the study. Fibula free flap is the most commonly utilised vascularised free flap reconstruction technique (80.76%). Ten out of eighteen studies reported no complications. Recurrence of osteonecrosis was registered in five cases (6.41%) after free flap reconstruction. The overall free flap success rate was 96.16%. Conclusions. Based on the limited data available in literature (Level 4 of the Oxford Evidence-based medicine scale), micro-osseous-vascular reconstruction of the jaws represents a valid treatment in patients with bisphosphonate-related osteonecrosis at stage III of the disease. However, additional data based on a larger cohort of patients are necessary to justify this type of intervention in patient affected by MRONJ.

1. Introduction

Bisphosphonates (BP) are antiresorptive drugs used in the management of conditions as diverse as osteoporosis and metastatic bone diseases. These drugs are widely administered and generally well tolerated by patients. In 2003, Marx et al. [1] first reported a nonhealing necrosis of the maxillofacial region in some patients taking BPs. In the last decade researchers have discovered that BPs not exclusively cause osteonecrosis of jaws, as other drugs, such as antiresorptive (bone-targeted) agents like denosumab, but also were found to cause it. In addition, monoclonal antibodies able to bind and selectively inhibit VEGF-A, specifically mTOR inhibitors, can also cause osteonecrosis of the jaw [2–6].

For this reason, in 2014 the bisphosphonate-related osteonecrosis of the jaw (BRONJ) nomenclature was changed by the position paper of the American Association of Oral and Maxillofacial Surgeons (AAOMS) special committee on Medication-Related Osteonecrosis of the Jaws (MRONJ) [7].