Medication related osteonecrosis of the jaws: Prospective cohort study analysing the aetiology, microbiology, and response to surgical debridement with adjunctive leucocyte and platelet rich fibrin (L-PRF)

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Declaration

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Summary

Background

Bisphosphonates and RANK ligand inhibitors are antiresorptive medications which are prescribed for the treatment of osteoporosis and bone metastases. These medications inhibit osteoclastic action thereby reducing bone turnover, which significantly improves quality of life in the context of oncology patients and greatly reduces the incidence of fragility fractures for osteoporotic patients. An adverse side effect that can develop as a result of these drugs is medication related osteonecrosis of the jaws (MRONJ), which can affect quality of life, resulting in profound morbidity.

Since the emergence of this disease entity in 2003, research efforts have been focused on enhancing our understanding of the aetiology, pathogenesis and management of MRONJ. However, treatment of this condition can be difficult, posing significant challenges for clinicians. Conservative management has been the mainstay for treating MRONJ, and in the palliation of symptoms. However, more recent studies adopting a surgical approach appear to yield superior curative rates.

The aim of this study was to identify common risk factors in the aetiology and pathogenesis of MRONJ, and evaluate the therapeutic benefits of leucocyte-platelet rich fibrin (L-PRF) as an adjunct to conservative surgical debridement of MRONJ in an out-patient setting. The microbiology of disease sites was also investigated. In addition, this study aimed to investigate the current trends in treatment strategies for MRONJ used by clinicians in the Republic of Ireland.

Materials and methods

Fourteen patients with a diagnosis of MRONJ were prospectively analysed specifically looking at clinical features, drug history, medical history, and social history to assess demographics and possible risk factors. The patients underwent conservative surgical debridement with adjunctive L-PRF at fifteen disease sites

under local anaesthetic (+/- conscious intravenous dental sedation) in an outpatient theatre facility. Culture and sensitivity testing was performed for all cases.
A questionnaire was designed and circulated to clinicians who were active in the diagnosis, and management of patients with MRONJ in secondary and tertiary care settings. The questionnaire explored aspects of what guidance clinicians apply to their practice, case-based scenarios on management, and their antibiotic prescribing protocols.

Results

In 93.3% (n=14) of disease sites there was a dental origin such as dental extraction or denture trauma identified as the initiating factor for MRONJ. Fourteen patients with fifteen disease sites were followed up for 6 months and curation was successful in 73.3% of disease sites (n=11). The microbes isolated from the samples were largely in keeping with the normal oral flora and sensitive to Penicillin. Similar microbes were isolated in oncology and osteoporosis patients, and in patients on bisphosphonates and RANK ligand inhibitors. The results of the questionnaire revealed no clear consensus on the management of MRONJ. The level of academic, and surgical training did not influence clinician's treatment strategies to treat cases conservatively or surgically. There was no standardised consensus on the choice of antibiotic, route of administration, dosage, temporal pattern or duration of antibiotic prescribing for non-surgical treatment, or surgical treatment.

Conclusion

The results of this study indicate that local factors such as dental extractions and denture trauma play a role in the aetiology of MRONJ. Conservative surgical debridement with L-PRF is a successful treatment option in selected cases of MRONJ in an out-patient facility. Penicillin continues to provide suitable antibiotic coverage against the pathogens isolated from MRONJ disease sites. However, further evidence-based guidance is required to assist clinicians in the management of MRONJ cases.

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Index of contents

Declaration ..... **II**

Summary ..... **III**

Acknowledgments ..... **V**

Index of tables ..... **XI**

Index of figures ..... **XII**

Terms and abbreviations ..... **XVI**

1 Introduction **1**

2 Literature review **4**

2.1 Medications associated with MRONJ 4

2.1.1 Bisphosphonates 4

2.1.2 Denosumab 8

2.1.3 Anti-angiogenics 9

2.2 Risk factors for MRONJ 11

2.2.1 Medication related risk factors 11

2.2.2 Local Factors 17

2.2.3 The role of patient demographics and systemic factors as risk factors 20

2.2.4 Genetic factors 23

2.3 Pathogenesis of MRONJ 24

2.3.1 Inhibition of bone resorption and remodelling 24

2.3.2 Infection 25

2.3.3 Inhibition of angiogenesis 26

2.3.4 Soft tissue toxicity 27

2.3.5 The immuno-compromised patient 27

2.4 Diagnosis of MRONJ 27

2.4.1 Clinical examination 28

2.4.2 Plain film radiographs 28

2.4.3 Specialised imaging 29

2.4.4 Biomarkers of disease risk 30

2.4.5 Histological examination 31

2.5 Staging of MRONJ 31

2.5.1 Clinical presentation, symptoms and radiographic features according to AAOMS Staging system 34

2.6 The microbiology of MRONJ 35

2.7 Treatment of MRONJ 37

2.7.1 Factors that influence the treatment approach 39
2.7.2 Treatment options 39
2.7.3 Difficulties encountered reviewing the literature 40
2.7.4 Measurement of healing 41
2.8 Non-surgical conservative treatment 42
2.8.1 Antibiotics for non-surgical management 45
2.8.2 The role of Chlorhexidine as a topical antimicrobial 46
2.8.3 Hyperbaric oxygen 46
2.8.4 Drug holiday 47
2.8.5 Pentoxifylline and α-tocopherol (PENTO) 48
2.8.6 Low level laser therapy 49
2.8.7 Teriparatide 49
2.9 Surgical Management 50
2.9.1 Bone removal techniques 51
2.9.2 Delineating necrotic bone from viable bone 52
2.9.3 Factors which affect outcome of surgical intervention 53
2.9.4 Evidence for using L-PRF as an adjuvant to surgery 58
2.10 Platelet concentrates 60
2.10.1 How are platelet concentrates prepared? 63
2.10.2 cPRP Vs L-PRF 63
2.10.3 Evolution of PRF 65
2.10.4 Centrifuge characteristics and the benefits of Intra-Spin Intra-Lock System 66
2.10.5 L-PRF Cellular Content and mode of action 70
2.10.6 Clinical effects of fibrin on tissue healing and cells of the body 78
2.11 Questionnaire Design 80
2.12 Aims and objectives 82
3 Materials and methods: 83
3.1 Part 1 83
3.1.1 The sample 83
3.1.2 Subjects 84
3.1.3 Ethics 85
3.1.4 Location 85
3.2 Part 1A: Patient characterization and identification of risk factors 86
3.3 Part 1B: Surgical procedure and preparation of L-PRF 87
3.4 Part 1C: Microbiology and culture and sensitivity testing 92
3.5 Post-operative assessment 92

3.5.1 Clinical assessment of healing 92
3.5.2 Resolution of pathological symptoms 93
3.5.3 Radiographic criteria for resolution 93
3.5.4 Acceptability of treatment 93
3.5.5 Full blood count result 94
3.5.6 Histopathology result 94
3.6 Part 2: Questionnaire 95
3.6.1 Ethical approval 95
3.6.2 Data Collection 95
3.7 Data analysis 97
4 Results 98
4.1 Part 1: Results of prospective cohort study 98
4.1.1 Subject demographics 98
4.1.2 Antiresorptive history 99
4.1.3 Precipitating factors 100
4.1.4 Site 101
4.1.5 Stage and symptoms 101
4.1.6 Radiographic signs 101
4.1.7 Smokers 102
4.1.8 Co-medication and immunosuppression 103
4.1.9 Previous treatment 104
4.1.10 Changes in antiresorptive drug regime 104
4.1.11 Surgical intervention 104
4.1.12 Response to treatment 106
4.1.13 Symptoms at 6 months 109
4.1.14 Radiographic review at 6 months 110
4.1.15 Early intervention 113
4.1.16 Visual analogue scale for surgical intervention 113
4.1.17 Topical and systemic antimicrobials 113
4.1.18 Histopathology 113
4.1.19 Microbiology 114
4.2 Part 2: Results from the questionnaire 117
4.2.1 Number of respondents 117
4.2.2 Knowledge of International or local guidance resources 117
4.2.3 Guidance used by clinicians 118
4.2.4 Which guidance is used by clinicians? 119

4.2.5 Independent treatment strategies 119
4.2.6 Current guidance relevant and comprehensive? 120
4.2.7 Problems with current guidance resources 121
4.2.8 Comfortable managing MRONJ? 122
4.2.9 Barriers to managing MRONJ 122
4.2.10 Case 1 Management 123
4.2.11 Case 2 Management 128
4.2.12 Case 3 Management 132
4.2.13 Case 4 Management 136
4.2.14 Case 1: Choice of antibiotic 141
4.2.15 Case 1: Duration of antibiotic prescribing 142
5 Discussion 143
5.1 Epidemiology 143
5.2 Risk factors and precipitating factors for MRONJ 144
5.2.1 Drug-related factors 144
5.2.2 Duration of antiresorptive 144
5.2.3 Patient risk factors 145
5.2.4 Local factors 146
5.3 Treatment of MRONJ 148
5.3.1 Treatment in an out-patient setting 151
5.3.2 Surgical outcomes depending on underlying disease 152
5.3.3 Surgical outcomes based on stage of disease 153
5.3.4 Outcomes in relation to maxillary or mandibular disease 154
5.3.5 Outcomes in relation to medical co-factors, and smoking habits 154
5.3.6 Outcome in relation to tobacco habits 155
5.4 Denosumab 156
5.5 Local anaesthetic 157
5.6 Microbiology 158
5.7 Questionnaire results 159
5.7.1 Case based management 160
5.7.2 Antibiotic prescribing 162
5.8 Limitations 167
5.9 Future work 169
6 Conclusions 170
7 Appendices 172
7.1.1 Appendix: 1 Patient information leaflet 172

7.1.2 Appendix: 2 Consent form for participation in the study 177
7.1.3 Appendix: 3 Ethical approval 178
7.1.4 Appendix: 4 Visual analogue scale 179
7.1.5 Appendix: 5 Copy of the questionnaire 180
7.1.6 Appendix: 6 Statistics for larger samples 191
8 References 192

Index of tables

Table 1: Bisphosphonates predisposing to MRONJ 6
Table 2: RANK-ligand inhibitors predisposing to MRONJ 8
Table 3: Anti-angiogenic medications predisposing to MRONJ 10
Table 4: Risk of developing MRONJ for osteoporotic patients on antiresorptive medications 13
Table 5: Risk of developing MRONJ for oncology patients on antiresorptive medications 15
Table 6: AAOMS (2014) Staging system for MRONJ 32
Table 7: Classification of MRONJ according to clinical and radiological features 33
Table 8 Clinical features, symptoms and radiographic signs according to disease Stage 34
Table 9 Treatment according to disease Stage 38
Table 10: Treatment options for MRONJ identified by Cochrane (2017) 40
Table 11: Outcomes for conservative non-surgical management 43
Table 12 Outcomes of different surgical protocols treating MRONJ 54
Table 13: Outcomes for surgical management of MRONJ 56
Table 14: Outcomes of different surgical protocols depending on Stage of disease 57
Table 15: Outcomes for surgery with adjunctive L-PRF 60
Table 16: Classification of platelet concentrates 62
Table 17: Preparation protocol for 2nd generation platelet concentrates. 66
Table 18: Macroscopic features of L-PRF using 4 different centrifuge systems 68
Table 19: Cellular content of L-PRF 71
Table 20: Subject demographics 98
Table 21: Antiresorptive drug history 100
Table 22: Clinical features of MRONJ within the patient sample 102
Table 23: Medical and social co-factors for MRONJ 103
Table 24: Factors relating to surgical intervention 106
Table 25: Clinical and radiographic outcome of surgical intervention 107
Table 26: Macroscopic results of culture and sensitivity testing 114
Table 27: Microscopic results of culture and sensitivity testing 116

Index of figures

Figure 1: Effects of drugs and hormones on bone turnover 4
Figure 2: Intracellular mevalonate pathway 7
Figure 3: Pharmacology of RANK-ligand inhibitors 8
Figure 4: Risk of MRONJ increases with duration of antiresorptive treatment 17
Figure 5: SDCEP (2017) Risk assessment tool for patient on, or with a history of antiresorptives 18
Figure 6: Percentage of isolates positive for Actinomyces 36
Figure 7: Percentage of samples which cultured various pathogens at MRONJ sites 36
Figure 8: Preparation protocols for cPRP, PRGF and PRF 63
Figure 9: Kinetics of growth factor release from L-PRP, L-PRF, and blood clot 65
Figure 10: Kinetics of growth factor release; TGFβ1 (A), PDGF-AB (B), VEGF (C) and BMP2 (D) from L-PRF and A-PRF membrane for 7 days *in-vitro* 69
Figure 11: Distribution of cells within L-PRF clot 70
Figure 12: Theoretical modelling of tri-molecular flexible branch junctions in fibrin architecture in L-PRF 72
Figure 13: Theoretical modelling of condensed rigid tetra-molecular branch junctions associated with fibrin adhesives and cPRP 72
Figure 14: Concentrations of growth factors were measured within the various components produced following centrifugation 75
Figure 15: Study flowchart 83
Figure 16: Illustrates venipuncture 88
Figure 17: Shows the collection of the blood sample using a 21 gauge butterfly needle 88
Figure 18: Tubes were balanced within the Intra-Spin L-PRF system, and removed after centrifugation 2700 rpm 88
Figure 19: The a-cellular plasma was the superior layer, the L-PRF clot in the middle, and red blood cells at the base of the tube 88
Figure 20: The clot was removed gently from the tube with fine tweezers using an aseptic technique 88
Figure 21: most red cells were removed from the clot carefully preserving the white buffy coat and a fine layer of red cells, which is rich in cytokines, platelets and leucocytes 88
Figure 22: The Xpression kit was used to compress the L-PRF clots 89

Figure 23: Membranes with a consistent thickness of 1mm were formed, which takes 5 minutes 89
Figure 24: Alternatively, a piston and cylinder assesmbly isused to make L-PRF plugs. 89
Figure 25: Exposed necrotic bone in the lower right mandible 90
Figure 26: Full thickness mucoperiosteal flap was raised 90
Figure 27: L-PRF plug was placed into defect 91
Figure 28: L-PRF membrane was placed over the defect 91
Figure 29: Primary closure of the soft tissues 91
Figure 30: Proportion of patients who required no additional local anaesthetic, and proportion requiring top-up anaesthetic. 105
Figure 31: Patient 5 OPG pre-operatively 110
Figure 32: Patient 5 OPG 10-days post-operatively 110
Figure 33: Patient 5 OPG 6-months post-operatively 110
Figure 34: Patient 7 OPG pre-operatively 111
Figure 35: Patient 7 OPG 10-days post-operatively 111
Figure 36: Patient 7 OPG 6-months post-operatively 111
Figure 37: Patient 11 Maxillary occlusal pre-operatively 112
Figure 38: Patient 11 periapical 10-days post-operatively 112
Figure 39: Patent 11 periapical 6-months post-operatively 112
Figure 40: Proportion of isolates identified macroscopically from 15 samples 114
Figure 41: Proportion of microbial species cultured from 15 samples 115
Figure 42:The breakdown of respondents by role 117
Figure 43: Awareness of various International, and local guidance resources available for the management of MRONJ 118
Figure 44: Number of respondents that follow a guideline, and number who do not 118
Figure 45: Number of respondents who follow a specific guidance 119
Figure 46: Strategies employed for the treatment of MRONJ by respondents who do not follow guidance 120
Figure 47: Attitude of respondents to current guidance resources available 121
Figure 48: Problems with current guidance resources cited by respondents 121
Figure 49: Attitude of respondents to managing patients with established MRONJ 122
Figure 50: Barriers identified by respondents to managing patients with MRONJ 123
Figure 51: Case 1 treatment strategy adopted by respondents 124
Figure 52: Case 1 Non-surgical antibiotic prescribed by respondents 124

Figure 53: Case 1 Non-surgical antibiotic dose prescribed by respondents 125
Figure 54: Case 1 Pre-surgical antibiotic prescribed by clinicians 126
Figure 55: Case 1 post-surgical antibiotic prescribed by respondents 127
Figure 56: Case 1 post-operative surgical antibiotic dose prescribed by respondents 127
Figure 57: Case 2 treatment strategy adopted by respondents 128
Figure 58: Case 2 Non-surgical antibiotic prescribed by respondents 129
Figure 59: Case 2 Non-surgical antibiotic dose prescribed by respondents 129
Figure 60: Case 2 Pre-surgical antibiotic prescribed by respondents 130
Figure 61: Case 2 post-surgical antibiotic prescribed by respondents 131
Figure 62: Case 2 post-surgical antibiotic dose prescribed by respondents 131
Figure 63: Case 3 treatment strategy adopted by respondents 132
Figure 64: Case 3 non-surgical antibiotic prescribed by respondents 133
Figure 65: Case 3 non-surgical antibiotic dose prescribed by respondents 134
Figure 66: Case 3 pre-surgical antibiotic prescribed by clinicians 134
Figure 67: Case 3 post-surgical antibiotic prescribed by respondents 135
Figure 68: Case 3 post-surgical antibiotic dose prescribed by respondents 136
Figure 69: Case 4 treatment strategy adopted by respondents 137
Figure 70: Case 4 non-surgical antibiotic prescribed by respondents 138
Figure 71: Case 4 non-surgical antibiotic dose prescribed by respondents 138
Figure 72: Case 4 pre-surgical antibiotic prescribed by respondents 139
Figure 73: Case 4 post-surgical antibiotic prescribed by respondents 140
Figure 74: Case 4 post-surgical antibiotic dose prescribed by respondents 140
Figure 75: Case 1 justification for choice of antibiotic prescribed non-surgical and surgical 141
Figure 76: Case 1 justification for duration of antibiotic prescribing for non-surgical and post-operative antibiotic prescribing 142

Terms and abbreviations

MRONJ	Medication related osteonecrosis of the jaws
BRONJ	Bisphosphonate related osteonecrosis of the jaws
DRONJ	Denosumab related osteonecrosis of the jaws
ARONJ	Antiresorptive related osteonecrosis of the jaws
Bp	Bisphosphonate
Deno	Denosumab
Zoledron	Zoledronic acid
Alendron	Alendronic acid
AAOMS	American Association of Oral and Maxillofacial Surgery
JIDA	Journal of the Irish Dental Association
SDCEP	Scottish Dental Clinical Effectiveness Programme
IV	Intravenous
SC	Subcutaneous
CT	Computed tomography
CBCT	Cone beam computed tomography
MRI	Magnetic resonance imaging
VAS	Visual analogue score
OPG	Orthopantomogram
FBC	Full blood count
RANKL	Receptor activator of nuclear factor-$_{k}$B ligand

1 Introduction

Osteonecrosis of the jaws (ONJ) is a rare but severe disease, which can affect the mandible or maxilla. A predisposing factor which has been implicated in the pathogenesis of ONJ is the therapeutic prescription of antiresorptive, and anti-angiogenic medications.[1] The term medication related osteonecrosis of the jaws (MRONJ) describes this phenomenon. It is a debilitating adverse drug-induced reaction, associated with medications prescribed for the treatment of metabolic bone disorders and some cancers. To distinguish MRONJ from other delayed healing conditions, the American Association of Oral and Maxillofacial Surgeons (AAOMS) outlined defining characteristics that must fit the criteria, which include:

1. Current or previous treatment with antiresorptive or anti-angiogenic medications
2. An area of exposed bone, or bone that can be probed through an intraoral or extraoral fistula(e) in the maxillofacial region that has been present for over 8 weeks
3. No history of radiotherapy to the jaws, or obvious metastatic disease to the jaws.[1]

The disease affects a small number of patients; however, it is none the less debilitating for those affected, resulting in exposed necrotic bone within the oral cavity, and progressive destruction of the maxilla or mandible. Patients may present with pain, loosening of teeth, superimposed infections, halitosis, exposed bone, fistulae, and even pathological fractures.[2] The impact of the disease burden can have a profoundly negative impact on the patient's quality of life, social confidence, and function. MRONJ also carries a burden of therapeutic cost, challenges for provision of and access to specialist care facilities.

The first reported cases of MRONJ were described separately by Marx and Migliorati in 2003 and were associated with bisphosphonate treatment.[3,4] However; some two hundred years ago industrial workers, exposed to phosphorous fumes and pastes in

the manufacturing of friction matches, presented with necrotic jaws. The condition was known as “phossy jaw”. The phenomenon quickly disappeared with the International “Berne” Convention in 1906, which prohibited the use of white phosphorous.[5] Its modern variant bisphosphonate related osteonecrosis of the jaws (BRONJ), resurfaced with the medical use of bisphosphonates in the 1990s, and in September 2004, Novartis, the manufacturer of Pamidronate and Zoledronic acid notified healthcare professionals that they carried a risk of inducing ONJ.[1, 5] Whilst the phenomenon of BRONJ gained momentum with an increase in numbers of cases being reported, so too did the confusion and conjecture on how to manage this new disease entity.

The AAOMS, in 2006 established an expert panel to review the literature surrounding BRONJ. This document has subsequently been superseded by the 2009, and 2014 versions to correlate with our evolving understanding of the pathogenesis, prevention and management of the disease.[1, 6] In 2010, Taylor *et al.,* described the first case of RANK Ligand inhibitor (Denosumab) induced osteonecrosis of the jaws, and coined the term antiresorptive drug-related osteonecrosis of the jaws (ARONJ) .[7] Some authors also refer to Denosumab related osteonecrosis of the jaws, as DRONJ.[8] The most recent literature classifies DRONJ and BRONJ as medication-related osteonecrosis of the jaw.[1, 2, 9] This term also encompasses anti-angiogenic medications, which have been implicated as a precipitating factor in causing ONJ in the most recent document published by the AAOMS, in 2014.[1] The term MRONJ appears to be the universally accepted term, at present.[1]

In 2018, there is no “gold standard” consensus for the treatment of MRONJ.[1, 10, 11] Interventions applied to managing this condition are diverse, controversial, and largely empirical. Three broad categories of treatment options have been described, which include; conservative treatment, surgical techniques, and various “add-on” treatments. Difficulties are encountered, when measuring treatment outcomes of various techniques, as these three approaches are often used in combination, at the same time, or in succession.[1, 10, 11]

Management of patients with MRONJ in Ireland is guided by a combination of local and International guidance papers; which includes a paper by Flint *et al.,* (2006),

Rogers *et al.,* (2010), Henry *et al.,* (2017), the positional paper by the American Association of Oral and Maxillofacial surgery (2014), and the SDCEP guidance (2017).[1, 12-15] Conservative management appears to be the mainstay of treatment recommendations outlined by these papers, and surgical intervention is only recommended for Stage 3 extensive disease or refractory cases.[1, 2] Experts in the field have expressed frustration with this position, and argue there is little evidence to support a conservative approach, and that these concepts are outdated and are not reflective of the literature that is currently available.[16]

Recent systematic reviews and research studies addressing treatment strategies for MRONJ have shown that surgical treatment may be more effective than conservative management, and for all stages of disease (Stages 0, 1, 2 and 3).[16-20] Prompt surgical intervention at an early stage of the disease process may also reduce the necessity for extensive surgical resections, and reduce the risk of disease extending into adjacent structures such as the maxillary antrum, floor of the nose or even a pathological mandibular fracture.[18]

Efforts to enhance wound healing is a concept that has been applied to various surgical specialities for the past 30 years.[21] Platelet concentrates have been developed to act as bioactive surgical additives, which are used in many fields of surgery. The process manipulates the clotting process producing an optimised blood clot, which may be applied to a wound site, as an autologous matrix optimising healing and promoting tissue regeneration. Recent research indicates that this technique may be beneficial in cases of MRONJ, and this study aimed to explore this concept.[22-24]

2 Literature review

2.1 Medications associated with MRONJ

Bone remodelling is a normal physiological process that occurs in health and disease. It is regulated by systemic and local factors, including endocrine, paracrine, and autocrine regulation, and extraosseous mechanical stress (Figure 1).[25] Bone remodelling is an equilibrium between bone resorption and deposition, and is principally regulated by osteoblasts, osteoclasts, and osteocyte cells. Certain disease conditions can affect this equilibrium, and antiresorptive medications are prescribed to counter these effects.

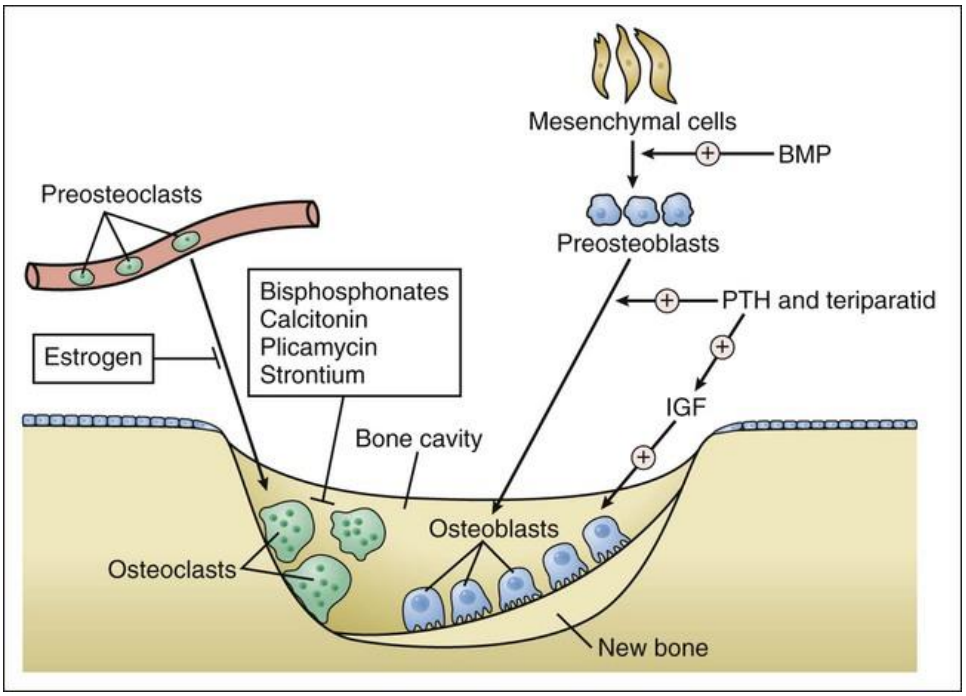


Figure 1: Effects of drugs and hormones on bone turnover[26]

2.1.1 Bisphosphonates

Bisphosphonates are prescribed in oral, and intravenous forms (IV). Oral bisphosphonates are approved for the management of osteoporosis, osteopenia, and other less frequent conditions, including Paget's disease, and osteogenesis

imperfecta.[1] Occasionally IV bisphosphonates are prescribed to patients with severe osteoporosis, or when the patient cannot tolerate the oral form. Intravenous bisphosphonates are prescribed to manage cancer-related conditions including; hypercalcaemia of malignancy, metastatic bone cancer in the context of solid tumours, such as breast, prostate, lung, and multiple myeloma.[1] In the absence of osteoclast inhibition, oncology patients are at risk of serious skeletal complications, including spinal cord compression, pain, and hypercalcaemia of malignancy. Antiresorptive treatments reduce the risk of these complications by 20-50%. [27] The risk of developing one of these complications outweighs that of developing MRONJ, which is not a life-threatening condition.

Bisphosphonates have a high affinity for calcium which accounts for their bone-targeting properties. They also have a high affinity for sites of bone with a rapid turnover evident by their increased uptake at tumour sites, bone grafts, growth plates, and normal healthy jaws. [28] The bi-dentate 3-D structure of bisphosphonates enables chelation of the calcium ions within hydroxyapatite bone mineral surface.[25] Bisphosphonates are rapidly absorbed from plasma, and deposited within the bone matrix resulting in a long half-life of 1 to 10 years within bone.[29]

Bisphosphonates can be classified as nitrogen containing, and non-nitrogen containing, and nitrogen dictates its pharmacological activity (Table 1). Nitrogen containing bisphosphonates have enhanced target protein binding, increasing their potency, and in turn increase the risk of inducing MRONJ.[1]

Bisphosphonate	Primary Indication	Nitrogen containing	Dose	Route
Alendronate (Fosamax)	Osteoporosis	Yes	10mg/day 70mg/week	PO
Risendronate (Actonel)	Osteoporosis	Yes	5mg/day 35mg/week	PO
Etidronate (Didronel)	Pagets	No	5-10mg/Kg/day	PO
Tiludronate (Skelid)	Pagets	No	400mg/day	PO
Ibandronate	Osteoporosis	Yes	2.5mg/day 150mg/month	PO
(Bonviva)			3mg/3months	IV
Clodronate (Bonefos, Loron, Clasteon)	Malignancy	No	1600-3200mg/day	PO
Pamidronate (Aredia)	Malignancy	Yes	90mg/3weeks	IV
Zoledronate (Reclast)	Osteoporosis	Yes	5mg/year	IV
Zoledronate (Zometa)	Malignancy	Yes	4mg/3weeks	IV

Table 1: Bisphosphonates predisposing to MRONJ [1, 30]

**Abbreviations: PO orally; IV intravenously*

Nitrogen-containing bisphosphonates interfere with the enzymatic pathways of the mevalonate pathway, which inhibits the production of cholesterol and isoprenoid lipids. Cholesterol is required for cell signalling pathways.[25] The absence of isoprenoid lipids causes apoptosis in osteoclasts by inhibiting the prenylation of small GTPase signalling proteins, which affects osteoclast morphology and function (Figure 2).[31, 32]

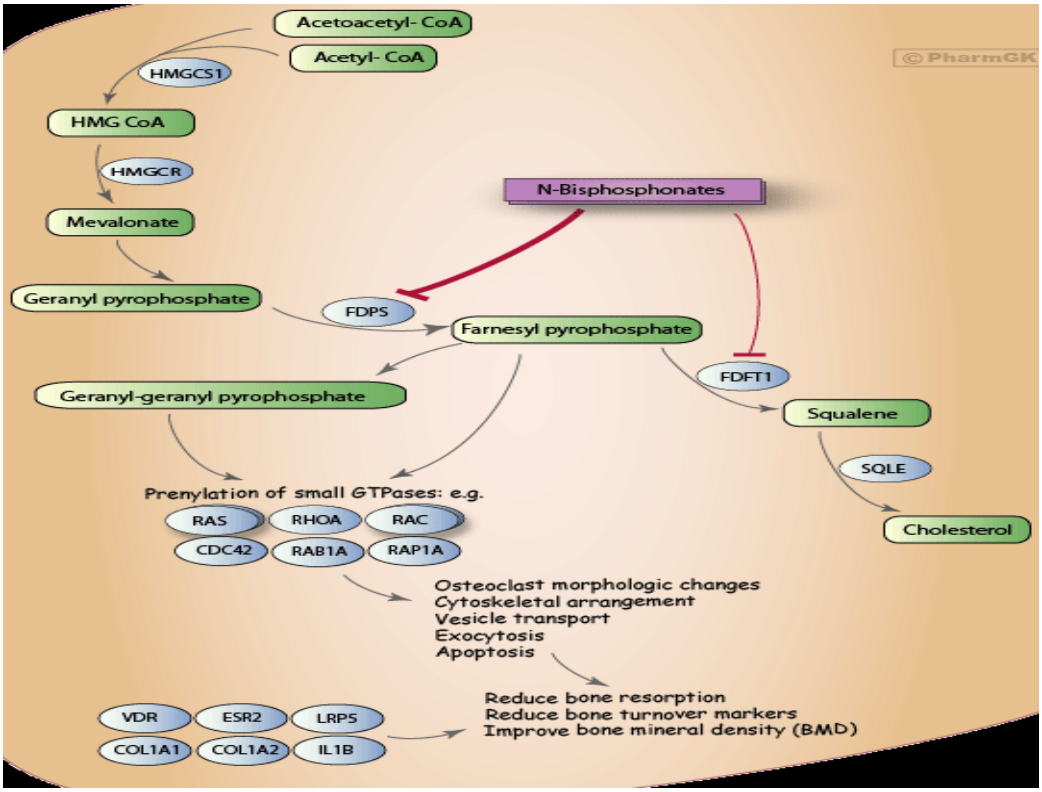


Figure 2: Intracellular mevalonate pathway[32]

There is also evidence to suggest bisphosphonates inhibit osteoclasts adhering to the bone matrix proteins via cell surface integrins, and it is this property that serves as a protective mechanism inhibiting tumour cells to adhere to the bone surface.[31] Bisphosphonates alter osteoclast morphology in two ways:

1. They alter their ability to form a “ruffled border” which is required to enable the osteoclast to adhere to the bone matrix
2. The osteoclast cytoskeletal structure is altered with loss of the actin ring structure

These two morphological changes are sufficient to inhibit bone resorption.[25]

Non-nitrogen containing bisphosphonates have a different pharmacological mechanism that induces apoptosis of osteoclast cells. The mechanism of action may involve the formation of cytotoxic metabolites in osteoclasts (non-hydrolysable analogues of ATP) or the inhibition of protein tyrosine phosphatases.[25, 31] This class of drug does not bind to hydroxyapatite as avidly as nitrogen containing bisphosphonates, are less potent, and therefore rarely cause MRONJ.[33]

2.1.2 Denosumab

Denosumab is a recombinant monoclonal antibody (IgG) that reduces bone turnover. It is approved for the treatment of patients with osteoporosis and patients with metastatic bone cancer from solid tumours (Table 2).[1] Its mode of action inhibits osteoclast activity, reduces bone resorption, and increases bone density.

Antiresorptive	**Primary Indication**	**Nitrogen Containing**	**Dose**	**Route**
Denosumab (Xgeva)	Bone metastases	No	120 mg/4 weeks	SC
Denosumab (Prolia)	Osteoporosis	No	60mg/6 months	SC

Table 2: RANK-ligand inhibitors predisposing to MRONJ [1]

Abbreviations: SC subcutaneous

It has a different mechanism of action to bisphosphonates. Denosumab targets and inhibits receptor-activated nuclear factor-kappa B ligand (RANKL), by mimicking the effect of osteoprotegrin on RANKL. RANKL is a cytokine which is present in osteoblasts, and has a key role in osteoclast function, activation, differentiation and overall survival. Denosumab interrupts the RANK/RANKL interaction between osteoblasts and osteoclasts resulting in decreased bone turnover (Figure 3).[25, 34]

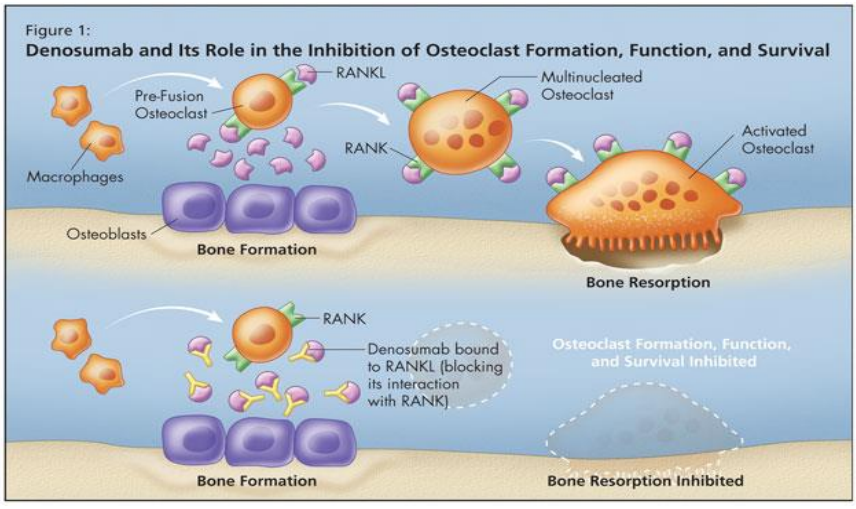


Figure 3: Pharmacology of RANK-ligand inhibitors [34]

In the context of bone metastases, bone resorption causes the release of growth factors which perpetuates tumour activity and in turn stimulates osteoblastic release of RANKL. Denosumab inhibits this positive feedback loop, and therefore prevents the progression of bone metastases. RANKL is also expressed in T and B lymphocytes, and it has been proposed that inhibition of RANKL can result in immunosuppression.[35] This may act as a co-risk factor increasing a patient's risk of MRONJ. Unlike bisphosphonates, Denosumab is not incorporated into the bone matrix, and hence its influence on bone resorption may be negligible after 6 months following cessation of treatment with Denosumab.[36] The European Medicines Agency report serum levels for Prolia (60mg/ 6months) decline with a mean half-life of 26 days over a period of 3 months. No accumulation or change in the pharmacodynamics were observed when administered at 60mg subcutaneously every 6 months, and 53% of patients had no measurable level of Denosumab detected at 6 months.[37] The European Medicines Agency report serum levels of Xgeva have a 2-fold accumulative effect on serum levels when administered at 120mg/ 4 weeks, with a mean half-life of 30 days.[38] Although the half-life for detectable serum levels of Denosumab range from 6-55 days for Prolia or Xgeva, the effect on bone turnover suppression may persist longer than that, as presented by Bekker *et al*., in (2004) in a randomised, single-dose, double-blind, placebo-controlled study.[39] They found bone turnover was suppressed up to 81% (3.0mg/Kg) at 6 months. This degree of bone turnover suppression is comparable to the most potent antiresorptives such as Zoledronic acid (54-65%) at 12 months, when administered as a yearly 4mg infusion. Although this dose is higher than the usual dose administered for osteoporotic patients (~1mg/Kg) and is closer to the oncology dose, it does highlight that the effects of Denosumab may not be eliminated at 6 months as the AAOMS (2014) report.[1]

2.1.3 Anti-angiogenics

These medications inhibit the process of neo-vascularisation, through inhibition of various signalling molecules required in the angiogenesis-signalling cascade.[1] Different medications target specific stages in the cascade, including vascular

endothelial growth factor (VEGF) signalling, rapamycin signalling and tyrosine kinase signalling.[10] It is likely that these medications interrupt stages in wound healing, or in the differentiation, and survival of osteoclasts.[1] Anti-angiogenics are also used in combination with bisphosphonates, and there is evidence to suggest this carries a higher risk of developing MRONJ.[40] They are prescribed for their anti-tumour properties in the management of gastrointestinal tumours, renal cell carcinomas, and neuroendocrine tumours (Table 3). At present the FDA have issued a MRONJ warning for Bevacizumab and Sunitinib only, but similar medications may very well carry the same risk.

Drug	**Mechanism of action**	**Primary indication**
Sunitinib (Sutent)	Tyrosine kinase inhibitor	GIST, RCC, pNET
Sorafenib (Nexavar)	Tyrosine kinase inhibitor	HCC, RCC
Bevacizumab (Avastin)	Humanised monoclonal antibody	mCRC, NSCLC, Glio, mRCC
Sirolimus (Rapamune)	Mammalian target of rapamycin pathway	Organ rejection in renal transplant

Table 3: Anti-angiogenic medications predisposing to MRONJ[1]

Abbreviations: GIST gastrointestinal stromal tumour; RCC renal cell carcinoma; pNET pancreatic neuroendocrine tumour, HCC hepatocellular carcinoma; mCRC metastatic colorectal carcinoma; NSCLC non-squamous non-small cell lung carcinoma; Glio Glioblastoma; mRCC metastatic renal cell carcinoma

In a bid to reduce the risk of MRONJ, the European Medicines Agency's Pharmacovigilance Risk Assessment Committee (2015) has proposed patients carry a patient reminder card, that they can present to health professionals, with the aim to reduce the risk of MRONJ.[41] This may be particularly beneficial for patients who receive subcutaneous injections/ IV bisphosphonates for the treatment of osteoporosis as they are administered infrequently, and are often omitted from their list of regular medications.

2.2 Risk factors for MRONJ

The AAOMS (2014) classified risk factors for MRONJ as follows:

- Medication related risk factors
- Local factors
- Demographic and systemic factors and other medication factors
- Genetic factors

2.2.1 Medication related risk factors

The therapeutic indication, the medication, the dose, and duration of treatment are parameters that must be considered when evaluating the risk of MRONJ, and disease frequency estimates.[42]

- Therapeutic indication can be subdivided into osteoporosis or malignancy.
- Medications can be categorised as bisphosphonate or non-bisphosphonate (anti-angiogenic/antiresorptive)

2.2.1.1 Risk of MRONJ in osteoporotic patients

2.2.1.1.1 Risk of MRONJ in osteoporotic patients exposed to oral bisphosphonates

The risk of MRONJ in patients exposed to oral bisphosphonates is low, and a Cochrane review in 2017 reported the incidence may not be substantially higher than the natural background incidence of the disease.[10] A survey of over 13,000 Kaiser Permanente members, reported the overall incidence of MRONJ in patients exposed to oral bisphosphonates was 0.1% (equivalent to a risk of 10 cases per 10,000) which increases to 0.21% (21 cases per 10,000) with exposure exceeding 4 years.[43] This is likely due to the cumulative effect of bisphosphonates being incorporated into the

bone matrix. A large systematic review by Khan *et al.,* in 2014 estimated an incidence of BRONJ in osteoporotic patients prescribed oral bisphosphonates ranging from 0% to 0.04% with the majority below 0.001%.[2] A systematic review by Dodson *et al*., (2015) concluded that the estimated risk of developing MRONJ for osteoporotic patients exposed to oral bisphosphonates was 10 times smaller (0.1%) than the risk for oncology patients.[42]

2.2.1.1.2 Risk of MRONJ in osteoporotic patients exposed to intravenous bisphosphonates

Dodson *et al*., (2015), reported for osteoporosis patients exposed to Zoledronate the risk of MRONJ was 0.02%, around 100 times less than the risk for oncology patients.[42] This was less than the oral form also, and largely due to the lower cumulative dose of one infusion per year. The AAOMS (2014) reported an incidence of 0.017%-0.04%, which was nearly equivocal to placebo groups. Furthermore, the risk of 1.7 cases per 10,000 at 3 years of exposure to Zoledronate apparently did not increase after 6 years of exposure to the medication.[1, 44]

2.2.1.1.3 Risk of MRONJ in osteoporotic patients exposed to Denosumab

The AAOMS (2014) reported an estimated risk of 0.04% (4 cases per 10,000) for osteoporotic patients exposed to Denosumab,[1] although Cochrane (2017)[10] reported a higher risk of 5.2 cases per 10,000 and Dodson *et al*., (2015) reported that it can affect up to 20 cases per 10,000.[42] DRONJ is a relatively new entity and our knowledge on disease incidence and risk factors is evolving over time. Table 4 presents some of the larger, well-designed studies, and it would appear Denosumab carries a higher risk of MRONJ than bisphosphonates in osteoporotic patients.

Author	Study design	Placebo	Zoledronate	Oral Bp	Denosumab
Sugimoto *et al.,* (2014)	RCT				0.1% (n=775)
Bone *et al.,* (2013) 6 years 2 years	RCT				0.2% (n=2342) 0.1% (n=2207)
Papapoulos *et al.,* (2012)	RCT	0% (n=3383)			0.04% (n=4549)
Grbic *et al.,* (2010)	Systematic review	0.02% (n=4945)	0.02% (n=5864)		
Malden and Lopes, (2012)	Epidemiological study			0.004% (n=900,000)	
Lo *et al.,* (2010)	Cross-sectional			0.1% (n=8572)	

Table 4: Risk of developing MRONJ for osteoporotic patients on antiresorptive medications

Abbreviations: RCT randomized controlled trial; oral Bp oral bisphosphonate

2.2.1.2 Risk of MRONJ in oncology patients

2.2.1.2.1 Risk of MRONJ in oncology patients exposed to intravenous bisphosphonates

Oncology patients are at a higher risk of developing MRONJ than osteoporotic patients due to the higher cumulative dose, and increased potency of IV antiresorptive drugs prescribed exerting a more profound osteoclastic inhibition. The AAOMS (2014) estimated the risk of MRONJ in oncology patients exposed to Zoledronate was low,

ranging from 0.7% to 6.7%.[1, 45, 46] When Level 1 evidence was considered the risk approximates to 1% (100 in 10,000 patients), which was roughly 50-100 times higher than patients treated with placebo (Table 5).[1] Dodson *et al*., (2015) estimated the risk was greater at 1-2% (100-200 per 10,000 patients) whilst still considering Level 1 evidence.[42]

2.2.1.2.2 Risk of MRONJ in oncology patients exposed to Denosumab

A systematic review of the literature by Boquete-Castro *et al.,* (2016) which included the results of 7 randomised controlled trials (n=8963) reported a MRONJ incidence of 1.7% (95% CI: 0.9-3.1) with a higher associated risk than bisphosphonates (not statistically significant) or placebo (statistically significant).[35] These were similar to the results reported by Dodson *et al.,* (2015) at 0.7% to 1.7%,[42] and the AAOMS (2014) 0.7%-1.9% (70-90 cases per 10,000 patients).[1] The AAOMS (2014) and Cochrane (2017) report the risk of developing MRONJ was equivocal for cancer patients exposed to Zoledronate and Denosumab.[1, 10]

2.2.1.2.3 Risk of MRONJ in oncology patients exposed to anti-angiogenics

The AAOMS (2014) and Dodson *et al*., (2015) estimated the risk of anti-angiogenic induced MRONJ at 0.2% (20 cases per 10,000 patients) based on three large prospective phase III trials (n=3,560) by Guarneri *et al*., (2010) (Table 5).[1, 40, 42] The risk significantly increased to 0.9%-2.4% when anti-angiogenics and Zoledronate were given concomitantly.[40] However, this figure was only slightly above the associated risk for Zoledronate alone.

Authors	***Study Design***	***Placebo***	***IV BP***	***Oral Bp***	***Deno***	***Bevaciz***	***Bevaciz & Zoledron***
Gnant *et al.,* (2015)	RCT	0% (n=903)	0% (n=900)				
Barrett-Lee *et al.,* (2014)	RCT		1.3% (n=697)	0.7% (n=704)			
Coleman *et al.,* (2014)	RCT	0% (n=1678)	1.7% (n=1681)				
Qi *et al.,* (2014)	System rv	0% (n=1450)	1.1% (n=2928)		1.7% (n=4585)		
Henry *et al.,* (2014)	RCT		1.1% (n=792)		0.8% (n=786)		
Jackson *et al.,* (2014)	RCT		3.7% (n=981)				
Chiang *et al.,* (2013)	Prospec cohort		0% (n=414)				
Van den Wyngaert *et al.,* (2013)	Prospec cohort		6% (n=298)				
Scagliotti *et al.,* (2012)	RCT		0.8% (n=400)		0.7% (n=411)		
Guarneri *et al.,* (2010)	System rv					0.3-0.4% (n=1076)	0.9% (n=233)
Stopeck *et al.,* (2010)	RCT		2.0% (n=1020)		1.4% (n=1013)		
Vahtstevranos *et al.,* (2009)	Prospec cohort		6.7% (n=1163)				
Mauri *et al.,* (2009)	System rv	0.02% (n=5382)	0.3% (n=3987)				

Table 5: Risk of developing MRONJ for oncology patients on antiresorptive medications [42]

Abbreviations: System rv systematic review; Prospec cohort prospective cohort; IV Bp intravenous bisphosphonate; oral Bp oral bisphosphonate; Deno Denosumab; Bevaciz & Zoledron Bevacizumab and Zoledronate

2.2.1.3 Duration of antiresorptive medication as a risk factor

The risk of developing MRONJ increases with duration of antiresorptive treatment regardless of the indication for treatment.[1, 2, 14] A randomised double-blind study by Henry *et al.*, in 2011 compared time as a risk factor for Zoledronic acid, and Denosumab in oncology patients. The risk of developing MRONJ was 0.6% for Zoledronic acid, and 0.5% for Denosumab at 1 year, 0.9% and 1.1% at 2 years, and 1.3% and 1.1% at 3 years, with the risk of developing MRONJ in the Denosumab group plateauing between 2 and 3 years.[47] These findings are comparable to a study by Saad *et al.,* in 2012 which pooled the results of three blinded phase three trials (n=5,723) where the overall numbers of MRONJ cases were 89 (1.6%). The incidence of developing MRONJ, comparing Zoledronate with Denosumab, was 0.5% and 0.8% at 1 year, 1.0% and 1.8% at 2 years, and 1.3% and 1.8% at three years respectively, with a similar plateau after 2 years in the Denosumab group. [48] This plateau may be due to Denosumab not being incorporated into the bone, unlike bisphosphonates which have a cumulative effect which increases over time.

Similarly, the risk of MRONJ increases with duration of oral bisphosphonate treatment. The prevalence of MRONJ increases over time from near 0.1% to 0.21% after ≥4 years of bisphosphonate treatment, with median duration of treatment in patients presenting with MRONJ/MRONJ symptoms at 4.4 years.[43] This is in agreement with the AAOMS (2014) (Figure 4), and the SDCEP (2017) risk assessment tool which classes oral bisphosphonate use ≥5 years in a high risk category.[1, 14] However, osteoporotic patients receiving yearly infusions of Zoledronate were not at an increased risk of developing MRONJ at 3 years compared to 6 years.[1, 44] This was likely due to the overall low cumulative dose.

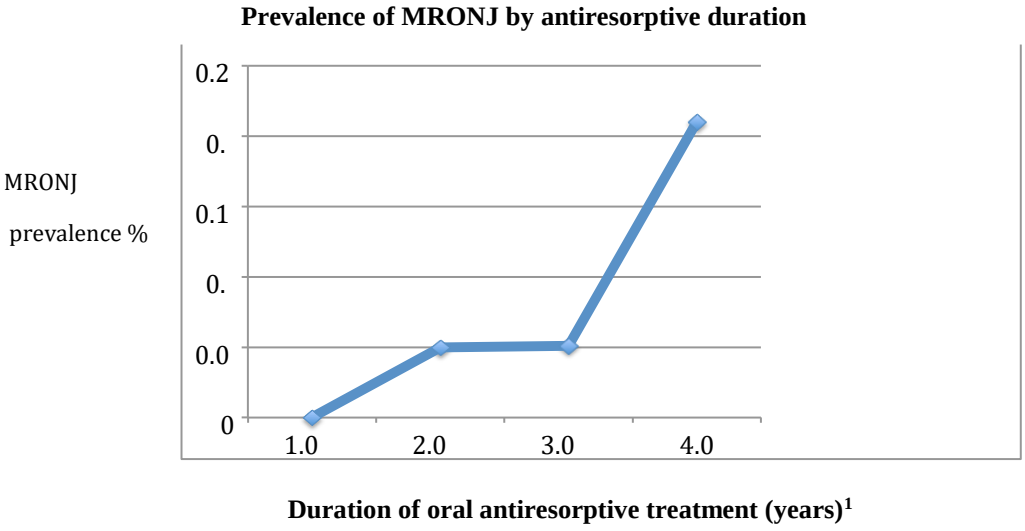


Figure 4: Risk of MRONJ increases with duration of antiresorptive treatment

2.2.2 Local Factors

2.2.2.1 Dental surgery

Dentoalveolar surgery is a major precipitating factor for developing MRONJ, with dental extraction being the causative factor in 52-77% of cases.[1, 35, 42] The operative trauma of the procedure has been implicated as the precipitating factor, and surgical extractions are thought to carry a higher risk than simple routine extractions. In addition, the normal sequence of wound healing requires bone resorption and remodelling to occur, however over-suppression of bone turnover inhibits this healing process. Intraoperative guidelines to promote healing at extraction sites, published by Rogers *et al.,* in 2010, and Henry *et al*., in 2017 advocated pre-operative and post-operative antibiotics, optimising oral hygiene, an atraumatic approach, use of plain local anaesthetic, avoiding raising full thickness mucoperiosteal flaps and tight sutures if possible which may reduce tissue perfusion.[12, 30] The SDCEP in 2017, published a useful guidance tool to quantify a patient's risk of developing MRONJ prior to undergoing dentoalveolar surgery (Figure 5).[14] It implicated bisphosphonates and Denosumab regardless of whether they are prescribed for oncological or metabolic bone disorders.

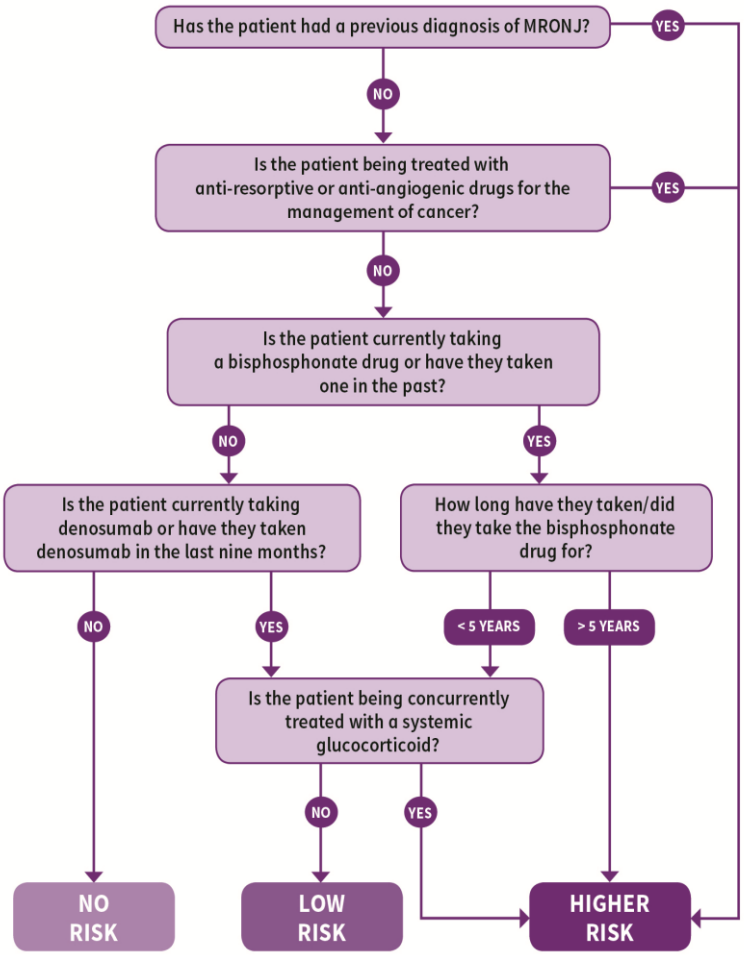


Figure 5: SDCEP (2017) Risk assessment tool for patient on, or with a history of antiresorptives[14]

Evidence suggests the risk is higher for oncology patients on IV bisphosphonates undergoing extractions ranging from 1.6% to 14.8% of cases.[1] A case control study by Kyrgidis *et al., in* 2008 reported a 16-fold increase in risk for oncology patients exposed to Zoledronate, whereas a longitudinal cohort study of 1,621 patients by Vahtsevanos *et al*., in 2009 found a 33 fold increased risk following a dental extraction.[46, 49] Both of these studies were retrospective, and it was unclear whether any interventions were undertaken to reduce the risk of developing MRONJ, for example pre- or post-operative antibiotics. Similarly, there appears to be an increased risk for oncology patients treated with Denosumab undergoing dental surgery. A systematic review of seven RCT's by Boquete-Castro *et al*., in 2016 examined the precipitating factor in 143 cases of MRONJ in oncology patients on Denosumab (total n=8963).[35] Dental extraction was the luxating factor in 66%-77% of the cases. There is little in the

literature describing the incidence of MRONJ in osteoporotic patients receiving Denosumab treatment. The risk of developing MRONJ following dentoalveolar surgery in patients exposed to oral bisphosphonates was low, however this increases with duration of treatment. A prospective study by Kunchur *et al.*, (2009) looked at 194 patients who underwent extraction of >1 tooth, of which one developed MRONJ giving an estimate of 0.5% risk.[50]

However, Dodson (2015) advocated caution when interpreting extraction as a casual precipitant, as tooth extraction may be incidental to the disease, and not the precipitant.[42]

2.2.2.2 Anatomic Features

The mandible is more often affected by MRONJ (73%), when compared with the maxilla (22.5%) or both 4.5%.[48] It has been proposed that this is because the blood supply in the maxilla is richer with cancellous bone, in comparison to dense cortical bone in the mandible. Studies also reported a higher risk of developing MRONJ in denture wearers.(7, 8) Vahtstevranos *et al., (2009),* looked at 1,621 patients who received IV bisphosphonates, and demonstrated that the use of dentures was associated with a two fold increase in risk with an adjusted odds ratio of 2.02; 95% CI, 1.03 to 3.9.[46] Local recurrent trauma at the site of a bony prominence was considered to be the likely cause. Patients often have a positive history of loose and painful dentures prior to presenting with MRONJ. MRONJ appears to have a predilection for the posterior lingual ridge at the level of the mylohyoid ridge. When the patient is edentulous, the lingual gingivae can be traumatised by normal oral function such as eating.[2] Furthermore, the thin layer of overlying squamous mucosa at this anatomical point may make it more susceptible. Khan *et al.,* (2015) in the Canadian Consensus paper also reported oral ulceration and benign sequestration can occur at this lingual site in the absence of antiresorptives. It is usually self-limiting but potentially could be captured in incidence data pertaining to drug related osteonecrosis of the jaw.[2] This was strongly opposed by an expert opinion paper submitted by Otto *et al.*, (2015) in opposition to that paper.[16]

2.2.2.3 Concomitant oral disease

The literature implicates periodontal disease and periapical pathology as risk factors for developing MRONJ.[1] A cross sectional study by Tsao *et al.,* (2013) reported increased numbers of MRONJ in patients with pre-existing periodontal disease, and suggested that periodontal disease and the associated bacteria are potentially implicated in the pathophysiology of MRONJ.[51] There is a positive history of preceding dental pathology in 50% of oncology patients diagnosed with MRONJ.[48, 52] This however must be interpreted with some caution, as extraction is often the treatment option for unrestorable caries, or periodontally compromised teeth, and therefore may act as a confounding factor in the relationship of concomitant oral pathology, and the pathophysiology of MRONJ.[1]

2.2.3 The role of patient demographics and systemic factors as risk factors

2.2.3.1 Demographic factors

Age and sex are reported as risk factors for MRONJ. [1] The incidence of MRONJ is more common amongst women than men however, this is more likely a reflection of the underlying condition being treated e.g. breast cancer and osteoporosis which are more common in females. MRONJ occurs in a more aged population, and this may be due to improved healing capacity in adolescents, differences in bone composition, fewer concomitant social/medical risk factors, or due to less invasive dental treatment at this life point. An observational study by Brown *et al.,* (2008) reviewed 42 paediatric patients with osteoporosis treated with IV bisphosphonates for a mean duration of 6.5 years. Eleven of these patients underwent invasive dental treatment, however there were no cases of ONJ following clinical and radiographic assessment.[53] However, the sample size is small, and the risk of BRONJ for adult osteoporotic patients receiving IV medications is low at 1.7 cases per 10,000. [1, 44]

2.2.3.2 Co-morbid conditions and concomitant medications as a risk factor

Co-morbid conditions such as a low haemoglobin levels amongst oncology patients (<10g/dL) was reported as a risk factor in developing MRONJ.[1,48,51,54] Although, Khan *et al*., (2015), implicated anaemia as a risk factor in osteoporotic patients and not oncology.[2] The AAOMS (2014) and Dodson (2015) concluded that the literature implicating anaemia as a risk factor for MRONJ was inconsistent.[1,42] This was unsurprising as there were so many confounding factors.

Diabetes mellitus is also a recognised risk factor for developing MRONJ, and this may be directly related to the impaired healing, and reduced immunity associated with the condition.[48,51] Khan *et al.*, (2015) reported it as a risk factor amongst oncology patients, although Dodson (2015) and the AAOMS (2014) concluded that there were inconsistencies in the reporting.[1,2,42]

Concomitant use of corticosteroids was associated with an increased risk of MRONJ.[48,51,54,42]Corticosteroids are used for decreasing the bodies inflammatory response, and it is possible that this may contribute to a patient's susceptibility in developing MRONJ. Corticosteroids suppress the inflammatory response by inhibiting synthesis of two important pro-inflammatory products, prostaglandins and leukotrienes. Corticosteroids prevent the transcription of pro-inflammatory genes, including interleukins (IL) 1B, IL-4, IL-5, and IL-8, chemokines, cytokines, granulocyte-macrophage colony-stimulating factor, and tumour necrosis factor-α genes.[55] Corticosteroids also suppress the cell-mediated immunity. They act by inhibiting genes that code for the cytokines most importantly IL-2, which is a potent T-cell growth factor.[55] Reduced cytokine production supresses the T-cell proliferation, and hence affects the immune response. An impaired immune and inflammatory response increases the susceptibility to bacterial infection. One of the earlier animal rat studies, mimicking bisphosphonate ONJ in oncology patients could only consistently induce ONJ when bisphosphonates were combined with dexamethasone.[56] A prospective animal study by Allen *et al.,* (2013) looked at ONJ in mature beagle dogs. Intravenous bisphosphonates were given at doses comparable to humans, and they underwent molar extractions at 7 and 8 months after the start of the study. One group were also given systemic dexamethasone. ONJ was not observed in the control group, but was

in the bisphosphonate only group, and the bisphosphonate and dexamethasone group. They concluded that corticosteroids do not prevent MRONJ. [57]

Concomitant use of anti-angiogenic agents with bisphosphonates poses an increased risk of MRON.[1] A well-designed study by Guarneri *et al.,* (2010) looked at 3,560 patients receiving anti-angiogenic bevacizumab therapy for locally recurrent, or metastatic breast cancer in two double-blind, randomised trials (AVADO and RIBBON-1) and a large, non-randomised, safety study (ATHENA).[40] The overall incidence of ONJ with bevacizumab was 0.3% to 0.4%. The risk increased to 0.9% to 2.4% in patients exposed to bisphosphonates and anti-angiogenic medications.

The type of cancer was also reported as a risk variable in developing MRONJ. A meta-analysis by Qi *et al.,* in 2014 looked at seven RCTs (n=8,963).[45] They reported an increased risk of MRONJ associated with those with metastatic prostate cancer (RR 3.358, 95 % CI: 1.573-7.166, P = 0.002), than in non-prostate cancers (RR 1.142, 95 % CI: 0.678-1.921, P = 0.618). It is unknown why the type of cancer is a risk factor, but it may be due to metastatic prostate disease is osteoblastic, whereas multiple myeloma and breast metastases are osteolytic. Another plausible factor is that the median follow-up period for prostate disease was 24 to 41 months, whereas non-prostate cancer was 3 to 24 months within the study. It was probable that a longer follow-up period resulted in an increased incidence.

A longitudinal cohort study by Veterans *et al.,* in 2009 also looked at the type of cancer, as a risk factors for MRONJ (n=1,621).[46] The incidence of ONJ was 8.5%, 3.1%, and 4.9% in patients with multiple myeloma, breast cancer, and prostate cancer, respectively.[46] However, the low incidence in the breast cancer cohort is most likely be as a result of being treated with Ibandronate which has a lower potency than Zoledronate, and Pamidronate, which were used in the prostate and multiple myeloma groups.

It is difficult to ascertain whether the cancer type is a risk for MRONJ, or whether the observation is due to heterogeneity across patient demographics and study design.

The literature has been inconsistent when reporting on tobacco use as a risk factor for MRONJ.[42] Tobacco use is associated with local tissue ischaemia, and suppression of

the innate, and host immune responses, resulting in pathology, and compromised wound healing. [58] There are many well designed studies to support the pathological role of tobacco use in periodontal disease, and increased implant failures in smokers. However, the evidence is poor for implicating smoking as a risk factor in MRONJ due to lack of prospective controlled trials with large sample sizes, and the considerable number of variables amongst patient populations. A case control study by Borromeo *et al.*, (2014) reported an association between smoking and MRONJ, with an increased odds ratio of 5.5 (95% CI 1.3-22.9) that was statistically significant (P=0.02).[59] A case control study by Kyrgidis *et al.*, (2008) with 40 patients in the test group and 40 matched controlled patients approached statistical significance possibly implicating smoking as a risk factor.[49] The small sample size may have prevented the study achieving statistical significance. A longitudinal cohort study by Vahtsevanos *et al.*, (2009) found no association between smoking and risk of MRONJ.[46] There is insufficient evidence to support or refute its role as a risk factor for MRONJ at this time.[1, 42]

2.2.4 Genetic factors

There has been some recent evidence to support a genetic predisposition to developing MRONJ. Single nucleotide polymorphisms located in regions of the genome, which encode for bone turnover, showed collagen formation and metabolic bone disorders, may be implicated.[1] A cohort study by Katz *et al.*, (2011) reported a 57% incidence rate of MRONJ in patients with multiple myeloma, when single nucleotide polymorphisms were present in five candidate genes (COL1A1, RANK, MMP2, OPG and OPN) which are responsible for bone turnover. Smoking and the type of bisphosphonate, combined with the five candidate genes were highly significant for developing MRONJ.[60] A more recent study by Yang and Katz, (2017) identified the SIRT1/HERC4 locus on Chromosome 10 to be associated with MRONJ, and the two promoter single nuclear polymorphisms may act as potential genetic markers for this association.[61] A genome study by Nicoletti *et al.*, (2012) included 47 cases of MRONJ, and identified a significant marker in the RBMS3 gene. Patients positive for this single

nuclear polymorphism were 5.8 times more likely to develop MRONJ (odds ratio, 5.8; 95% confidence interval, 3.1-11.1).[62] It is apparent from these studies that there may be a germ line sensitive to antiresorptives that places some individuals at a greater risk of developing MRONJ. However, heterogeneity across patient demographics and confounding factors make it difficult to assess the true relationship.

2.3 Pathogenesis of MRONJ

The pathophysiology of MRONJ is a contentious issue amongst clinicians and researchers with many hypotheses being proposed to explain the unique localisation of MRONJ to the jaws. Among these hypotheses are altered bone remodelling, or over suppression of bone resorption, inhibition of angiogenesis, recurrent microtrauma, suppression of the innate or acquired immune system, vitamin D deficiency, toxicity of bisphosphonates to soft tissues, and infection.[1] Certain factors which are unique to the oral cavity include increased rates of bone turnover, the presence of teeth and thin mucosa overlying bone. No single hypothesis has been accepted, and animal studies show evidence that it may well be a combination of these factors.

2.3.1 Inhibition of bone resorption and remodelling

Antiresorptives affect bone turnover through inhibition of osteoclast differentiation, function and increased osteoclast cell death with an overall reduction of bone resorption and remodelling. The antiresorptives exact their effects on all skeletal bones, however it is only alveolar bone that is affected by MRONJ and this may be attributed to the rapid physiologic turnover of bone in the jaws; and hence a higher concentrations of antiresorptives are incorporated in the bone.[1] Over-suppression of bone resorption and remodelling is generally the most accepted hypothesis. A longitudinal 3-year study by Allen and Burr in 2008 looked at the histology of bone samples from beagle dogs, who received daily bisphosphonates compared with controls. The test group showed significantly lower bone turnover and increased rates

of osteonecrosis.[63] The significant role of bone remodelling inhibition in the pathogenesis was affirmed with the similar incidence of MRONJ with antiresorptives, such as Denosumab. Furthermore, increasing doses, potency, and duration of antiresoptives were associated with a more profound inhibitory action, and an increased risk of MRONJ, which supported this hypothesis. There was also evidence from animal studies that anabolic bone drugs, such as Teriparatide, can favour healing in patients undergoing dental extractions, with a prior history of IV bisphosphonates, thus reducing the risk of MRONJ.[1] Contrary to this, a study by Ristow *et al.*, (2014) assessed bone turnover rates in 45 female oncology patients using bone scintigraphic images, comparing bisphosphonates, Denosumab and a control group prior to treatment, and during the course of treatment at 12 and 24 month time intervals.[64] They reported bone turnover was not overtly changed by bisphosphonates or Denosumab, and that over-suppression of bone turnover was unlikely to cause MRONJ. This suggested that there are other factors at play in the pathogenesis, and that interruption of the mucosal integrity may be implicated in the pathogenesis.

2.3.2 Infection

Infection has been implicated in the pathogenesis of MRONJ for some time. An increased incidence of MRONJ has been reported in immunocompromised patients, and those with local oral risk factors, such as poor oral hygiene.[1] The difficulty arises in identifying whether the infection is the cause, or the consequence of bone exposure. The sequence leading to a dental extraction is usually preceded by periodontal disease, or periapical infection, which is a bacteria driven process, which may contribute to the pathogenesis of MRONJ. A large retrospective study by Hoff *et al.,* in 2008 identified poor oral health and dental extractions as risk factors for developing MRONJ in oncology patients.[65] Similar findings were reported in a large systematic review by Boquete-Castro *et al.*, (2016) which found a positive correlation with poor oral hygiene and dental extractions with developing MRONJ in patients on Denosumab; ranging from 55-77% in 143 cases of MRONJ (total n=8963).[35] However, failure to heal due to over-suppression of bone turnover following a dental extraction

is a confounding factor to the role of poor oral hygiene resulting in infected teeth being extracted. The success of preventative oral screening programmes prior to commencing intravenous bisphosphonates was highlighted by Ripamonti *et al.,* in 2009 with an overall reduction in the incidence of MRONJ from 3.2% to 1.3%.[66] However this reduction in MRONJ incidence may be spurious, and not related to improved local factors, but as a result of any poor prognosis teeth being extracted prior to commencing bisphosphonates. Studies looking at the histological features of bone from patients with MRONJ reported that inflammatory cells, bacteria or their products, may have a large, direct lytic effect on bone tissue, challenged by bisphosphonates resulting in sequestra, regardless of whether infection was the cause, or consequence of bone exposure.[67, 68]

2.3.3 Inhibition of angiogenesis

Angiogenesis is the formation of new blood vessels from the existing vasculature, through a process of growth, migration, and differentiation of cells, and is a crucial step in the sequence of wound healing.[1] The process is regulated by signalling molecules such as vascular endothelial growth factor (VEGF) which is central to promoting angiogenesis. Classically osteonecrosis occurs with an interruption of the vascular supply, and therefore inhibition of angiogenesis is a proposed hypothesis for MRONJ. Zoledronic acid is a potent anti-angiogenic drug, which results in decreased circulating VEGF.[1] A study by Santini *et al., (2003)* reported up to 34% decrease in circulating serum levels of VEGF, which persisted at 21 days post IV Zoledronic acid infusion in oncology patients.[69] Other anti-angiogenic drugs such as kinase inhibitors, and monoclonal antibodies, which specifically target VEGF, may be linked to MRONJ, although inhibition of angiogenesis has not been linked to Denosumab which indicated that the pathogenesis is likely multifactorial.[1]

2.3.4 Soft tissue toxicity

Soft tissue toxicity has been reported with bisphosphonates, although the affinity for bone is much greater. An *in-vitro* study by Landesburg *et al.*, (2008) revealed reduced wound healing, and proliferation of oral keratinocytes when exposed to pamidronate.[70] However, bisphosphonates were excreted via the kidneys within a few hours of administration, and therefore the concentration within soft tissues is minimal. The extent to which tissue toxicity plays a role in the pathogenesis is unclear, and soft tissue toxicity has not been reported with Denosumab.[1]

2.3.5 The immuno-compromised patient

Immuno-compromised patients are associated with an increased risk of ONJ, due to suppression of the adaptive regulatory T cells, and activation of the inflammatory T-helper-producing interleukin 17 cells.[71] Oncology patients, undergoing concurrent chemotherapy, may also be at a higher risk due to immunosuppression combined with high dose bisphosphonates. This has been shown by animal studies.[71]

2.4 Diagnosis of MRONJ

Patients may be considered to have MRONJ, if all the following criteria are fulfilled:

1. Current or previous treatment with antiresorptive or anti-angiogenic medications,
2. Exposed bone, or denuded bone which is palpated with a dental probe intra- or extra orally in the maxillofacial region which has persisted for more than 8 weeks; and
3. No history of radiation to the jaws or obvious metastatic disease to the jaws.[1]

2.4.1 Clinical examination

Patients at risk of MRONJ can also present with other clinical conditions that must not be mistaken for MRONJ. The differential diagnosis includes alveolar osteitis, sinusitis, gingivitis/periodontal disease, periapical pathology, fibro-osseous lesions resulting in secondary sequestration, chronic sclerosing osteomyelitis and sarcoma.[1, 2]

A detailed patient history and clinical examination are the most sensitive diagnostic tools for MRONJ, with the aid of adjunctive special tests. An area of exposed bone that fits the AAOMS (2014) criteria and fails to respond to appropriate treatment is usually diagnostic of MRONJ.[1] These sites of exposed bone may be an incidental finding, and may remain symptom free for weeks, months or even years.[1] However, more often patients develop symptoms of inflammation in the surrounding tissues, and may encounter problems prior to a clinical diagnosis of MRONJ. Symptoms include pain, mucosal swelling, tooth mobility, dusky erythema of overlying tissues, draining sinuses, ulceration or sensory deficit to the associated branch of the trigeminal nerve.[1] In most cases patients will describe invasive dentoalveolar surgery at the site, but in some cases, may arise spontaneously. Secondary infection is common, and if left untreated may result in extraoral fistulae, which can mimic the appearance of malignancy. Extensive involvement in the mandible can also result in pathological fractures, whereas in the maxilla it may cause oroantral fistulas, or oronasal fistulas.

2.4.2 Plain film radiographs

Intraoral and panoramic radiographs are useful as a screening tool for MRONJ. They are easily accessible, inexpensive, and deliver a low dose of radiation to the patient. They are useful for diagnosing gross caries, which plays a preventative role to minimise future invasive treatment.

Dental radiography can visualise pathology of the mandible, maxilla, and relevant adjacent structures such as maxillary antra, nasal cavity, inferior dental canal, lower border of the mandible, and mental foramina.

Long-term antiresorptive treatment results in radiographic features such as thickening of the lamina dura, increased trabecular density of alveolar bone and thickening of the mandibular canal/ sinus floor cortication as a result of inhibition of bone turnover.

The pathogenic radiographic features of established MRONJ include:

- Widening of the periodontal ligament space
- Incomplete healing of extraction socket
- Osteolysis
- Sequestrum formation
- Periosteal bone formation.[2]

2.4.3 Specialised imaging

Computed tomography (CT) and cone beam computed tomography (CBCT) imaging are superior to conventional radiography from a diagnostic point of view, but they are associated with increased cost and larger doses of radiation. MRONJ often presents with diffuse osteosclerosis, sites of osteolysis, cortical erosion, and bone sequestration. Pathology can be diagnosed in an earlier phase than with conventional radiographs, and they can provide accurate detail on disease extension and boundaries, especially relevant when it involves adjacent structures such as the maxillary antra.[2] This may be helpful in surgical planning, although typically CT underestimates disease extension. A prospective study by Stockmann *et al.,* (2010) looked at the detectability of MRONJ in 24 patients prior to surgical intervention.[72] This was assessed using three imaging techniques; OPG, CT, and MRI. The detectability was 54% for OPG radiographs, 92% for MRI scans, and 96% for CT scans respectively. Intraoperatively CT and MRI were found to grossly underestimate the extension of disease. MRI has the benefit of evaluating soft tissue extension, and disease extension within the marrow spaces with superior accuracy than CT/CBCT. These detailed investigations may be more relevant to Stage 2 and 3 disease, or where surgical

intervention is planned, but they may not necessarily change the treatment or disease outcome.[2] Other techniques less commonly used are bone scintigraphy and positron-emission tomography (PET), which can analyse the osteoblastic and inflammatory activity of the process, and have a higher specificity and sensitivity than other techniques in the early diagnosis of MRONJ. Poor resolution of anatomic detail, high doses of radiation and access prohibit their routine use in the diagnosis of MRONJ.[73]

2.4.4 Biomarkers of disease risk

MRONJ is a disease associated with the use of antiresorptive medications, and some authors have suggested biomarkers that quantify bone turnover may be indicative of prognosis. The serum bone turnover marker; C-terminal telopeptide (CTX), has been suggested as a biomarker and useful in predicting an individual's risk of developing MRONJ prior to invasive dental surgery, or in the assessment of MRONJ severity. A study by Marx concluded that increasing levels of CTX following cessation of bisphosphonates is associated with increased bone turnover and that this relationship is casual and can quantify a patient's risk.[74]

Marx's criteria:

- CTX<100pg/mL=High risk
- CTX 100-150pg/mL=Moderate risk
- CTX >150pg/mL=Low risk

However, the hypothesis was never formally tested as there was no control arm in the study which continued bisphosphonate treatment. Furthermore, there was no correlation between CTX levels and disease severity. Other publications have reported that CTX as a biomarker was neither accurate nor reliable in predicting the risk of MRONJ.[2, 30] In the HORIZON trial one patient in the Zoledronic acid test group (n=5,903) developed MRONJ, and one developed MRONJ in the placebo control group. In the test group 43% of the patients had CTX levels <100pg/mL and according to Marx's criteria would have been categorized as "high risk", yet the incidence of MRONJ

is no higher than the control group.[44] Overall the literature would suggest low CTX levels are associated with recent antiresorptive treatment, however data does not support CTX levels being used as a predictor of risk in developing MRONJ, or when is the optimum time to treat MRONJ with a surgical intervention. Other biomarkers such as N-telopeptide, alkaline phosphatase, and osteocalcin have reportedly failed to predict a patient's risk of developing MRONJ.[2]

2.4.5 Histological examination

Histological analysis is not required to make a diagnosis of MRONJ, and nor is it diagnostic in isolation. However it is good practice to send samples to exclude other pathology, especially metastatic disease.[1] A histological study by Carmagnola *et al., (2013)* examined specimens from patients with MRONJ (n=14), and osteoradionecrosis (ORN) (n=2). Histometric and morphometric analysis found common histological features between the samples. These included; loss of normal bone architecture which lacked a proper Haversian system and proper marrow spaces, sites of necrosis with non-mineralised tissue, empty osteocytic lacunae adjacent to areas of hyper-cellularity, resorption pits with rare osteoclast-like cells, and the presence of bacteria and of an inflammatory infiltrate. They concluded that histologically MRONJ has similar features to ORN, and osteomyelitis.[67]

2.5 Staging of MRONJ

A staging system is important to accurately describe the presentation of pathology and allows clinicians to appropriately stratify patients. For the purposes of research, it allows clinicians to communicate scientifically, and may help to guide management strategies. In 2006, the AAOMS proposed a staging system based on the severity of disease, which was subsequently updated in 2009 (Table 6).[6] The updated 2009 classification included a Stage 0 category, which describes the non-specific symptoms, clinical, or radiographic anomalies experienced by patients on antiresorptive drugs.

These non-specific finding are found in 50% of patients who go on to develop Stage 1,2 or 3 disease or in patients who have a history of Stage 1, 2 or 3 disease but have since healed.[1] Disease stage is not static, and MRONJ lesions can regress or progress.

Staging of medication related osteonecrosis of the jaws

At risk—no apparent necrotic bone in patients, who have been treated with oral or intravenous bisphosphonates
Stage 0—no clinical evidence of necrotic bone, but may present with non-specific clinical findings, radiographic changes, and symptoms
Stage 1—exposed and necrotic bone, or fistulas that probes to bone in patients, who are asymptomatic, and have no evidence of infection
Stage 2—exposed and necrotic bone, or fistulas that probes to bone associated with infection, as evidenced by pain, and erythema in the region of exposed bone with or without purulent drainage
Stage 3—exposed and necrotic bone, or a fistula that probes to bone in patients with pain, infection, and ≥ of the following: exposed and necrotic bone extending beyond the region of alveolar bone (i.e., inferior border and ramus in mandible, maxillary sinus, and zygoma in maxilla), resulting in pathologic fracture, extraoral fistula, oral-antral or oral-nasal communication, or osteolysis extending to the inferior border of the mandible or sinus floor

Table 6: AAOMS (2014) Staging system for MRONJ[1]

However, the AAOMS classification does not incorporate radiological features into its classification, and nor does it advocate radiographic signs alone should be used to define a case of MRONJ as this could lead to overestimation of disease.[1] Radiographs are advised as an adjuvant in diagnosis and classifying the Stage of disease.[6] In 2009, Wilde *et al.*, used a staging system similar to that proposed by the AAOMS in 2009, but which included a Stage 4 category which divided the AAOMS Stage 3 disease into a mild and severe form (Table 7).[73]

Stage	Clinical and radiologic signs
0	No exposed necrotic bone
1	Asymptomatic exposed necrotic bone or single intraoral fistula
2	Exposed necrotic bone associated with pain and infection
3	Exposed necrotic bone associated with pain, infection with swelling and abscesses, multiple intraoral fistulas, and extended osteolysis in the radiologic findings
4	Exposed necrotic bone associated with pain, infection with swelling and abscesses, pathologic fracture, naso-oral fistula, extraoral fistula, or osteolysis extending to the inferior border

Table 7: Classification of MRONJ according to clinical and radiological features[73]

A literature review by Gavalda and Bagan in 2016, on Staging classifications concluded that radiographic assessment, particularly CT formed an important part in the diagnosis of disease extension and should formulate part of the Staging process.[75] Furthermore this may be helpful in treatment planning, especially when surgery is considered. There are least eight other classification systems discussed in the review article, all variations of the AAOMS (2009) Staging system, but the key issues of contention were the lack of consideration for radiologic features, and whether Stage 0 disease should be omitted and MRONJ be classified into three stages regardless of whether bone is exposed or not.[75] Other classifications systems by Franco *et al.*, (2014), and McMahon *et al.,* (2007) classified MRONJ by the extent of bone exposure in centimetres.[76, 77] However, Gavalda and Bagan (2016) concluded there was no single Staging system used by all clinicians, and the AAOMS (2014) classification appeared to be the system most frequently used.[6, 75]

2.5.1 Clinical presentation, symptoms and radiographic features according to AAOMS Staging system

Stage	Symptoms	Clinical findings	Radiographic findings
0	Odontalgia Dull, aching pain, in the mandible which radiates to the TMJ Sinus pain +/- inflammation, and thickening of sinus wall Neurosensory disturbance	Intact mucosa with no exposed bone Loosening of teeth Periapical/periodontal sinus not due to pulpal necrosis, and the sequelae of caries	Alveolar bone loss Persistence of un-remodelled extraction socket Osteosclerosis Thickening of the lamina dura Narrowing of the PDL
1	Usually asymptomatic	Exposed necrotic bone/ a fistula that probes to bone No evidence of infection	Similar to Stage 0, localised to alveolar bone region Sequestrum
2	Usually symptomatic, with pain, swelling, foul taste, halitosis	Exposed necrotic bone, or fistula that probes to bone Evidence of infection +/- suppuration Adjacent, or regional soft tissue inflammatory swelling	Similar to Stage 0, localised to alveolar bone region Sequestrum
3	Usually symptomatic, with pain, swelling, foul taste, halitosis Neurosensory disturbance Oronasal regurgitation Draining extra-oral fistula	Exposed necrotic bone, or fistula that probes to bone with evidence of infection, and one or more of the following: • Exposed necrotic bone extending beyond alveolar bone • Pathologic fracture • Extra-oral fistula • Oro- antral or nasal fistula • Osteolysis extending to the lower border of the mandible or antral floor	Similar to Stage 0, localised within or beyond alveolar bone region Sequestrum Oro-antral/nasal fistula Pathological fracture

Table 8 Clinical features, symptoms and radiographic signs according to disease Stage[1,2]

2.6 The microbiology of MRONJ

A study by Sedghizadeh *et al.,* in 2008 was one of the first studies to identify the presence of mixed-species microbial biofilms in MRONJ cases using scanning electron microscopy.[68] A biofilm is a microbially derived complex community of microorganisms bound together in an extracellular matrix, enabling communication and expression of an altered phenotype. This regulates growth rates, gene transcription and antimicrobial resistance. They observed bacterial morphotypes varying from 2-15 within the biofilms, and isolates included Fusobacterium, bacillus, Actinomyces, Staphylococcus, Streptococcus, Selenomonas, Candida and 3 distinct types of treponemes. Gram-positive and Gram-negative organisms were identified, which included some aerobes, but anaerobes were dominant. Co-aggregation was also observed whereby cell-to-cell recognition of genetically distinct morphotypes reside together. [68] It is likely the longer the bone is exposed to the oral cavity, a thicker and more complex biofilm develops. The microbes identified were consistent with those isolated from the oral cavity in health, and particularly in pathologic states such as periodontal disease, bacterial driven periapical periodontitis and oral candidiasis. The formation of a thick biofilm can help bacterial colonies evade systemic antibiotics and result in refractory cases. [68] In these situations, topical antimicrobial mouth-rinses such as Chlorhexidine, play a significant role in reducing plaque biofilms, and improving the efficacy of systemic antibiotics.

A systematic review by Hinson *et al.*, (2014), which included 55 articles on 814 cases of MRONJ looked at the microbial flora cultured from samples.[78] They reported 248 (68.8%) of which had histological samples had microscopic evidence of *Actinomyces* colonization (Figure 6). Actinomyces is a chronic granulomatous pathogen that is one of the most common genera in the oral cavity, which facultatively enters deeper tissues when the mucosal surface is interrupted.[79] They form thick aggregates of branching filamentous bacilli, which make it difficult for systemic antibiotics to penetrate and often necessitate prolonged high dose courses of treatment +/- debridement. Actinomyces are usually sensitive to Penicillin and beta-lactams,

however they are intrinsically resistant to Metronidazole and have reduced sensitivity to Clindamycin .[79]

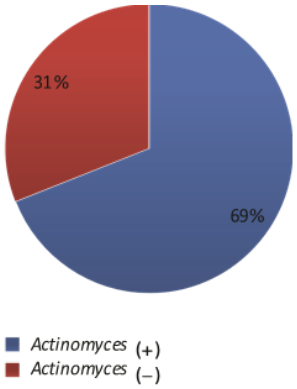


Figure 6: Percentage of isolates positive for Actinomyces[78]

Of the 166 cases which obtained cultures, 60.48% reported growth and 39.6% had no growth. Excluding *Actinomyces*, Streptococcus was the most abundant organism grown. Other organisms grown included *Candida*, *Staphylococcus*, *Klebsiella*, *Eikenella*, *Haemophilus*, *Fusobacterium*, and *Escherichia* (Figure 7). Mixed oral flora was reported in 43 of the cases. It was also noted that in 10% of immunocompromised patients, Candida species were isolated, and the authors recommended an anti-fungal medication for refractory cases.[78]

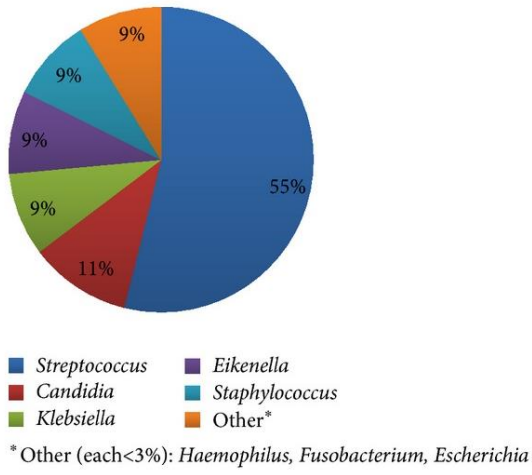


Figure 7: Percentage of samples which cultured various pathogens at MRONJ sites [78]

Actinomyces is often implicated as one of the prevalent pathogens isolated, and these observations are based on microscopic isolation.[1, 78] Increasingly a more accurate method of gene sequencing called 16s ribosomal RNA is used to study the taxonomy,

and phylogeny in complex microbiomes.[79] A study published by the National Institute of Health by Xiaojie *et al.*, (2012) did not observe these high numbers of Actinomyces species when using molecular 16s rRNA techniques to identify bacterial species.[80] Furthermore, they compared the effects of antibiotic on the bacterial profile of MRONJ cases. One group were given systemic antibiotics for 2 weeks and the other group had no prior antibiotic therapy. Using 16s rRNA they observed no significant differences in bacterial diversity in MRONJ samples, indicating systemic antibiotics may have limited efficacy on the bacterial populations associated with MRONJ.[80] Such an observation may explain the low resolution rates observed with medical management alone, and may support a surgical approach.

2.7 Treatment of MRONJ

For patients with established MRONJ, the aim of treatment is to control infection, limit disease progression, and promote healing within the tissues.[1, 10, 11] Some authors believe this cannot be achieved without the removal of all necrotic bone and achieving primary closure at the site.[18] There are concise guidance papers issued by the Journal of the Irish Dental Association (2010, 2017), SDCEP accredited by NICE (2017), and AAOMS (2014) for the prevention of MRONJ.[1, 12, 14, 30] However, there are no universally accepted treatment guidelines for managing established MRONJ, and in the absence of clearly defined protocols, there is a consensus to adopt a more conservative approach. The Journal of the Irish Dental Association published an article in 2006, outlining principles of treatment for patients with established MRONJ.[13] They advocated conservative management only and surgery should be avoided, however this concept for treatment is outdated. The AAOMS (2014) proposed a standard management protocol based on the Stage of disease, and this is the most widely adopted protocol (Table 9).[1] These are the same principles of treatment adopted by the Royal College of Surgeons (2014), and indeed the guidance article is written by the same lead author as the AAOMS paper.[36]

MRONJ Staging	Treatment Strategies
At risk category	▪ No treatment indicated ▪ Patient education
Stage 0	▪ Systemic management, including the use of pain medication and antibiotics
Stage 1	▪ Antimicrobial mouth rinse ▪ Clinical follow-up on a quarterly basis ▪ Patient education, and review of indications for continued bisphosphonate therapy
Stage 2	▪ Symptomatic treatment with oral antibiotics ▪ Oral antimicrobial mouth rinse ▪ Pain control ▪ Debridement to relieve soft tissue irritation, and infection control
Stage 3	▪ Antimicrobial mouth rinse ▪ Antibiotic therapy and pain control ▪ Surgical debridement/resection for longer term palliation of infection and pain

Table 9 Treatment according to disease Stage[1]

** Mobile sequestrum should be removed, regardless of disease stage without exposing uninvolved bone. Symptomatic teeth within exposed, necrotic bone may be extracted as it is unlikely to worsen the situation*.

However they do explicitly mention in the disclaimer that the position paper "is not intended to set any standards of care". In 2015, Khan *et al.*, published a systematic review and international consensus, which advised a similar management protocol as

the AAOMS staging dependent system.[2] However, a letter to the editor submitted by experts in the field Otto and Marx, (2015) felt that the consensus paper by Khan *et al.*, (2015), did not provide a balanced and fair comparison of the outcomes of different treatments, both surgical and non-surgical.[16] The consensus paper suggested "conservative treatment should be the mainstay of care", however Otto and Marx felt that they provided little evidence to support this comment in the review. In addition it was proposed that insufficient consideration was given to systematic reviews meeting PRISMA guidelines that reported a surgical approach can be superior to conservative treatment.[2, 16, 20, 81]

2.7.1 Factors that influence the treatment approach

There are multiple factors that may influence the rationale for one treatment option over another. These include; age, sex, disease status (oncology/osteoporotic patient), Stage and size of MRONJ lesion, antiresorptive exposure, medical and pharmacological comorbidities, such as diabetes and use of systemic steroids.[82] These factors can influence the progression of MRONJ and influence an individual's response to treatment.

Another factor which may influence a clinician's decision process is the patient's life expectancy, and if an oncology patient is palliative, and asymptomatic, surgical debridement may not be in the patient's best interest. However, if a patient's quality of life is affected, and they are unresponsive to conventional non-surgical treatment, clinicians are more likely to intervene surgically at an earlier stage.

2.7.2 Treatment options

Treatment is often a combination of one or more non-surgical interventions, combined sometimes with a surgical intervention. The table below shows the different treatments reported in the literature, which were identified in a Cochrane review looking at interventions for managing MRONJ (Table 10).[10]

Non-surgical treatment options	Surgical treatment options
Antiseptic mouth rinses	Sequestrectomy (conservative surgery)
Systemic antibiotics	Debridement (conservative surgery)
Hyperbaric oxygen	Resection rim/segmental (extensive surgery)
Pentoxifylline and α-tocopherol (vitamin E) with antibiotics	
Low-level laser therapy (LLLT) (argon, carbon dioxide, helium/neon, neodymium-doped yttrium-aluminium-garnet)	
Teriparatide	
Autologous platelet concentrates (PRP, PRGF, L-PRF)	

Table 10: Treatment options for MRONJ identified by Cochrane (2017) [10]

2.7.3 Difficulties encountered reviewing the literature

In 2016, a Cochrane review set out to establish the efficacy and safety of interventions aimed at treating MRONJ. They concluded that classical "wound healing" conservative treatment and surgery, were the treatments most often employed for treating BRONJ; however there was a lack of evidence from RCTs to guide treatment of MRONJ.[11] In 2017, a similar Cochrane review was completed with only two RCTs included in the analysis, specifically looking at hyperbaric oxygen, and fluorescence-guided bone surgery to improve healing in established MRONJ. [10] There was insufficient evidence in either of the studies, to claim or refute the benefits of the interventions. Small samples, poor study design, and heterogeneity across samples, and interventions result in low quality evidence and make it very difficult to systematically review the literature and draw meaningful conclusions. Realistically, this can only be overcome by multi-centric collaboration, or referral to single designated specialised unit.[11]

2.7.4 Measurement of healing

The Cochrane review in 2017 reported there was no standardised scale for measuring healing of MRONJ.[10] Resolution of lesions is based on clinical examination, imaging, or both. Healed, is generally defined as mucosal healing, with continuous oral epithelium over an area of exposed bone.[10] In addition, some authors included the criteria of lack of symptoms, and lack of radiographic signs of pathology. The follow-up period is variable within the literature, with some authors advising a follow-up period of at least one year.[10] In a large systematic review by Fliefel *et al.,* (2015) they recorded 7 different outcome variables, which included mucosal healing, bone exposure, pain, change in signs and symptoms, improvement in Stage, reduction in lesion size and number, and infection control.[81] The heterogeneity in terms of data reporting added to the difficulties in determining best practice. The evaluation of wound healing using scales has been used in OMFS; such as Landry's Healing Index, the Early Wound Healing Index, and three other variations of Landry's index with modifications.[83] However, these scales assess healing in the inflammatory, and proliferative phase, which extends up to 3 weeks, they do not evaluate the remodelling phase which is pertinent to MRONJ cases, where soft tissue maturation, and bone remodelling occurs.

2.8 Non-surgical conservative treatment

Nearly all patients with established MRONJ are managed conservatively first and foremost, with the aim of halting disease progression, preventing additional disease sites, and to promote healing. Conservative treatment involves optimising oral hygiene, smoking cessation advice, saline and antimicrobial topical mouth rinses, and systemic antibiotic therapy as indicated.[2] These interventions are sometimes combined with adjuvant "add-on" treatments. The principles of conservative management continue to be the mainstay for recommendations from the Canadian International Task Force on ONJ (2015), the Irish Dental Association (2006), Royal College Surgeons (2014), and the AAOMS (2014) for all Stages of disease except Stage 3.[1, 2, 13, 36] Conservative management is the least invasive, and can result in two sequelae; complete resolution of the lesion which can often take months or even years, or progression of disease. Palliation of symptoms and living with persistent disease, can have a negative impact on quality of life, and cost implications. In addition, multiple courses of antibiotics give rise to issues of increased bacterial virulence, resistance and the negative side effects associated with antibiotics.[84]

A systematic review by Rupel *et al.,* (2014), meeting PRISMA guidelines, reviewed the therapeutic approaches in managing BRONJ.[20] They identified 14 different combinations of treatment modalities in 858 BRONJ patients within 22 studies. Two hundred patients were treated with a conservative non-surgical approach. The overall response to medical management with regards to healing was 36% (Table 11). They also concluded medical management was beneficial in arresting disease progression and causing minor improvement in lesions.[20]

Type of treatment	Author, year	% healed	Overall % & number of patients healed
Antibiotic therapy	Montebugnoli *et al.,* (2007) Thumbigere-Math *et al.,* (2009) Scoletta *et al.,* (2010) Ferlito *et al.,* (2011) Martins *et al.,* (2011) Moretti *et al.,* (2011) Mucke *et al.,* (2011) Freiberger *et al.,* (2012) Vescovi *et al.,* (2012)	0% 0% 87% 0% 33% 24% 100% 33% 19%	36% (n=50/138)
Antibiotic therapy & LLLT	Vescovi *et al.,* (2012)		30% (n=11/37)
Antibiotic therapy & HBO	Freiberger *et al.,* (2012)		52% (n=13/25)

Table 11: Outcomes for conservative non-surgical management[20]

Abbreviations: LLLT low level laser therapy; HBO hyperbaric oxygen

The systematic review only included one RCT, with the rest of the studies Level 3 and 4, within the hierarchy of evidence. They also excluded articles which were not published in English. The duration of follow-up is short, with some studies reporting on a follow-up period of 1 month. This is too short to assess the outcome of an intervention. They also conceded that heterogeneity amongst the studies limits their analysis to descriptive statistics, and hence were unable to perform a meta-analysis. However; the sample size is large, and this helps to strengthen the evidence from this review.

A systematic review by Fliefel *et al.,* (2015) which met PRISMA guidelines, looked at the results from 97 studies reporting on 4,879 cases of BRONJ between 2003 and 2014. The mean duration of bisphosphonate therapy was 38.2 +/-15.7 months. Two hundred and eighty-six patients were treated medically; 45.1% showed complete healing, 18.2% showed partial healing, 8.0% had stable lesions, 2.8% had progressive

lesions, 18.2% had regressive lesions, 7.0% had recurrent lesions, and 0.7% had negligible or no healing. Firstly, there is confusing overlap in the outcome measures. Furthermore, overall complete healing is relatively high, in comparison to other previous studies. One of the studies included in this systematic review was by Marx *et al.,* (2005). He reported 90.1% of 119 cases were pain free with medical management only, and this contributes to a large proportion of the cases (107 of the 129) that were included in the systematic review by Fliefel *et al.,* (2015).[81, 85] However, it failed to report whether the MRONJ lesions resolved, or whether symptoms were simply palliated. There have been reported difficulties in reproducing some studies by Marx *et al.,* (2005), and this could potentially result in misleading results from this systematic review. This systematic review's main strength is the large sample size; however, the studies included are mostly retrospective, Level 3 and 4 evidence. Heterogeneity across the studies, precluded meta-analysis of the studies.

A well designed systematic review, and meta-analysis by El-Rabbany *et al.*, in 2017, looked at the effectiveness of different treatments for MRONJ.[17] They completed a comprehensive, repeatable search of the available literature, resulting in the inclusion of 13 studies. The studies included were a single RCT, one non-randomised controlled trial, and eleven prospective cohort studies, which have a stronger level evidence, than the previously discussed systematic reviews. Treatment options included bisphosphonate drug holidays, hyperbaric oxygen therapy, medical management, teriparatide, PRGF, and conservative surgical debridement and aggressive surgical debridement. They concluded that compared to medical treatment with local antimicrobials, with or without systemic antimicrobials, surgical treatment offered higher odds of complete resolution. Meta-analysis revealed two studies with 76 participants; unadjusted odds ratio of 3.55 (95% confidence interval 1.12 to 11.19). They also concluded there was insufficient evidence for alternative conservative treatments such as drug holidays, Teriparatide, PRGF and hyperbaric oxygen. This systematic review was robust in its appraisal of the selected studies, and although the studies were deemed medium to high risk of bias using the Cochrane Risk of Bias Tool, they were all prospective studies. The review also highlighted that follow up periods

were generally too short, which makes it difficult to assess the outcome of an intervention, and the potential for recurrence at that site.

A study by Vescovi *et al.,* (2014) compared non-surgical treatment with conservative surgical debridement (n=181).[86] Non-surgical management resulted in 12.5% Stage 1 and 25% Stage 2 disease sites healing. Conservative surgical debridement resulted in 93% Stage 1, and 76% Stage 2 disease sites healing. This revealed that irrespective of disease Stage, surgical outcomes are superior to non-surgical management.

2.8.1 Antibiotics for non-surgical medical management

A systematic review by Bermudez-Bejarano, *et al.*, (2017) systematically reviewed the different antibiotic protocols used for conservative treatment of MRONJ induced by oral antiresorptives and IV bisphosphonates separately.[87] They concluded there was no consensus on which was the most appropriate antibiotic, at what dosage, and for which total treatment time. The antibiotics mostly used for patients taking oral or intravenous bisphosphonates included; Penicillin, Amoxicillin, Co-amoxiclav, Metronidazole, and/ or a combination of both. Other combinations included Penicillin G and IV Metronidazole, Levofloxacin and Metronidazole, Piperacillin and Tazobactam, or Imipenem and Cilastatin. Clindamycin is usually prescribed in the event of a Penicillin allergy. There is no consensus for total treatment time, with treatment times varying from 7 days, 10 days, 15 days, 3-4 weeks, or until the site had fully healed. Furthermore, there was no consensus on whether antibiotics should be administered orally or parenterally. Sparse clinical data and lack of randomised trials make it impossible to identify the most suitable protocol.[87] . The AAOMS identify Penicillin as the first line antibiotic based on microbial sensitivity tests, and for those with Penicillin allergy; quinolones, Metronidazole, Clindamycin, Doxycycline and Erythromycin have been used with success. [1] However, they do not identify doses and temporal patterns. Heterogeneity in prescribing for medical management of MRONJ make it impossible to draw conclusions regarding the most successful protocol.

2.8.2 The role of Chlorhexidine as a topical antimicrobial

Chlorhexidine was first used in medicine in 1953, and is a broad spectrum bisbiguanide antiseptic.[88] Loe and Schiott (1970) showed Chlorhexidine's effect on bacterial biofilms is dose dependant, and the optimum daily dose is 20mg twice daily, which was the equivalent to 10mls of 0.12% Chlorhexidine mouthwash.[89] Research showed rinsing for 30 seconds was acceptable, although 60-second regimes were also advocated.[90] The positively charged cationic Chlorhexidine binds to negatively charged bacterial cell wall affecting the osmotic equilibrium of the microbial cell. The effects are dose dependent, at low concentrations it has a bacteriostatic effect, and at higher concentrations it is bactericidal.[88] Chlorhexidine digluconate is cytotoxic against a wide host of microorganisms including gram-positive and gram-negative bacteria (aerobes and anaerobes) and fungi.[88] An *in-vitro* study by McBain *et al.*, (2003) looked at the vitality and susceptibility of oral bacteria to Chlorhexidine. They found a significant reduction of total anaerobes and aerobe counts ($P<0.05$), with fewer reduction in gram-negative anaerobes. Chlorhexidine caused a marked decrease in Prevotella and Selenomonas, while the inhibition Streptococcus and Actinomyces varied greatly between individuals.[91] It has been accepted that bacteria have a role in the pathogenesis and progression of MRONJ, and topical antiseptic mouthwashes form an important part in the management of MRONJ to reduce secondary infection, help to arrest disease progression. Overzealous mechanical removal of biofilms at disease sites with toothbrushes is generally not advised as it can traumatise the mucosa further, and so chemical removal is beneficial to MRONJ cases.

2.8.3 Hyperbaric oxygen

The literature to support hyperbaric oxygen as a treatment option mostly consists of case reports. The therapeutic benefits reported include antimicrobial action, enhanced angiogenesis, mobilising stem cell migration, and supporting stages crucial to wound healing. It may also increase the partial pressure of oxygen in the tissues, resulting in improve oxygenation of tissues, and stimulate osteoclast differentiation

which is important for healing, and normal bone metabolism.[92] A study by Freiberger *et al,* (2012), was the first prospective, non-blinded RCT analysing treatment outcomes for MRONJ, by comparing medical management with adjunctive HBO, with medical management alo.ne.[93] Forty-six patients were included in the study; 25 in the test group and 21 in the control. Complete healing occurred in 14 of 25 HBO-treated patients (52%) versus 7 of 21 controls (33.3%). The HBO group also had reduction in pain especially in advanced stages of disease. They concluded that HBO appeared to be a useful adjunct to medical management alone. Due to the small sample size, there was no statistical difference between the control and intervention group, and a Cochrane review in (2017) found the level of evidence to be low, concluding there was insufficient evidence to claim or refute the therapeutics benefits of HBO after reviewing this study.[10, 93] Rupel *et al.*, (2014) concluded its value as an adjunct to surgical debridement is uncertain, and further well designed prospective studies are needed.[20]

2.8.4 Drug holiday

The benefit of interrupting antiresorptive medications, also known as a "drug holiday" is unclear and the theoretical benefits are based on the pharmacodynamics of medications rather than evidence based.[17] Bisphosphonates are incorporated into the bone matrix, particularly with prolonged treatment, and high potency IV bisphosphonates treatment; hence these patients are unlikely to benefit from a short-term interruption of the medication. Furthermore, the benefit of continuing antiresoptives for oncological reasons, are likely to outweigh those for interrupting the medication for the purpose of treating MRONJ, and this decision should only be made by the patient's oncologist. However, if a long-term interruption is feasible, it may help to stabilise existing lesions, and reduce the risk of new disease sites.[6] Patients on oral bisphosphonates may benefit clinically from a drug holiday, and discontinuation for 6-12 months may result in spontaneous sequestration, or resolution following surgical debridement.[6] A systematic review by El-Rabbany *et al.*, (2017) examined the results of three prospective cohort studies investigating the

benefits of a drug holiday, and concluded the effects of a bisphosphonate holiday are inconclusive.[17] One study reported cessation of bisphosphonates did not influence resolution, another showed a significant higher rate of improvement with bisphosphonate cessation prior to surgery, whereas the third study showed no benefit of cessation prior to surgery. Mixed results, missing relevant data, and methodological heterogeneity in the studies precluded quantitative data analysis. Interestingly, RANK ligand inhibitors do not bind to bone, and their antiresorptive effects are reportedly diminished within 6 months of ceasing treatment, however the benefit of a drug holiday in the management of DRONJ is unknown.[1, 25]

2.8.5 Pentoxifylline and α-tocopherol (PENTO)

Pentoxifylline improves blood perfusion of tissues by reducing blood viscosity and improving erythrocyte flexibility, microcirculatory flow, and tissue oxygen concentrations.[11] It also has an anti-tumour necrosis factor effect, which reduces the inflammatory response, and decreases tissue fibrosis. Tocopherol is an antioxidant which may influence platelet aggregation, and reduce tissue fibrosis.[11] The use of PENTO in the management of osteoradionecrosis was pioneered by Delanian *et al.*, (1999), however she also advocated its use at an ONJ symposium in London in 2017.[94] There are only case series to support the use of PENTO in the management of MRONJ. The largest case series included seven patients treated with PENTO for a mean duration of 16.8 months. All patients experienced a reduction in symptoms, radiographically there was evidence of new bone formation, and 3 out of 9 lesions fully resolved.[95] The Cochrane review in 2017 cited PENTO as a treatment that needs further well designed studies to investigate its therapeutic benefit as a treatment, or as an adjunct to other treatment.[10]

2.8.6 Low level laser therapy

Low level laser therapy (LLLT) is a technique used as an adjunct to conservative management, or surgical debridement. The materials used in laser therapy include; Er:YAG, Nd:YAG, ErCrYSGG and Nd:YAP. LLLT is thought to have a bio-stimulant effect, which improves the healing process, increases the inorganic content of bone matrix, increases osteoblastic differentiation, and increases neoangiogenesis.[96] This is achieved by reducing pro-inflammatory cytokines and increasing anti-inflammatory growth factors, and cytokines.[11] A study by Vescovi *et al.*, (2012) which was the largest study assessing the therapeutic benefits of lasers reviewed the clinical outcomes of 139 MRONJ sites that were treated with various techniques in a single centre.[96] Group 1 received medical therapy only (n=28), Group 2 medical therapy and LLLT (n=32), Group 3 medical therapy and conventional surgery (n=17), Group 4 medical therapy, LLLT and conventional surgery (n=33) and Group 5 medical therapy, Er:YAG laser surgery and LLLT (n=29). Complete healing was observed in 17.8% of Group 1, 28% of Group 2, 64% of Group 3, 74% of Group 4, and 89.6% of Group 5.[96] In terms of complete resolution, it would appear that high powered laser assisted treatment, and surgical debridement may improve overall outcomes. However, LLLT alone resulted in poor outcomes (28%) when compared to the outcomes of medical management alone as reported by Rupel *et al.*, (2014) at 36% curation.[20, 96] A review by Cochrane (2017), advocated further research in the future to ascertain their therapeutic benefits of lasers.[10]

2.8.7 Teriparatide

Teriparatide is an osteoanabolic drug which has been approved for the treatment of postmenopausal osteoporosis, male osteoporosis, glucocorticoid-induced osteoporosis, and off label for fracture healing, dental stability, and MRONJ.[10] The osteoanabolic properties are greatest in the first 9 months, and following this initial period, osteoclastic action also begins.[92] Teriparatide also promotes bone mineral density, strength, and bone healing. These therapeutic properties have been shown

to help bone regeneration in intraoral bone defects.[92] More recently several case reports have used Teriparatide successfully as an adjunct, or as a definitive treatment for MRONJ.[92, 97] A prospective study by Pelaz *et al.*, (2014) which compared Teriparatide (n=4) versus sequestration and application of PRGF (n=5) for the management of MRONJ.[98] All of the PRGF group healed, whereas only one healed in the Teriparatide arm. This observation was not statistically significant; however, the poor sample size may account for this. A literature review by Spanou *et al.*, (2015), revealed there may be some evidence to support the use of Teriparatide in MRONJ cases, however the evidence is based on small case series.[92] Cochrane (2017) reported more prospective RCT need to further investigate the therapeutic benefits of Teriparatide before any conclusions can be reached.[10] Teriparatide is generally contraindicated in patients with a history of malignancy, or previous radiotherapy due to the increased risk of osteosarcoma. A rat study revealed 45% of the animals developed osteosarcoma at the highest tested dose level of Teriparatide, and subsequently a "black box" warning was issued by the FDA.[99] There have been a small number of cases arising in humans on Teriparatide, and for this reason it is not suitable for patients with a previous malignancy, active bone metastases, or skeletal radiation.[2] Despite the high cost of the medication, Teriparatide is often prescribed to patients with osteoporosis in the first instance in Ireland, which may inadvertently help to prevent MRONJ developing in the first place. It may also benefit patients with established MRONJ, as they can continue treatment of their osteoporosis if their antiresorptive medication is interrupted.

2.9 Surgical Management

Initial treatment recommendations have favoured conservative management, and discouraged surgical treatment until disease progression has resulted in mobile sequestrum formation or in advanced Stage 3 disease.[1, 2] The philosophical rationale for managing MRONJ with surgery is based on a basic surgical algorithm, which requires surgical debridement to remove necrotic tissue as it cannot be resurrected,

otherwise it will persist as a chronic non-healing wound.[100] More recently clinicians are increasingly opting for early surgical intervention, and demonstrating successful outcomes.[9 , 81 , 101-103]

Surgery can be divided into:

1. Conservative surgery:

   A conservative approach includes sequestrectomy and or superficial surgical debridement/ ostectomy with removal of all sharp edges. Full thickness mucoperiosteal flaps are raised conservatively. This procedure may not always result in complete removal of all necrotic bone or in primary closure at the site.

2. Extensive surgery:

   Extensive surgical protocols include marginal resections, decortication, extensive saucerisation or segmental resection of all necrotic jaw until fresh bleeding bone is reached. It also aims to round off all bony edges which reduces dead space and enables primary closure with the periosteum in close association with the bone.[18 , 20]

Reviewing the literature is confusing as it is not always clear from the methodology whether it was conservative debridement or extensive surgery. In addition, the surgical procedure and treatment protocols vary widely among studies, and thus a direct comparison of the clinical efficacy among these procedures is difficult.

2.9.1 Bone removal techniques

Methods of necrotic bone removal include conventional rotary surgical drills, saws, piezo surgery, or lasers. Surgical drills, and saws are conventionally used due to access of equipment, and speed of bone removal. Provided there is ample water coolant to avoid iatrogenic overheating of the bone, this method has been used with success.

Piezo surgery is an atraumatic method of targeted bone cutting by means of ultrasonic vibrations at a frequency (25-29kHz). It provides good irrigation for good visibility,

spares soft tissue damage of adjacent structures, and the water-cooling feature has a micro-cavitation effect which bursts bacterial cell walls, resulting in their destruction.[104] Evidence to support the use of piezo surgery in MRONJ cases is limited to small case series, and bone removal is slow, and time consuming.

High power lasers may be used for extensive surgical debridement. A beam of light generated by laser or light-emitting diodes, is focused on the MRONJ site and delivers energy to vaporise necrotic bone until healthy bone is reached.[11] The laser penetrates the hard tissue for 0.1mm providing a reliable safety margin. Sectioned bone surfaces are left regular and micro-perforations can be made to improve neovascularisation. Furthermore, the laser is bactericidal. A systematic review by Rupel *et al.,* (2014) reported curative rates of 84% with extensive surgery, and 85% with laser assisted surgery.[20] However, these studies have small sample numbers, therefore further robust research is required to evaluate the benefits of one technique over another.

2.9.2 Delineating necrotic bone from viable bone

The primary goal of treating MRONJ with surgery is to remove as much bone as necessary, and as little as possible.[16] The delineation of necrotic bone from viable bone is key to the success of the intervention. This is largely decided by the surgeon's subjective impression, with the removal of necrotic, grey sclerotic bone down to bleeding viable bone margins. Other methods include tetracycline fluorescence guided bone removal, and the VELscope system which uses auto-fluorescence to identify viable bone. In a randomised controlled trial by Ristow *et al.*, (2017) they compared auto-fluorescence with tetracycline fluorescence guided bone removal in 40 cases of MRONJ.[105] Twenty patients were randomly assigned to each group. There was no significant difference between the two groups with 18/20 in the auto-fluorescence group and 17/20 in the tetracycline fluorescence group healed at 8 weeks. This is one of the few randomized controlled trials looking at treatment of MRONJ, however it would have been beneficial to have a control group using the surgeon's clinical judgement only. Furthermore, the small sample size, may have contributed to a lack of measurable effect. The Cochrane review in 2017 deemed the

quality of evidence as low, and there was insufficient evidence to support one treatment over the other at present.[10]

2.9.3 Factors which affect outcome of surgical intervention

2.9.3.1 Extensive surgery Vs Conservative surgical debridement

Wilde *et al.,* (2011) was one of the earlier pioneers of extensive surgical intervention. He proposed full thickness mucoperiosteal flaps should be raised and extended beyond necrotic bone to disease-free margins. The resection should extend horizontally, and inferiorly until disease free bleeding bone is reached, sharp edges smoothed, and primary soft tissue closure achieved. Using this technique, success rates of 88% were achieved, with a median follow-up of 60 weeks irrespective of interruption of bisphosphonate treatment.[101] Improved outcomes have been reported with extensive resections when compared with conservative management and limited conservative debridement.[2] A well-designed prospective study by Mucke *et al.*, (2011) looked at the outcome of treatments, and which parameters influenced recurrence of MRONJ in 108 patients. They reported significant factors that influenced recurrence were the method of treatment; with a higher recurrence rate with a non-surgical conservative approach (p=0.001), and larger resections associated with fewer recurrences (p<0.0001).[106] Improved outcomes were also observed by Graziani *et al.*, (2012) when extensive surgery (68%) was compared with conservative surgical debridement (49%).[107]

More recently a well-designed study by Pichardo *et al.,* (2016) published the results of 74 Stage II/III bisphosphonate related cases of MRONJ, which were treated with extensive surgery based on a surgical protocol for osteomyelitis.[18, 108] The patients were followed-up for 6-96 months. They reported curation in 93.2% of the cases. Of the 69 patients who showed resolution, 21 required further treatment prior to complete resolution. Six required further systemic antibiotics, and 13 patients required a minor second surgery, of which some were completed under GA

(sequestrectomy/ curettage). Furthermore, the author also published the results of a study where 11 patients with Denosumab-related osteonecrosis of the jaws were managed with extensive surgery. Curation was achieved in nine of the eleven cases, and the other two patients died from their underlying illness.[8] However, the authors did not discuss the interval from the last dose of Denosumab to the time of surgery. This is relevant as Denosumab is not incorporated into the bone, and hence the effects have been reported as negligible after a 6-month drug holiday.[1] It would appear that DRONJ responds well to surgery also, and future studies should examine the timing of the intervention with the aim of optimising the timing of surgery taking the pharmacodynamics of Denosumab into account.

A systematic review by Rupel *et al.,* (2014) reported 78% of MRONJ cases (516 of 658 cases) healed with a surgical intervention.[20] Table 12, presents the results of this study's systematic review, with extensive surgery (84%), and laser surgery (85%) providing better outcomes than conservative surgery (75%). Heterogeneity across the included studies did not permit meta-analysis, and this was one of the main limitations of this systematic review.

	Number of patients healed/Number of patients treated	**Outcome of intervention (%)**
Conservative surgery	298/400	75%
Extensive surgery	172/204	84%
Laser surgery	46/54	85%

Table 12 Outcomes of different surgical protocols treating MRONJ

Fliefel *et al.,* (2015) systematically reviewed the outcomes of different treatment interventions. He reported poorer outcomes for minimally invasive surgery with 301 of 776 (39.2%) completely healing, compared to 207 of 252 (82.1%) which were treated with major surgical treatment.[81] Furthermore, the systematic review by El-Rabbany *et al.*, (2017) reported extensive surgery achieved complete resolution quicker than conservative surgery ($p<0.001$).[17] Superior results with extensive surgery

was presumably due to removal of all the necrotic bone, which cannot be resurrected, and extensive resections may enable the surgeon to get primary closure with more ease and less tension.

However, a study by Nisi *et al.*, (2018) achieved 91.8% healing using a conservative surgical approach (n=53).[109] The patients were followed up for two years. All the patients were osteoporotic patients treated with oral bisphosphonates. This may indicate that oral bisphosphonate induced MRONJ responds better to conservative surgery than oncology patients.

Most studies consistently report superior curative rates for surgery than those attained with non-surgical medical management (36%) (Table 13).[20]

Author	**Study design**	**Medication**	**Surgery Type**	**Healed**	**Follow up (months)**
Pichardo *et al.,* (2016)[18]	Cohort study n=74	Oral & IV Bp	Extensive	93.2%	6-96
Pichardo *et al.,* (2016) [8]	Cohort study n=11	Denosumab Prolia n=4 Xgeva n=7	Extensive	81%	6
Carlson & Basile, (2009) [110]	Cohort study n=74	Oral & IV Bp	Extensive	91.6% Maxilla 100%	12
Stockmann *et al.,* (2010)[111]	Cohort study n=50	IV Bp	Extensive	89%	12
Bedogni *et al.*, (2011) [19]	Cohort study n=30	IV Bp	Extensive	90.6%	24
Schubert *et al.*, (2012) [112]	Cohort study n=50	Oral & IV Bp	Extensive	86.5%	Minimum 3 months
Jacobsen *et al.*, (2012)[113]	Cohort study n=64	Oral & IV Bp	Conservative	78%	Unknown

Graziani *et al.*, (2012) [107]	Comparative study n=347	Oral & IV Bp	Conservative n=227 Extensive n= 120	49% 68%	12
Nisi *et al.*, (2018)[109]	Retrospective case series n=53	Oral Bp	Conservative	91.8%	24
Vescovi *et al.*, (2014)[86]	Retrospective case series n=181	Oral & IV Bp	Conservative (laser/drills)	Stage 1: 93% Stage 2: 76%	Minimum 6 months

Table 13: Outcomes for surgical management of MRONJ

It would appear treating MRONJ with a more aggressive surgical protocol produces more consistent, predictable outcomes. In many of the studies patients were reportedly lost to follow-up, due to mortality from their underlying oncological illness, and therefore it may be that the success of surgical intervention was in fact underestimated. However; extensive surgery was more often associated with requiring a general anaesthetic, extended in-patient stays, bone defects that may be difficult to restore patients with functional prosthetic dentures, and the usual complications that arise with larger surgical procedures which may include sensory nerve deficits, scarring, and jaw fracture.

2.9.3.2 Stage of disease

Previously surgery was only indicated for advanced Stage 3 disease, and refractory cases, and this may have been in part due to the ability to palliate symptoms of most Stage 1 and 2 disease, with topical, and systemic antimicrobial agents. However, there is evidence to support early intervention in less advanced stages of disease. A retrospective study by Vescovi *et al.*, (2014) reported 93% curation in 25 Stage 1, and 76% Stage 2 disease sites which were treated with conservative surgical debridement.[86] These cases were refractory to 6 months of non-surgical conservative

treatment (topical and systemic antimicrobials +/- hyperbaric oxygen, ozone therapy and low level laser therapy). This study was well designed, and its methodology was clear and reproducible. It also highlighted surgical intervention was not specifically reserved for Stage 3 disease only, as previously advocated. The systematic review previously discussed, by Rupel *et al.*, (2014) looked at outcomes of treatment relating to different Stages of disease as classified by the AAOMS.[20] The outcomes for a conservative non-surgical approach markedly diminish with increasing Stage, however the outcomes for extensive, and laser surgery remained consistently effective in advanced stages (Table 14).[20] Stage 1 and 2 disease respond well to conservative surgical measures, but this markedly reduced when conservative surgery was used to treat Stage 3 disease, this may be due to difficulties achieving primary closure, or large segments of necrotic bone involved that cannot be resurrected.

Stage	**Non-surgical approach**	**Conservative Surgery**	**Extensive surgery**	**Laser surgery**
0			89% n=8/9	
1	33% n=5/15	72% n=26/36	87% n=27/31	100% n=17/17
2	24% n=13/55	79% n=149/189	96% n=114/119	83% n=29/35
3	0% n=0/7	27% n=3/11	81% n=50/62	100% n=4/4

Table 14: Outcomes of different surgical protocols depending on Stage of disease[20]

2.9.3.3 Drug history, site and underlying disease as factors

A study by Carlson and Basile (2009) treated 95 sites of MRONJ with extensive surgery. Of the 27 sites treated in the oral bisphosphonates group 26 fully healed (96.3%).[110] In the IV bisphosphonate group 61 of the 68 sites healed (89.7%). Furthermore, all maxillary resections irrespective of oral or IV bisphosphonate history healed. Of the

eight refractory cases which occurred in the mandible, one of the patients was taking oral bisphosphates and the other 7 were on IV bisphosphonates. This study indicated that maxillary resections may have better outcomes than mandibular, and patients on oral bisphosphonates are more likely to heal than patients who are on IV bisphosphonates. Similar findings were presented by Nisi *et al.*, (2018) who observed 91.8% curation (n=53) when oral bisphosphonate induced necrosis of the jaws was treated with conservative surgery with a 2 year follow up.[109] It would appear surgical treatment for oral bisphosphonate induced disease was more predictable than the outcomes in oncology patients.

Wutzl *et al.,* (2012) reported surgical success rates are better in patients with multiple myeloma, and osteoporosis, than in those with solid tumours.[114] This observation may be explained by a dose related factor for osteoporosis, but multiple myeloma, and solid tumours usually have similar antiresorptive dose regimes. This was a small retrospective study (n=41), and heterogeneity within the patient sample may have introduced bias. Larger sample sizes are required to investigate the relevance of the type of cancer on treatment outcomes.

A prospective cohort study by Lee *et al.*, (2014) looked at other patient risk factors that may affect healing outcomes. They reported smoking and systemic steroids may increase the overall time for complete resolution of MRONJ lesions with conservative and surgical interventions.[55]

2.9.4 Evidence for using L-PRF as an adjuvant to surgery

The principle of using autologous platelet concentrates as an adjunct to surgery, is to accelerate soft tissue, and bone healing.[115] It is generally accepted that extensive surgery yields superior outcomes than conservative debridement, however it is associated with higher surgical morbidity. The rational of applying L-PRF is to improve surgical outcomes with conservative surgery, while reducing the surgical morbidity associated with extensive surgery. A literature search of PubMed in October 2017 yielded five studies using specifically L-PRF in the management of MRONJ, of which

most of these studies use an extensive surgical protocol (Table 15). When the results of the larger studies >15 patients were considered, extensive surgical management with L-PRF, as an adjuvant treatment, had a curative rate of 83.7% in 74 patients.[22-24] The largest study was a prospective single-group cohort study by Kim *et al., (*2014) who treated a total of 34 patients with MRONJ using L-PRF and monitored healing clinically. Twenty-six patients showed complete resolution at 1 month, 6 had delayed healing with resolution at 4 months and 2 patients failed to resolve at 4 months. There was only one study which used a conservative surgical debridement protocol, of which the two cases included failed to heal.[116] It is difficult to draw conclusions from these studies alone due to the study design, small patient samples, different surgical protocols, and operator variables. In addition, there are many different platelet therapy systems with different protocols, and literature reviews often do not differentiate between the systems despite purporting unique biological healing properties. A systematic review by Fliefel *et al.,* (2015) reported on the use of cPRP and BMP2 growth factors in 92 patients. They reported 81.5% completely healed, 2.2% showed partial healing, 6.5% had stable lesions, 8.7% had regressive lesions, and 1.1% had a recurrent lesion.[81] A systematic review by Del Fabbro *et al.,* (2015) reported platelet concentrates (1st and 2nd generation) are effective at treating MRONJ. They reported 91.6% of 143 patients treated with surgery and platelet concentrate had complete healing.[115] A prospective study by Mozzati *et al.,* (2012) looked at 176 patients on IV bisphosphonates who required a dental extraction. The test group (n=91) had PRGF placed in the socket. Five cases of MRONJ developed, all of which were in the control group (n=85).[117] This would infer platelet concentrates improve post-operative healing, and may have a role in prevention of MRONJ also. The Cochrane review in 2017, advised further research into “add-on” adjuvant treatments such as platelet derived growth factors as a topic for future research questions, but at present there is insufficient evidence to advocate or refute the therapeutic benefits.[10]

Author	Study Design	Treatment	Antiresorptive medication	Sample size (n)	Healed
Kim *et al.,* (2014)[22]	Cohort study	Extensive surgery & L-PRF	Oral & IV Bp	34	76.4%
Soydan and Uckan (2014)[118]	Case report	Unspecified & LPRF	IV Bp	1	100%
Maluf *et al.,* (2016)[116]	Case report	Conservative surgery & L-PRF	Deno	2	0%
Norholt *et al.,* (2016)[24]	Cohort study	Extensive surgery & L-PRF	IV Bp/ Deno	15	93.3%
Park *et al.,* (2017)[23]	Cohort study	Extensive surgery & L-PRF	Oral & IV Bp	25	88%

Table 15: Outcomes for surgery with adjunctive L-PRF

2.10 Platelet concentrates

Platelet concentrates are bioactive surgical additives which are used to optimise wound healing. They are prepared from a whole blood sample, mostly through the process of centrifugation, which separates the blood elements, which can be applied therapeutically.[119] Platelet concentrates have been used in various medical fields; including oral and maxillofacial surgery, refractory leg ulcers, orthopaedics and sports medicine to stimulate, improve, and accelerate the healing process.[81, 119, 120] The concept of manipulating wound healing, using a regenerative medical preparation, was first introduced by Matras in 1970, using a fibrin glue to improve dermal wound healing in a rat model.[119] The concept behind this was that fibrin formed the first matrix of the healing process. Fibrin glues are still being used as surgical adjuvants for haemostasis. Between 1975-1978, Rosenthal *et al.,* recognised that platelets are critical in various stages of the wound healing process and looked to harness these supportive biological properties. He combined platelets, with the concept of a conventional fibrin glue, resulting in an upgraded product called a “gel foam”. These

were the first platelet-rich plasma concentrates (cPRP) in the sense that we are familiar with nowadays.[119] The technique of using cPRP in oral and maxillofacial surgery was introduced later by Whitman and Marx in the 1990s, and the concept gained momentum and evolved over time.[21] There are now more than 10 systems available with varying in-house protocols, centrifugation, and separation techniques.[121] All of these products were termed "first generation" cPRPs. In 2001, Choukroun developed a new type of platelet concentrate, platelet rich fibrin (PRF) for the specific use in oral and maxillofacial surgery. It had a very different biological and structural profile from the conventional cPRPs, and was therefore termed a "second generation" platelet concentrate.[121] The PRF technique trapped leucocytes and platelets within a polymerised fibrin matrix forming a strongly activated gel form. The science within these concentrates appears to lie within the cellular content, architecture, and release of growth factors, although the need for more consideration into their structure was advocated.[119] This debate over the importance of composition led to a classification system for platelet concentrates by Dohan Ehrenfest in 2009.[119] The two other main classification systems were the PAW (platelet, activation and white cells) and a system by Mishra *et al.*, (2012) which was limited and only covered the PRP families.[119] The classification by Ehrenfest encompassed first and 2nd generation concentrates, and was based on two important parameters; cellular content (particularly leucocytes) and fibrin architecture (Table 16).[119]

Platelet concentrate	Composition	Form	Additives	Generation
Pure platelet-rich plasma (P-PRP) (commercially known as PRGF)	Rich in platelets. Lacks leucocytes. Low-density fibrin network.	Liquid / activated gel	Bovine thrombin & calcium chloride	1st generation platelet concentrates
Leucocyte- and platelet-rich plasma (L-PRP)	Rich in platelets. Leucocytes present. Low-density fibrin network.	Liquid / activated gel	Bovine thrombin & calcium chloride	1st generation platelet concentrates
Pure platelet-rich fibrin (P-PRF)	Rich in platelets. Lacks leucocytes. High-density fibrin network.	Activated gel	Trisodium citrate	2nd generation platelet concentrate- debatable due to addition of trisodium citrate required
Leucocyte and platelet rich fibrin (L-PRF)	Rich in platelets. Leucocytes present. High-density fibrin network.	Activated gel	None	2nd generation platelet concentrates

Table 16: Classification of platelet concentrates[119]

2.10.1 How are platelet concentrates prepared?

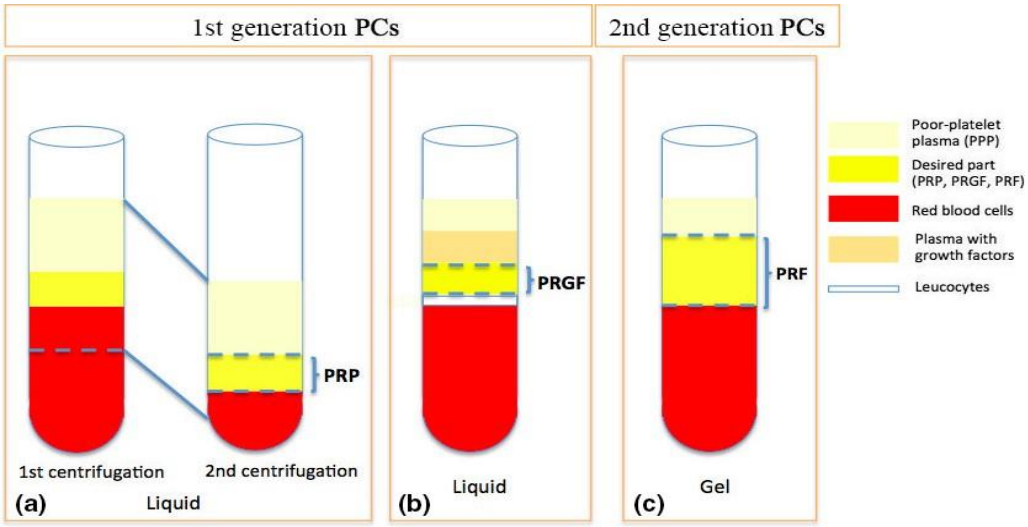


Figure 8: Preparation protocols for cPRP, PRGF and PRF. [122]

There are different preparation techniques for cPRP, PRGF, both 1st generation types of concentrate, and PRF, which is a 2nd generation concentrate (Figure 8). Figure 8(a) describes platelet-rich plasma (cPRP) preparation: after the initial centrifugation, the platelet-poor plasma, the "yellow" part called buffy coat and a few red blood cells are carefully collected (pipetting) and centrifuged again in order to obtain the PRP. Figure 8(b) describes PRGF preparation: following centrifugation, the blood is divided in five layers; undesirable layers are removed by pipette and discarded; and the most concentrated part with growth factors (PRGF) is collected. Figure 8(c) outlines PRF preparation: following centrifugation, the fibrin clot is in the middle of the tube, which is ready to be used.[122]

2.10.2 cPRP Vs L-PRF

First generation platelet concentrates have gone out of vogue recently.[123] The processing is time consuming, and involves a two-stage technique sensitive process with large cumbersome equipment. This is in comparison to a simplified cost-effective process of producing L-PRF; which requires no biochemical handling and is simply the patient's own blood.

One of the main differences between cPRP and L-PRF is the gelling mode.[21] Preparation of cPRP requires the addition of thrombin, and anticoagulant, which results in a rapid polymerisation of fibrin, whereas L-PRF requires no additives, and therefore occurs in a slower process resembling physiologic conditions. The addition of bovine thrombin also carries a very small risk of disease transmission and there is evidence in the literature to suggest bovine thrombin has toxic effects on cells of the body.[123] The polymerisation process has a large impact on the biological, and mechanical properties of the fibrin matrix.[21] The L-PRF fibrin matrix resembles that which occurs physiologically, and facilitates cell migration, and proliferation, optimising wound healing, while the slow polymerisation enhances cytokine, and cell trapping within the fibrin network, which results in functional cells, with enhanced and sustained growth factor release. This is in contrast with cPRP, whereby a massive platelet activation results in rapid release of cytokines, which are poorly bound within the fibrin matrix, and therefore growth factor release occurs for a very limited time.[21]

A study by Dohan Ehrenfest *et al.,* (2017) highlighted the impact that different centrifuge characteristics and protocols had on cell viability and growth factor release. He reported that some of the centrifuge systems used to produce cPRP, are associated with high levels of vibrations, which can compromise cell viability, reduce growth factor release, and result in overall small poor-quality clots.[124] Although cPRP and L-PRF contain similar levels of platelets; 95% and 97% respectively, the weak polymerisation within the gel causes cPRP to rapidly dissolve.[125] An *in-vitro* study compared the slow release of growth factors and matrix molecules from L-PRF and pure-platelet rich plasma (P-PRP).[121] Both concentrates were placed in culture medium for one week, whilst measuring the slow release of transforming growth factor-β1 (TGF), platelet-derived growth factor-AB (PDGF) and vascular endothelial growth factor (VEGF), thrombospondin 1, fibronectin and vitronectin at specific time intervals. These were 20 minutes, 1h, 4h, 24h, 72h, 120h, and 168h. The results revealed the L-PRF membrane remained intact and continued to release a large quantity of growth factors over the 7 days, whereas the P-PRP released growth factors for the first few hours and had completely dissolved by day 3. A similar study comparing L-PRF and L-PRP reported L-PRF released growth factors for longer time

with greater concentrations of TGFβ1, and stronger induction of cell migration. Figure 9 presents the kinetics of growth factor release from L-PRP, L-PRF, and blood clot (BC). Release of TGF-ß1, VEGF, IGF-1, PDGF-AB, and IL-1ß from cultured L-PRF, L-PRP, or BC was determined at each time point (8 hours-28 days). [126]

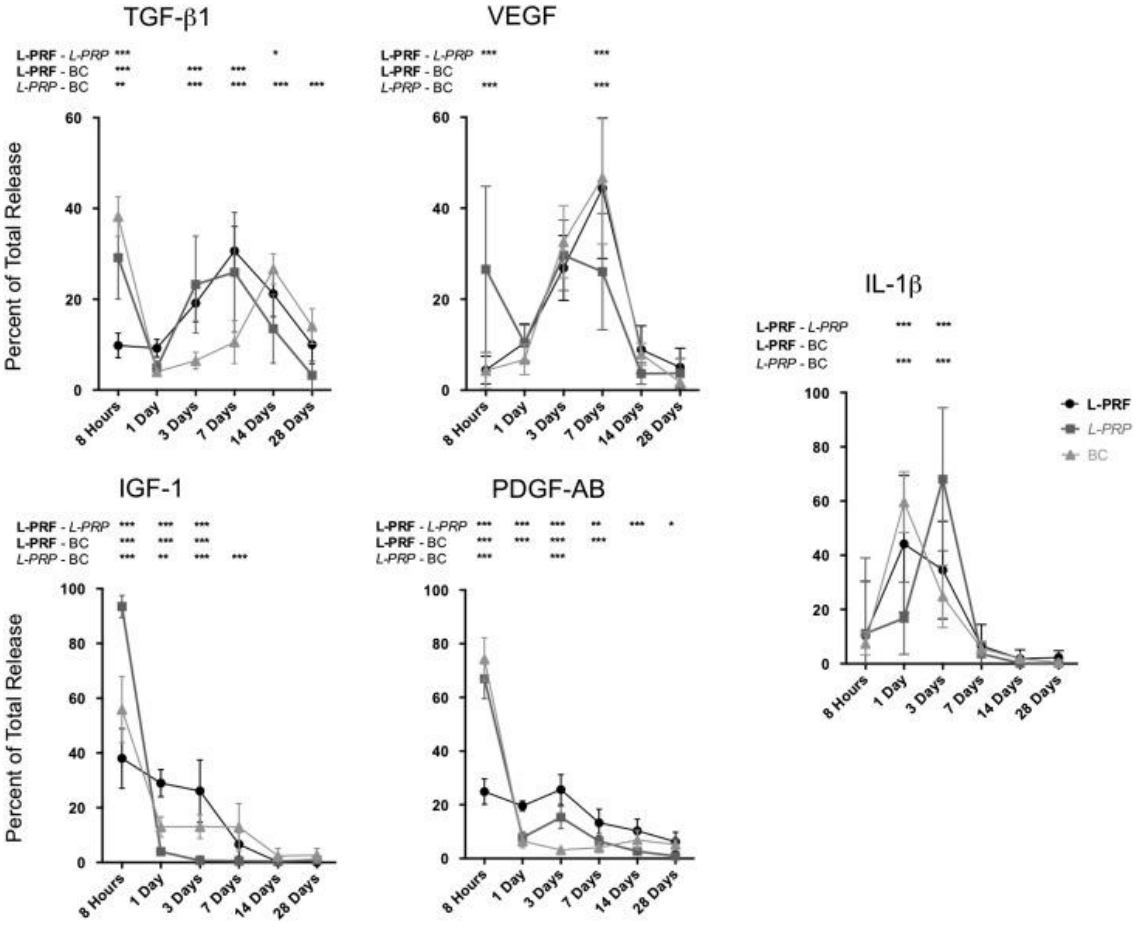


Figure 9: Kinetics of growth factor release from L-PRP, L-PRF, and blood clot.[126]

2.10.3 Evolution of PRF

The initial Choukroun protocol for PRF preparation was developed in 2001, and since then there have been comparable protocols developed; all purporting superior macroscopic features, growth factor release, and healing properties. It has been shown that cells accumulate towards the bottom of the L-PRF clot, and to reduce this phenomenon authors proposed reducing centrifuge times. This may decrease cell-pull

down by reducing centrifugation g-forces.[127] To the contrary Dohan-Ehrenfest *et al.,* (2017) reported centrifugation at too low a level resulted in poor separation of the blood products and the leucocytes were not activated.[124] Table 17 presents the various second generation concentrates and their preparation protocols.

Platelet concentrate	**Developed by**	**Rotations per minute**	**G-force**	**Time (minutes)**
PRF	Choukroun *et al.,* (2001)	3,000	708g	10
L-PRF		2,700	400g	12
A-PRF	Choukroun and Ghanaati *et al.,* (2013)	1,500	208g	14
A-PRF+	Choukroun and Ghanaati *et al.,* (2014)	1,300	60g	8

Table 17: Preparation protocol for 2nd generation platelet concentrates.[128]

A study by Fujioka-Kobayashi *et al.,* (2017) reported increased growth factor release from A-PRF and A-PRF+ compared with conventional L-PRF.[129] However, a study by Dohan Ehrenfest *et al.,* (2014) reported L-PRF had superior growth factor release, leucocyte trapping and larger clots, which did not dissolve prematurely, when compared to A-PRF.[124, 130]

2.10.4 Centrifuge characteristics and the benefits of Intra-Spin Intra-Lock System

The original L-PRF was developed as an unrestricted access protocol, and hence there are various systems using different centrifuges and protocols.[124] Assuming that these systems are all slightly different, so too will the final product. The unique fibrin architecture and cellular content are responsible for the biological signature, and their clinical applications. Specifically, mechanical characteristics during centrifugation such as vibration of the centrifuge during acceleration, and vibration shocks during acceleration can grossly affect the fibrin crosslinks, cellular viability, and overall quality

of the L-PRF.[124] The ideal centrifuge system would in theory have a reasonable speed to enable blood separation, activate the cells, and importantly that no vibration nor resonance damages the cellular content. There are multiple centrifuge systems available, which are being used to prepare L-PRF (some marketed for off label usage). Evidence has shown that the final L-PRF product varies with different machines.[124, 130-132] The Intra-Spin L-PRF system (Intra-Lock, Boca-Raton, FL, USA) is the only system with CE/FDA clearance available on the market and uses original preparation protocols. It is difficult to make an evidence-based decision on which system to use as the companies have not published on the influence their system has on growth factor content, cells and fibrin architecture. To clarify this a 3-part series of papers were published on the impact of the centrifuge characteristics, and centrifugation protocols of four models of commercially available table centrifuges marketed for producing L-PRF.[130-132] The 4 different tested centrifuges were the Intra-Lock system, and 3 other non-FDA/CE approved systems; 1310 Salvin, LW-UPD8, and centrifuge A-PRF12. The first study evaluated the vibrations produced by the 4 different systems. They reported increasing levels of vibrations with increasing rotation speeds for all systems. They also observed significant differences in the level of vibration at each rotation speed for the 4 different systems. The results for vibrations during centrifuge for LW-UPD8, Salvin, and A-PRF were 5.2, 6.3 and 6.8 respectively times higher than the Intra-Lock system in half load configuration.[124, 131] When radial vibrations increase above a certain threshold, there is a risk of resonance occurring within the tubes which can damage the cell content in the blood sample. All the systems except the Intra-Lock system are largely above this threshold, and it was concluded Intra-Lock system was by far the most stable.[124, 131]

In the second paper the authors looked at the macro- and microscopic effect of centrifuge characteristics on the cell and fibrin architecture of the L-PRF clot, and membrane for all 4 systems. Blood samples were collected from 8 healthy volunteers, which were spun to a standardised 2700rpm (400g) centrifuge force, to isolate only the centrifuge vibration parameter. Macroscopically, the Intra-Lock system maintained the lowest temperature during centrifugation, produced the heaviest clots, and the largest amounts of exudate (Table 18). The greatest clots, and

membranes in width and length, were produced by the Intra-Lock and Salvin systems.[124]

Variable	IntraSpin Mean (SD)	A-PRF Mean (SD)	Salvin Mean (SD)	LW Mean (SD)
Final T° of tube (°C)	27.5 (0.66)	28.83 (0.67)	28.8 (0.66)	27.88 (0.57)
Clot weight (g)	2.09 (0.19)	1.38 (0.24)	1.73 (0.27)	0.74 (0.15)
Membrane weight (g)	0.62 (0.15)	0.48 (0.17)	0.6 (0.19)	0.3 (0.25)
Exudate weight (g)	1.47 (0.13)	0.9 (0.21)	1.12 (0.27)	0.44 (0.26)
Clot length (mm)	35.69 (3.43)	26.56 (4.25)	35.25 (4.1)	20.12 (4.29)
Clot width (mm)	12.81 (0.75)	10.93 (1.08)	13.06 (0.94)	9.12 (1.13)
Membrane length (mm)	34.81 (2.95)	26.81 (3.38)	34.43 (2.87)	21.5 (2.39)
Membrane width (mm)	12.25 (0.71)	10.37 (0.92)	11.93 (0.78)	9.12 (0.64)
Weight ratio(%) clot/blood sample 10 ml	20.94 (2.4)	13.98 (2.6)	17.42 (2.63)	7.41 (1.45)

Table 18: Macroscopic features of L-PRF using 4 different centrifuge systems.[124]

Using light and scanning electron microscopy, the membranes were evaluated microscopically. The Intra-Lock system produced superior membranes with robust networks of polymerized fibrin with dense fibrin fibres, and the cells population were all alive and of normal morphology. In contrast were the 3 other systems, which produced PRF-like membranes with a weakly polymerised fibrin gel composed of narrow fibrin fibres, and the cells present were abnormal in shape appearing squashed and smaller. The entire cell population appeared abnormal, and possibly destroyed.[124, 132]

The third article in the series evaluated how changes in the L-PRF protocol (reducing centrifuge speeds) influenced its biological signature, by comparing the growth factor content, and slow release of factors between L-PRF and modified A-PRF independently from the characteristics of the centrifuge.[124] The original L-PRF protocol described blood samples collected in Intra-Spin 9ml glass coated tubes and immediately centrifuged at 2700rpm (400g) for 12 minutes. The A-PRF protocol differs from this by collecting the blood samples in 10ml glass tubes and centrifuging the samples at 1500rpm for 14 minutes. Of note, it has previously been shown that the preparation of L-PRF is not compromised by the use of glass tubes or glass coated plastic tubes.[133] The concentrations of growth factors TGFβ-1, PDGF-AB, VEGF and BMP2 released by the membranes were compared at 7 experimental times; 20 minutes, 1h, 4h, 24h, 72h, 120h, and 168h *in-vitro* using ELISA kits (Figure 10). They concluded the slow release

of TGFβ-1, PDGF-AB and VEGF from the L-PRF membrane was always significantly stronger ($p<0.001$) at each experimental interval than the A-PRF membrane. The L-PRF membrane continued to release growth factors up to day 7 the final examination interval, whereas the A-PRF had completely dissolved in the medium between day 1 and 3. Furthermore, the L-PRF membrane secreted BMP2, whereas the A-PRF membrane did not despite the company marketing it purports it does. Platelets do not produce BMP2, and therefore the living leucocytes in the L-PRF are the source of this growth factor. The A-PRF clots and membrane were at least 30% smaller than the L-PRF, which would indicate 1500rpm forms an inadequate gradient in centrifugation resulting in poor separation of the blood components. As a result, small membranes are produced with weak biologically signatures and a poorly polymerised fibrin network causing the membrane to rapidly dissolve.[124, 130]

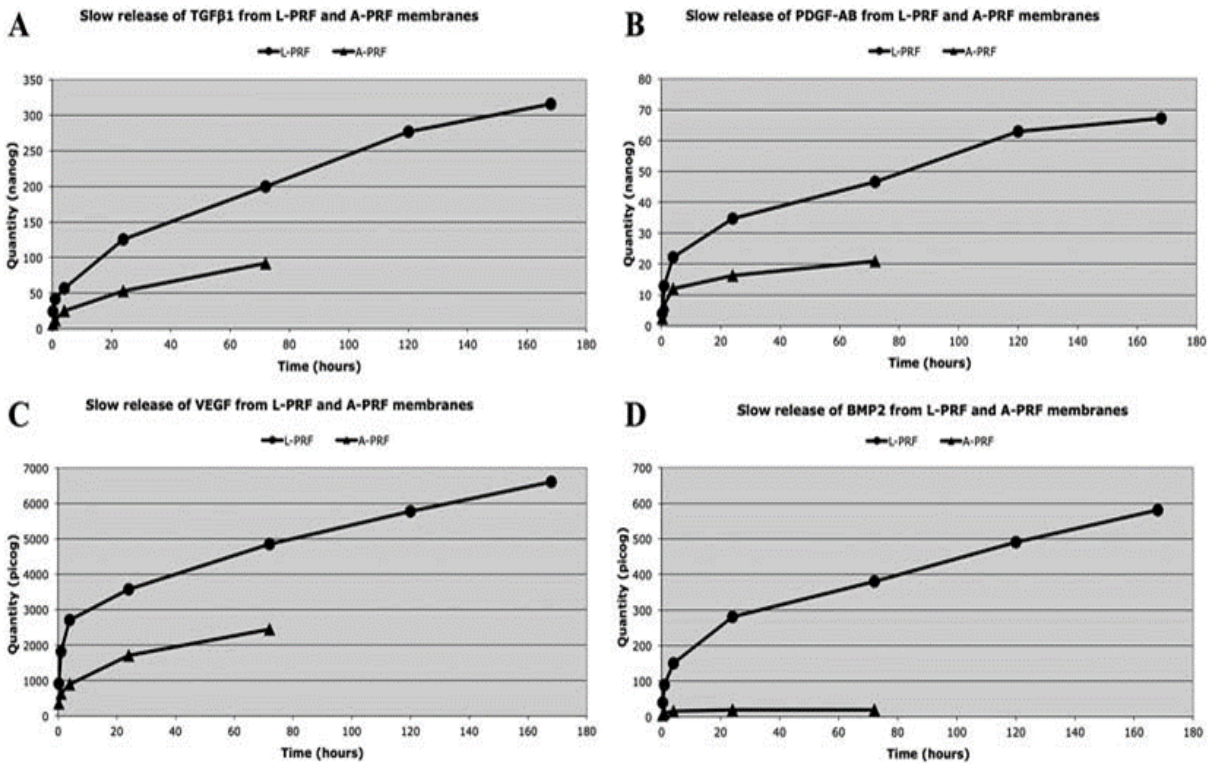


Figure 10: Kinetics of growth factor release; TGFβ1 (A), PDGF-AB (B), VEGF (C) and BMP2 (D) from L-PRF and A-PRF membrane for 7 days *in-vitro*. [124]

Values are expressed as the cumulative mean quantity of molecules over specific time intervals.

2.10.5 L-PRF Cellular Content and mode of action

L-PRF is a naturally derived concentrate of platelets, fibrin, and leucocytes. Photonic microscopy revealed uneven distribution of platelets, and leucocytes, within the clots. A macroscopic (whitish) buffy-coat is present between the L-PRF clot and red blood cells. It is at this point in the first 2mm of the buffy-coat that most of the leucocytes and platelets are concentrated, and beyond half of the fibrin clot none are seen (Figure 11).[133] Therefore when harvesting the L-PRF clot it is important to retain this whitish layer, and hence preserve a small amount of the red blood cell layer to maximise collection of platelets and leucocytes.

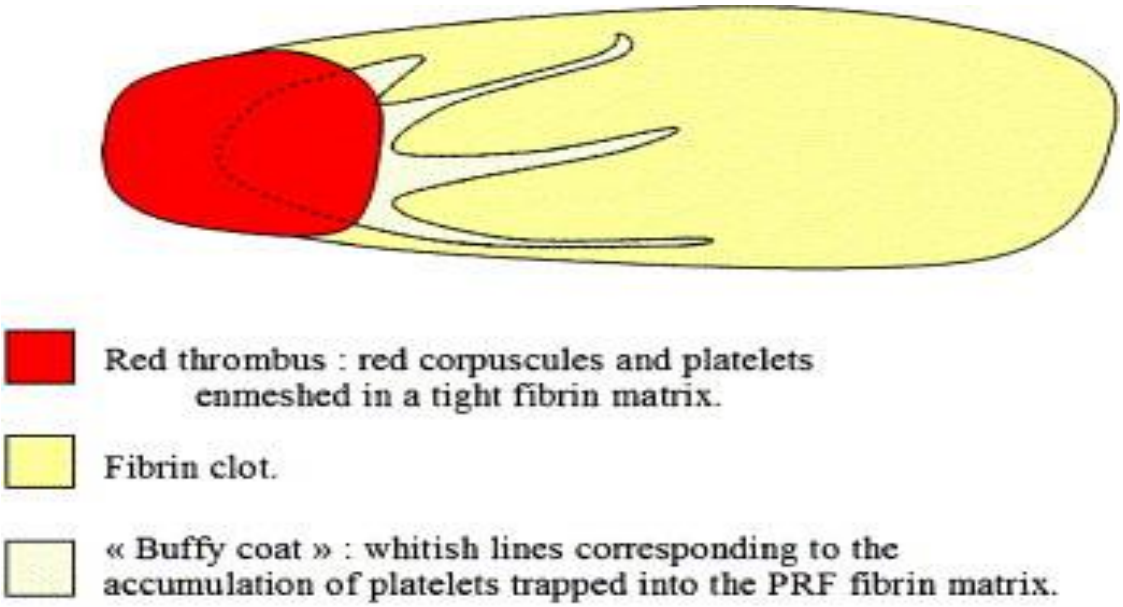


Figure 11: Distribution of cells within L-PRF clot.[134]

At a recent global conference on "Enhanced natural healing with L-PRF" in Leuven, Brussels, a researcher Ana Castro, who has published prolifically on L-PRF presented her interim results on L-PRF cellular content of 9 healthy patients (Table 19).

Cells	**L-PRF exudate**	**L-PRF membrane**
RBC	1.2 +/- 1.0%	40.1 +/- 18%
Platelets	0.9 +/- 0.8%	96.3 +/-3.5%
WBC	2.6 +/-1.5%	74.4 +/- 9.5%
Neu	1.4 +/- 2.1%	64.3 +/-13.1%
Lym	4.4 +/- 4.1%	79 +/- 23.1%
Mono	3.7 +/- 11.1%	87.5 +/- 17.1%
Eos	2.1 +/- 2.5%	33.1 +/-30.9%
Baso	1.1 +/- 2.0%	79.6 +/-25.2%

Table 19: Cellular content of L-PRF

[Cells in membrane] = [initial blood sample]-[remaining+exudate]

Abbreviations: RBC red blood cells; WBC white blood cells; Neu neutrophils; Lym lymphocytes; Mono monocytes; Eos eosinophils; Baso basophils

These results are similar to those of Dohan Ehrenfest *et al.,* (2010) for platelet content; 97%, but the more recent results have a higher leucocyte content 74.4% Vs 50%.[133] This may be due to the previous PRF protocol of 3000rpm for 10 minutes versus a newer widely accepted protocol of slower polymerisation at 2700rpm for 12 minutes resulting in enhanced cell trapping.

2.10.5.1 Fibrin

The slow polymerisation process of fibrinogen to fibrin during centrifugation resembles physiologic settings and is crucial in forming a 3-dimentionsal organised network. The fibrin fibrillae are organised with flexible tri-molecular junctions, compared to the condensed rigid tetra-molecular branch junctions associated with fibrin adhesives and cPRP (Figure 12 and 13).[21] Physiologic levels of thrombin result in

a fine and flexible network of fibrin, which facilitates cytokine enmeshment, and cellular migration. This structure also gives the matrix flexibility and strength, enabling the membranes to be adapted, and even sutured to surgical sites. This process is time sensitive, and quick handling during blood collection avoids diffuse polymerisation of the fibrin resulting in small clots.[21]

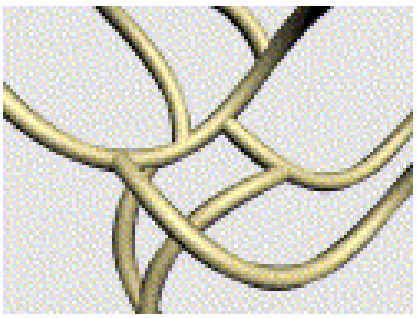


Figure 12: Theoretical modelling of tri-molecular flexible branch junctions in fibrin architecture in L-PRF.[21]

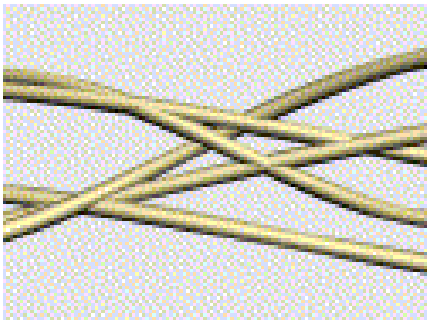


Figure 13: Theoretical modelling of condensed rigid tetra-molecular branch junctions associated with fibrin adhesives and cPRP.[21]

2.10.5.2 Platelets

Platelets are discoidal, anuclear, and are formed from megakaryocytes within bone marrow. They have a circulating lifespan of 8-10 days. They play a fundamental role in the prompt response to injury with thrombosis promoting clot formation, and in the initial stages of wound healing by regulating the proliferative response of mesenchymal cells by stimulating cell migration and proliferation within the fibrin

clot.[134] Platelets contain substantial amounts of granules which are released at the time of activation. The primary inflammatory mediators released are:

- Dense granules
  - Serotonin
  - Calcium
  - ADP
- α-granules
  - Cationic proteins
  - Fibrinogen and coagulation proteins
  - Platelet derived growth factor (PDGF) and transforming growth factor β (TGFβ)
- Lysosomes
  - Acid hydrolases
- Thromboxane A_2

2.10.5.3 **Platelet growth factors**

2.10.5.3.1 Transforming growth factor (TGFβ)

TGFβ is released from platelet α-granules during degranulation and mediate tissue repair, immune modulation, and extracellular matrix synthesis.[127] It is a powerful fibrosis agent that stimulates synthesis of matrix molecules, such as collagen I, and fibronectin.[134] Bone morphogenic proteins (BMPs) are a subgroup of the TGF family; with TGFβ-1 being the prominent isoform which regulates inflammation, angiogenesis, re-epithelialisation and connective tissue formation.[127] It also has a fundamental role in osteogenesis, and can upregulate VEGF which stimulates angiogenesis.

2.10.5.3.2 Platelet derived growth factors (PDGFs)

PDGF is a fundamental regulator for the migration, proliferation, and survival of mesenchymal cells. They are abundant in platelet α-granules. [127] Absence and presence of specific receptors can induce or inhibit different cell lineages, including osteoprogenitor cells, fibroblast, smooth muscle cells, and glial cells.[127, 134] This is fundamental to the process of physiologic healing with neovascularization and collagen production. It has a very short half-life, and the L-PRF matrix helps to support its slow release over time.[127]

2.10.5.3.3 Insulin-like growth factors (IGFs)

IGFs are positive regulators for cell proliferation, and differentiation of various type of cells. They enable cells to evade matricidal apoptotic stimuli, by inducing survival signals. This can occur in health and unfortunately pathology.[134] Increased concentrations of circulating IGFs have been linked with an increased risk for several common cancers including breast, prostate, lung, and colorectal.

2.10.5.3.4 Vascular endothelial growth factor (VEGF)

VEGF is released from thrombocytes and macrophages following injury to stimulate angiogenesis. It has a role in tissue remodelling. Incorporation of recombinant VEGF into bone biomaterials has been shown to stimulate osteogenesis.[127]

2.10.5.3.5 Epithelial growth factor (EGF)

EGF is a family which is fundamental to chemotaxis and angiogenesis of endothelial cells, and stimulating mitosis of mesenchymal cells. It hastens wound healing, and increases wound tensile strength.[127]

Histologic studies confirm that platelets are concentrated in the lower part of the fibrin clot following centrifugation and remain most concentrated at the junction between the red blood cells and the L-PRF clot. Furthermore glycosaminoglycans (hyaluronic acid, heparin) are also enmeshed within the fibrin mesh. Alcian blue staining confirmed, this trapping as the GAGs follow the fibrillary network.[134] These GAGs have a strong affinity for platelet cytokines, and provide a supporting structure, into which cell migration can occur.

2.10.5.3.6 Evidence for growth factor trapping within PRF clot

A study by Dohan Ehrenfest *et al.,* (2006) investigated the platelet-associated features of PRF.[134] They compared the quantity of PDGF-BB, TGFβ-1, and IGF-I within the platelet-poor plasma supernatant (PPP) with that of the PRF clot exudate serum (Figure 14).

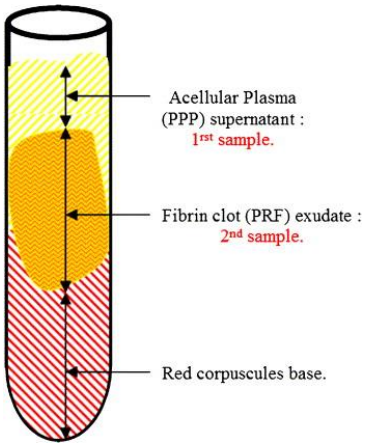


Figure 14: Concentrations of growth factors were measured within the various components produced following centrifugation.[134]

Secondly, they compared the quantity of growth factor found within the PRF clot exudate with established cPRP values originating from 5 different systems. Results found no statistical difference between cytokine concentrations within the PPP supernatant and those from the PRF clot exudate. They also found PDGF-BB and TGFβ-1 concentrations were significantly lower from the PRF clot exudate than all 5 cPRP protocols, except for IGF-I which was higher. They deduced from these results that the

PRF cytokines remain enmeshed within the fibrin meshes and fibrin polymers. Small soluble cytokines would presumably collect at the top part of tube in the PPP supernatant but instead are enmeshed in the PRF fibrin matrix, and remain intimately incorporated as they are not found within the PRF clot exudate either. The reason why IGF-I levels are higher in both PPP supernatant and PRF clot exudate is because it is naturally a circulating molecule, however IGF-I released during platelet activation would have been trapped in a similar mechanism as the other cytokines. Slow progressive polymerisation close to physiologic conditions is conducive with a homogenous 3-dimentional fibrin matrix, that is conducive with cytokine trapping, which are released slowly upon initial cicatricial remodelling, and not prematurely.[134]

2.10.5.4 **Leucocytes**

L-PRF is composed of at least 50% leucocytes.[133] Leucocytes play a fundamental role in wound healing owed to their anti-infectious properties, and immune regulation. Leucocytes are nucleated cells that can be classified into 5 different cell types; neutrophils, eosinophils, basophils, lymphocytes, and monocytes. They act as a fulcrum in positive and negative feedback systems in the cellular and cicatrisation stages of wound healing, through complex extracellular signalling pathways and a dynamic array of surface receptors.[135] This is achieved through the secretion of inflammatory cytokines, principally interleukins (IL); IL-1β, IL-6, and tumour necrosis factor alpha (TNF-α), and healing cytokines; IL-4 and VEGF.[127, 135]

2.10.5.4.1 Inflammatory cytokines

- (IL)-1β: produced by activated macrophages, neutrophils, endothelial, fibroblasts and keratinocytes. It has a significant role in mediating inflammation, particularly in stimulating T-helper lymphocytes. In combination with TNF-α, it can cause osteolysis with activation of osteoclast and osteoblast inhibition.

- IL-6: is largely produced by activated monocytes, fibroblasts, and endothelial cells. It acts as an amplifier to immune cells and stimulates differentiation of B lymphocytes and activates T lymphocytes.
- TNF-α: is an inflammatory cytokine released in response to bacterial endotoxin. It can activate monocytes, upregulates cellular phagocytosis, and stimulates remodelling by activating fibroblasts.

2.10.5.4.2 Healing cytokines

- IL-4: is produced largely by activated T cells. It has a fundamental role in supporting healing by moderating inflammation, by stimulating collagen production, and reducing matrix metalloproteinases (MMPs).
- VEGF is a vascular growth promoter, which stimulates, and supports vascular neogenesis.

2.10.5.4.3 Evidence for leucocyte cell trapping within PRF clot

A study by Dohan Ehrenfest *et al.,* (2006) quantified the concentration of these previously established inflammatory and healing cytokines within various parts of a PRF clot collection tube.[135] From this, they were able to observe how leucocytes responded during PRF preparation. They found no statistical significance between cytokine concentrations within the PPP supernatant and those within the PRF clot. They also found significantly higher concentrations of cytokines within the PRF clot exudate and the PPP supernatant, compared with those of plasma and sera samples. This would infer that the source can only be leucocytic, and during PRF processing these cytokines are released during leucocyte degranulation. Like other studies which revealed cytokine trapping during slow polymerisation, these inflammatory and healing cytokines become enmeshed within the fibrin and act as an "immune organising node".[134 , 135]

2.10.6 Clinical effects of fibrin on tissue healing and cells of the body

The fibrin within L-PRF supports and facilitates three aspects central to wound healing:

- Angiogenesis
- Immunity
- Epithelial cover

2.10.6.1 Angiogenesis

Neovascularisation requires a 3-dimensional matrix that allows cellular migration, division and phenotypic change of endothelial cells.[136] L-PRF simulates physiologic fibrin polymerisation, and *in-vitro* models by Nehl and Hermann, in 1996, showed neovascularisation was grossly influenced by the rigidity of the matrix.[137] It is reasonable to assume a system that mimics physiologic settings would be more conductive than rapidly polymerised fibrin found in PRP and fibrin glues. Factors which stimulate angiogenesis are enmeshed within the fibrin matrix; including fibroblast growth factor-basic (FGFb), VEGF, angiopoietin, and PDGF.[136] Fibrin can stimulate αvβ3 expression, which is an important process in angiogenesis. The expression of this factor by endothelial cells enables cells to bind to fibronectin, fibrin, and vitronectin.[136]

2.10.6.2 Immunity

Fibrin and fibrinogen degradation products help to modulate immunity by recruiting neutrophils, and regulate phagocytosis of neutrophils. They upregulate the expression of CD11c/CD18 receptors, which regulate the adhesion of neutrophils to endothelium, fibrinogen and transmigration of neutrophils.[136]

2.10.6.3 Wound coverage

The fibrin matrix facilitates wound coverage at sites of injury by affecting the metabolism of epithelial and fibroblast cells. Epithelial cells at a wound edge lose their basal and apical polarity, which cause lateral extension that project towards the wound edge to cover the defect.[136] Cells can then migrate within this transitory matrix of fibrinogen, fibronectin, tenascin and vitronectin. Furthermore fibrin, fibronectin, PDGF and TGF-β play a key role in integrin expression and the proliferation and migration of fibroblasts. Fibroblasts move within the fibrin clot via proteolytic activity, and this is optimised with cross-linked α-chains between the fibrin fibrils.[136] This cannot be said for fibrin glue and PRP, which are polymerised rapidly. The formation of collagen begins following fibrinolysis in the sequence of healing.

These properties combined with L-PRFs potential to trap stem cells provide the perfect medium for optimum wound healing. Mesenchymal cells derived from the circulating blood, provide a source of undifferentiated cells that can develop into different cell types.[136] However, most of these studies are *in-vitro* and further *in-vivo* studies are required to assess tissue responses to L-PRF.

A prospective cohort study by Pinto *et al., (*2017) demonstrated the beneficial properties of L-PRF as a regenerative medical strategy for refractory leg ulcers.[120] Forty-four consecutive patients with 49 wounds, all refractory to standard treatment for ≥3 months underwent weekly L-PRF application. All wounds showed significant improvements with 83.6% completely resolving. There were no adverse events recorded. Other clinical applications include socket preservation, oral surgery, periodontal surgery, implantology, and as a bone grafting material.

2.11 Questionnaire Design

Questionnaires are a useful tool to collect data on a population's knowledge, beliefs, attitude and behaviour.[138] However, poor methodology in the study design can result in poor quality data, and misleading conclusions. The first aim is to identify the research question, and what information you are aiming to collect. Using previously validated and published studies reduce the risk of error.[139] Additionally, the results can be compared with other previously published studies. However, there are currently no validated questionnaires within the literature specifically looking at clinician's treatment patterns for MRONJ. When there are no previously validated questionnaires the researcher must formulate a questionnaire. This can compromise the validity of the research, and studies have shown that general practitioners when asked how they treat a particular clinical condition often differ significantly from actual clinical practice.[140] Techniques to improve the validity include:[138]

- Researchers who are not familiar with the topic may often hold qualitative focus groups to help predict the possible respondent's response.
- Using a standardised questionnaire and recording the data in a uniform manner
- Pilot the questionnaire on an appropriate audience

Questions can be presented as closed-ended tick box categories, visual analogue scales, symbols, rating scales which usually produce quantitative data. This data is easier to analyse, but gives the respondent less freedom. Alternatively, questions can be open-ended which often produce qualitative data which allows creative expression but can be difficult to analyse.

Questionnaires fail when participants do not understand the questions, get bored or cannot answer the questions, and piloting the questionnaire can help to identify these pitfalls at the preliminary stages of design.

Research has identified methods to improve respondent response rates:[141]

- Include an introductory letter with the aims and means of the study explained
- The topic must be relevant and interesting to the audience

- Questions should be short, concise, and to the point
- Layout should be; Font 12, avoid double sided paging
- Simple layout and appealing to look at
- Respondent feels they are a stake holder in the study
- Questions phrased to hold the respondent's attention
- Postal questionnaire-include a pre-stamped and addressed envelope

2.12 Aims and objectives

1. Identify the common risk factors in the aetiology and pathogenesis of MRONJ within a sample of patients
2. To evaluate the therapeutic benefits of leucocyte-platelet rich fibrin (L-PRF) as an adjunct to conservative surgical debridement of MRONJ in an out-patient setting
3. To assess the pathogens isolated from MRONJ sites, with culture and sensitivity tests
4. To investigate the current trends in treatment strategies for MRONJ used by clinicians in the Republic of Ireland with a questionnaire.

3 Materials and methods:

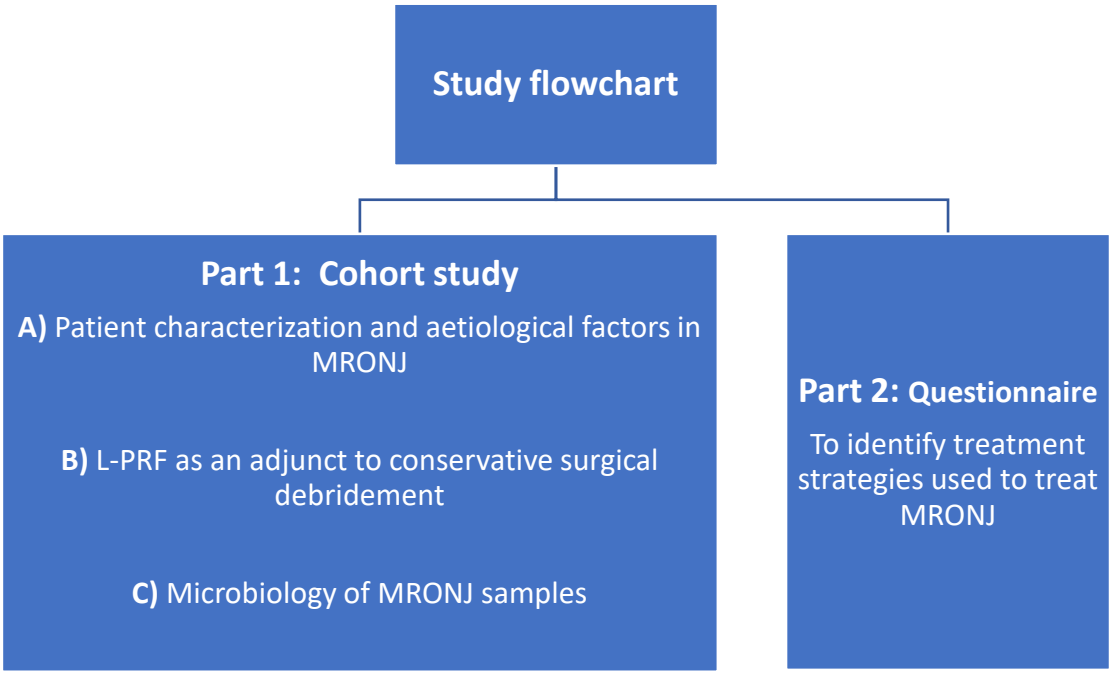


Figure 15: Study flowchart

3.1 Part 1

3.1.1 The sample

The sample population was recruited from a cohort of patients newly referred, and current patients of the Oral and Maxillofacial Surgery Department of the Dublin Dental University Hospital, with a diagnosis of MRONJ. Members of the Irish Association of Oral Surgery were also asked to refer any suitable cases for inclusion in the study. Recruitment of subjects began January 2016 and ceased in December 2017 (24 months). The designated gatekeeper provided potential study patients with information leaflets, which explained the study, and the benefits and risks associated with treatment (See Appendix 1). Patients were given 14 days to synthesise the information, prior to deciding to participate. Written consent to participate in the

study was attained in all cases (Appendix 2). Prior to therapy, verbal and written consent was also attained on the day of surgery as per hospital policy to ensure that all the information had been assimilated. Letters were issued informing the patient's medical practitioner of their participation in the study.

3.1.2 Subjects

Fifteen patients with sixteen disease sites were enrolled in the study. A total of 14 patients with 15 disease sites completed all stages of follow-up to the end-point.

One patient was excluded from the study, as intraoperatively the disease extended into the maxillary antrum, and was heavily infected. The infected Schneiderian membrane was removed, copious irrigation completed and L-PRF was placed over the defect and primary closure achieved. Subsequently a large sequestrum was shed, and the patient has achieved mucosal coverage at the site.

3.1.2.1 Inclusion criteria

The following inclusion criteria applied to the sample population:

1. Consenting patients attending the Oral and Maxillofacial Surgery Department of the Dublin Dental University Hospital for the management of MRONJ.
2. Stage 0, 1, and 2 disease were included in the study. Selected cases of Stage 3 disease were included when there were areas of osteolysis extending beyond alveolar bone but not resulting in pathological fracture, oro-antral communication, oro-nasal communication, or osteolysis extending to the inferior border of the mandible or sinus floor.
3. Patients over the age of 18 with the capacity to give informed consent.
4. Patients suitable for treatment under local anaesthetic with or without intravenous conscious dental sedation, with no health conditions, precluding them from the operative surgical procedure.
5. Patients who are willing to cooperate with requirements of the study protocol.

3.1.2.2 Exclusion criteria

The following exclusion criteria applied to the sample population:

1. Patients who have received radiation therapy to the jaws.
2. Patients with extensive Stage 3 disease resulting in pathological fracture, oro-antral communication, oro-nasal communication, or osteolysis extending to the inferior border of the mandible or sinus floor.
3. Patients with a health condition precluding them from the operative procedure
4. Cases that required a general anaesthetic.
5. Patients who do not want to participate in the study, or who are unable to give informed consent or unwilling to follow the study protocol.

3.1.3 Ethical approval

Ethical approval was obtained from the Trinity College Dublin, Tallaght Hospital / St James's Hospital Joint Research Ethics Committee. The study was initially designed as a randomised controlled trial, but due to MRONJ being a relatively uncommon condition, and difficulties recruiting sufficient numbers, the design of the study was changed to a prospective cohort study. The Trinity College Dublin Tallaght Hospital/St James's Hospital Joint Research Ethics Committee was informed of changes in the study design (See Appendix 3).

3.1.4 Location

All aspects of the study were completed in the Department of Oral and Maxillofacial Surgery, at the Dublin Dental University Hospital.

3.2 Part 1A: Patient characterisation and identification of risk factors

The first assessment appointment was performed by a Specialist Oral Surgeon and a resident in oral surgery. The appointment was used to record baseline patient characteristics, patient risk factors, and disease characteristics to assess demographics and possible aetiological factors. The following details were recorded:

1. A detailed patient history was taken, which included; medical history, social history, history of complaint, history of recent dental treatment / ill-fitting dentures, and symptoms.
2. Specifically details of the type of bisphosphonate / monoclonal antibody / anti-angiogenic medication, the dose, the duration of treatment, timing of the last dose, mode of administration, and indication for treatment were recorded.
3. Co-existing conditions such as diabetes, immunosuppression, social factors including smoking and alcohol intake, and iatrogenic factors, including the use of steroids, immunosuppressants, and chemotherapy were recorded.
4. If they had previously had any treatment for MRONJ, these details were recorded.
5. The presence of any infection, pain, and paraesthesia was recorded.
6. The site and size of the exposed necrotic bone were recorded where applicable. The size of the area was measured in millimetres.
7. A clinical photograph was taken.
8. An orthopantomogram or an intra-oral radiograph was taken to quantify the extension of the lesion and identify sequestra.
9. The cases were staged according to the AAOMS Staging Classification.[1] Radiographs were used to quantify extension of osteolysis; within alveolar bone was defined as Stage 2 disease, and large areas beyond alveolar bone was classified as Stage 3 disease.[18]
10. All patients were initially treated conservatively, which included antibiotics if signs of acute infection were present (Amoxicillin or Clindamycin in patients with a Penicillin allergy), warm saline mouthwash after each meal, and

Chlorhexidine 0.12% mouthwash twice daily (using a Monojet syringe, where indicated). Patients were given oral hygiene instructions and smoking cessation advice.

11. The patient was booked to have their treatment under local anaesthetic +/- intravenous conscious dental sedation (using Midazolam) in the sedation theatre within 6 weeks of the initial assessment.

3.3 Part 1B: Surgical procedure and preparation of L-PRF

The operative phase of the study was to investigate the effect of L-PRF as an adjuvant to conservative surgical debridement, and to collect microbiological samples from sites of the oral cavity affected by MRONJ.

1. On the day of surgery pre-operatively, patients were given oral antibiotics (Co-amoxiclav 625mg /Clindamycin 600mg) and Chlorhexidine 0.12% mouth rinse.
2. The L-PRF preparation was undertaken by a single skilled operator, who attended a workshop on L-PRF preparation in Leuven Belgium, which was held by the founders of the technique. Blood samples were collected pre-operatively in 9-ml plastic, glass coated tubes without anticoagulant, which were immediately centrifuged at 2,700 rotations per minute (rpm) for 12 minutes at room temperature (Figure 16 and 17). If the patient was taking an anticoagulant, the blood was centrifuged for an additional 6 minutes. This was performed rapidly to prevent initiation of the coagulation cascade. One of the blood samples was sent for full blood count analysis to St James's Hospital Haematology laboratory. Depending on the size of the bony defect to be filled 1-8 blood tubes were collected for L-PRF preparation. The L-PRF was prepared using the Intra-Spin L-PRF system and kit as per protocol (Intra-Lock, Boca-Raton, FL, USA). The L-PRF Intra-Spin Intra-Lock system was the only FDA/CE approved system (Figures 18 and 19). Depending on the size and shape of the defect the L-PRF clots were prepared into membranes or plugs using the

Expression kit (Figures 20-24). The L-PRF has a viability of 2.5 to 3 working hours, provided they are re-hydrated with exudate.[142]

Fig: 16

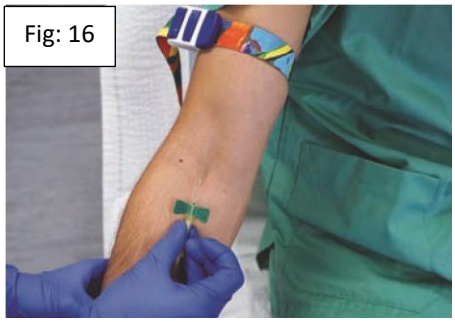


Fig: 17

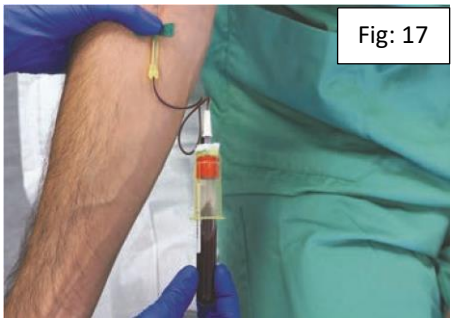


Figure 16: Illustrates venipuncture

Figure 17: Shows the collection of the blood sample using a 21 gauge butterfly needle

Fig: 18

Fig: 19

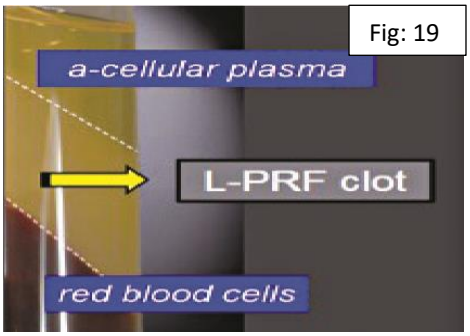


Figure 18: Tubes were balanced within the Intra-Spin L-PRF system, and removed after centrifugation 2700 rpm

Figure 19: The a-cellular plasma was the superior layer, the L-PRF clot in the middle, and red blood cells at the base of the tube

Fig: 20

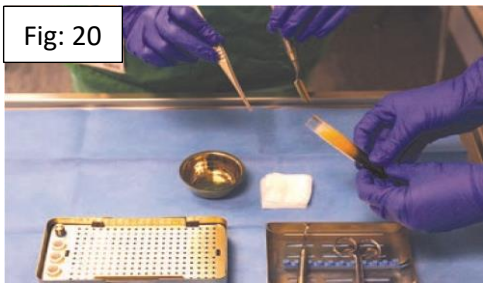


Fig: 21

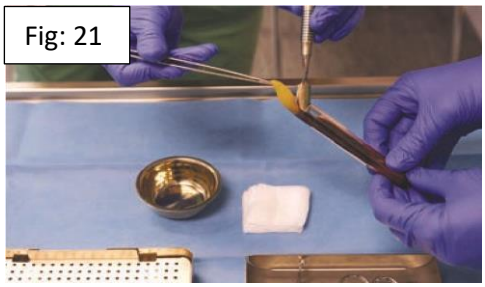


Figure 20: The clot was removed gently from the tube with fine tweezers using an aseptic technique

Figure 21: most red cells were removed from the clot carefully preserving the white buffy coat and a fine layer of red cells, which is rich in cytokines, platelets and leucocytes

Fig: 22

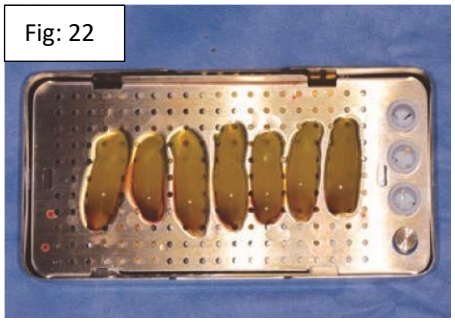


Fig: 23

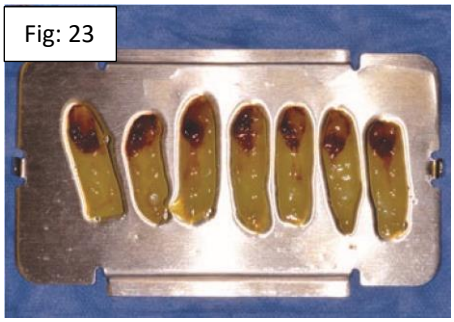


Figure 22: The Xpression kit was used to compress the L-PRF clots

Figure 23: Membranes with a consistent thickness of 1mm were formed, which takes 5 minutes

Fig: 24

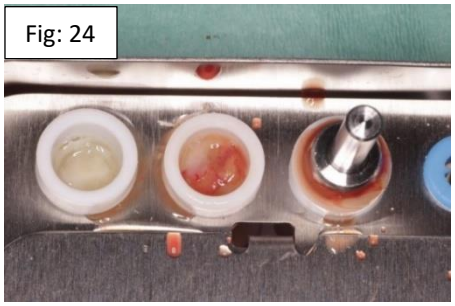


Figure 24: Alternatively, a piston and cylinder assesmbly isused to make L-PRF plugs.

3. Treatment was performed by one of two Specialist Oral Surgeons, alongside one resident oral surgeon. The surgeons were briefed and agreed to the study design and standardised surgical procedure. This included raising conservative full thickness mucoperiosteal flaps, removal of mobile sequestra, extraction of non-vital teeth at the site and removal of necrotic bone with surgical handpieces until fresh bleeding bone was achieved unless there was a risk of compromising vital adjacent structures. Sharp bone spurs were removed with Rongeurs or a surgical handpiece. Relieving incisions were made to achieve tension free primary closure where possible, where this was not achievable the sites healed by secondary intension. Treatment was performed with block or infiltration anaesthesia with plain local anaesthetic without adrenaline (Scandonest 3% Mepivacaine). If supplementary local anaesthetic was required, further plain local anaesthetic was given. If persistent pain was experienced, local anaesthetic with adrenaline 2% lidocaine 1:80,000 epinephrine was administered to enable completion of treatment.

4. The surgical procedure was performed under aseptic conditions in a theatre environment with standard surgical draping. A full thickness mucoperiosteal flap was reflected to expose the bone defect (Figures 25 and 26). Any mobile sequestra were removed and sent for histopathology (in Formalin) to St James's Hospital Histopathology Laboratory. Delineating margins of necrotic bone was by visual inspection, identified by lack of bleeding, greyish colour and sclerotic texture. Using a rotary drill (40,000rpm) with copious sterile saline irrigant; a conservative ostectomy was performed to remove necrotic bone until fresh bleeding bone margins were attained. Limited or no ostectomy with rotary instruments was performed at sites which were in close proximity to sensory nerves, or where the debridement would breach the floor of the nose or maxillary antrum. Sharp bony edges were smoothed using a surgical hand-piece or Rongeur bone cutter. Irrigation with 40ml of sterile normal saline 0.9% was used to minimise bacterial contamination and eliminate debris.

Fig: 25

Fig: 26

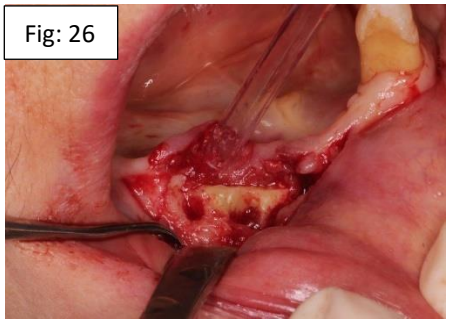
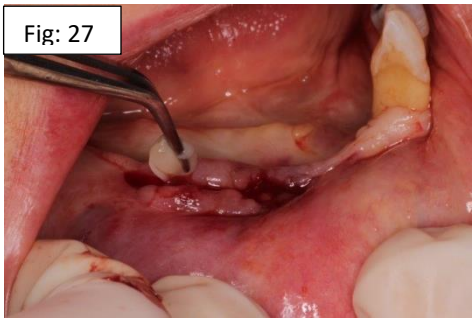


Figure 25: Exposed necrotic bone in the lower right mandible

Figure 26: Full thickness mucoperiosteal flap was raised

5. L-PRF membranes, plugs or a combination or both were used to fill the defect (Figures 27 and 28). The L-PRF was positioned so that the site of maximal concentration of cytokines was in proximity to the bone and soft tissue interface and closed primarily (Figure 29). In situations where tension-free primary closure was not feasible, additional relieving incisions were made to achieve primary closure. However, in some sites primary closure was not achieved, and so the L-PRF membrane was placed over the defect as a barrier membrane, which aimed to facilitate epithelial migration across the wound.



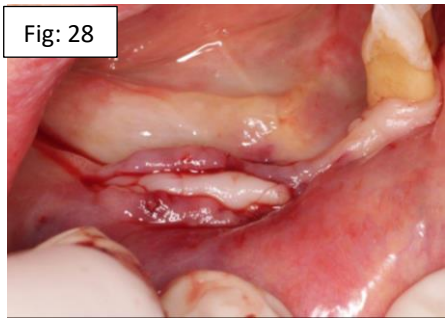


Figure 27: L-PRF plug was placed into defect

Figure 28: L-PRF membrane was placed over the defect

Figure 29: Primary closure of the soft tissues

6. Patients were prescribed oral antibiotics (Co-amoxiclav 625mg three times daily or Clindamycin 300mg twice daily with Quest Mega 8 Biotix probiotics once daily) for 7 days post-operatively and advised on maintaining good oral hygiene with warm saline mouthwash after each meal and Chlorhexidine 0.12% mouthwash twice daily starting 36 hours post-operatively. The patient was also advised on a standardised post-operative analgesic regime using oral Paracetamol and oral Ibuprofen (provided there was no contraindication to taking these medications):
    - Paracetamol 1g (500mg x 2 tablets) every 4 hours with a maximum of 4g in 24 hours together with
    - Ibuprofen 400mg (200mg x 2 tablets) every 4 hours with a maximum of 2.4g in 24 hours to be taken with food
7. All patients were discharged from the Oral and Maxillofacial theatre unit between 30 minutes to 1 hour following completion of the procedure when they had adequately recovered. Patients who underwent intravenous conscious sedation were discharged to the care of a responsible escort.

3.4 Part 1C: Microbiology and culture and sensitivity testing

Samples for microbiology testing were collected intraoperatively to examine the microbial pathogens that colonised sites in the oral cavity affected by MRONJ, and for antibiotic sensitivity testing.

Special attention was taken not to contaminate the swab with saliva from other sites of the oral cavity. Sterile Sarstedt® (80.1361) transport swabs with Amies transport medium (without charcoal), were used to collect samples intra-operatively. Transport media improve the recovery rate of pathogens during transport, especially when samples are not processed within 1 hour of collection, which applied to this study. These swabs are licenced for oral use and are suitable for collecting aerobes and anaerobes. If suppuration was present a swab of the pus was taken. Swabs were also taken from the necrotic bone after the full thickness periosteal flap had been raised. Additionally, where possible necrotic bone fragments were sent for culture and sensitivity in dry pots and sent to St James's Hospital Microbiology laboratory, as per normal procedure. Samples were transferred from Dublin Dental University Hospital to the microbiology laboratory within 30 minutes to avoid desiccation of the sample. St James's Microbiology laboratory use Matrix Assisted Laser Desorption Ionisation-Time of Flight method for the analysis of all samples. The results of these tests were reviewed at the first post-operative review at day 10 to confirm appropriate antibiotic coverage.

3.5 Post-operative assessment

3.5.1 Clinical assessment of healing

Patients were clinically assessed at 10 days post-operatively for suture removal, and subsequently at 1 month, 3 months, and 6 months. Clinical photos were taken at each appointment, and where exposed bone persisted it was measured in millimetres.

Curation was classified as the presence of continuous closed oral epithelium with no visually exposed bone, or probable sinus tract intra- or extra-orally. This was the primary measured outcome for resolution.
This was further classified as:

- **Early resolution:** At 1 month no exposed or necrotic bone at the site, full coverage by mucosa and the absence of pain or signs of infection.
- **Delayed resolution:** An area of exposed bone present at 1 month but resolved at 3 months.
- **No resolution:** Persistence of exposed bone or a probable sinus tract at 6 months

3.5.2 Resolution of pathological symptoms

Patients were asked to describe the presence, or absence of pathological symptoms at the post-operative surgical site at each review appointment. These details were documented within their file.

3.5.3 Radiographic criteria for resolution

At day 10 post-operatively an orthopantomograph / hemi-OPG / intraoral periapical radiograph was taken as a baseline to monitor the disease process. At the 6-month end-point of the study a similar radiographic was taken and compared to the baseline image to assess for persistent signs of pathology, bony infill, or disease worsening and progression. This was assessed by two clinicians; a Specialist Oral Surgeon and oral surgery resident.

3.5.4 Acceptability of treatment

At day 10 post-operatively, acceptability of the surgical treatment was measured using a validated modified Visual Analogue Scale (VAS) questionnaire (See appendix

4).[138] The VAS was presented as a horizontal line, anchored with two verbal descriptors at the extremes with numerical values, and the subject is asked to point at the description that best describes their perceived emotional response to the operative surgical procedure. The results of patients who underwent treatment under local anaesthetic were considered separately to those who received conscious dental sedation, as the amnesic effect of Midazolam may have acted as a confounding factor.

3.5.5 Full blood count result

The results of the full blood count were reviewed at day 10 post-operatively, specifically looking at haemoglobin levels. The results were compared against normal reference ranges (Female 11.5-16.4 g/dL and Male 13.5-18 g/dL)

3.5.6 Histopathology result

The histology results for the bone sample were reviewed at 1 month post-operatively to confirm a diagnosis of MRONJ and exclude metastatic disease.

3.6 Part 2: Questionnaire

The purpose of this part of the research was to gain information on the different treatment modes that are being adopted by clinicians, prescribing protocols, and whether they adhere to the AAOMS (2014) treatment by Stage guidance.[1] This information was collected using a questionnaire.

3.6.1 Ethical approval

Ethical approval was obtained from the School of Dental Science, Trinity College Dublin Research Ethics Committee for the questionnaire component.

3.6.2 Data Collection

The questionnaire was constructed as there are no previously validated questionnaires available. The questionnaire was designed as a mixed questionnaire, using both closed and open-ended format questions (See Appendix 5). The closed format questions included tick box categories and rating scales using a 5-point Likert scale. The questionnaire was piloted on two clinicians; a Consultant in Oral and Maxillofacial Surgery and a Specialist Oral Surgeon to identify any design pitfalls at the outset. The circulated questionnaires were standardised, printed in colour, font 12 and single sided. Questionnaires were posted/emailed to Consultants in Oral and Maxillofacial Surgery, Specialist Oral Surgeons, Specialist Registrars, and Non-consultant Hospital Doctors (NCHD) practicing in the Republic of Ireland. Posted questionnaires included a stamped, pre-addressed envelope. The names were accessed from specialist register lists and sent to oral and maxillofacial units in Ireland. A total of 70 questionnaires were distributed and returned anonymously via a gatekeeper. The questionnaire investigated various aspects of the management of MRONJ and comprised of a series of four anonymised clinical cases, which were included in this study. The respondent was asked to comment on how they would manage each case.

The data collected were assessed in relation to the role of the respondent; under the title NCHD, Registrar/Specialist registrar, Specialist Oral Surgeon, and Consultant in Oral and Maxillofacial Surgery.

3.7 Data analysis

All the collected data was transferred onto an Excel© (Microsoft Corporation) worksheet.

- Descriptive statistics were used to describe patient demographics, drug history, medical co-factors, and risk factors. Mean, median, standard deviation, and subject numbers were reported where appropriate.
- The outcomes of the surgical intervention were mostly limited to descriptive statistics due to the small number of patients within the study. Categorical variables according to outcome of surgery were analysed using Fisher's Exact test as the numbers were small (≤5 counts). The level of significance was set at 5% (P=0.05). If larger numbers were achieved non-parametric tests for larger samples would have been applied (Appendix 6).
- Descriptive statistics were used to present the results of microbiology testing.
- The results of the questionnaire were analysed and demonstrated using descriptive statistics. The data from the groups of respondents were collapsed into staff (Consultant OMFS and Specialist Oral Surgeons) and trainees (NCHDs/ SpR/Registrar) due to the small numbers. Chi-square (≥5 counts) or Fisher's Exact (≤5 counts) were used to assess the categorical variables. The level of significance was set at 5% (P=0.05).

4 Results

4.1 Part 1: Results of prospective cohort study

4.1.1 Subject demographics

All 14 subjects were white Caucasian, with a total number of 15 disease sites (Table 20). The mean age at presentation was 69.7 (SD +/-2.04) with a range of 58-82 years. Ten were female, and four were male, with a gender ratio of 2.5:1. The mean age of the females was 68.6, and 72.5 for the males. The subjects had a mean weight of 60.9Kg (SD+/-2.97Kg). The subjects had a mean BMI of 22.45 (SD +/-1.09).

Patient	**Ethnicity**	**Age**	**Sex**	**ASA**	**BMI**
1	Caucasian	69	F	I	15.9
2	Caucasian	68	F	II	17.6
3	Caucasian	73	F	II	30.9
4	Caucasian	78	F	II	21.3
5	Caucasian	61	M	II	19.6
6	Caucasian	73	F	II	19.6
7	Caucasian	67	M	I	22.5
8	Caucasian	58	F	I	20.8
9	Caucasian	61	F	II	28.6
10	Caucasian	80	M	II	20.7
11	Caucasian	76	F	II	27
12	Caucasian	61	F	II	22.5
13	Caucasian	82	M	III	22.5
14	Caucasian	69	F	II	22.8

Table 20: Subject demographics

Abbreviations: F female; M male

4.1.2 Antiresorptive history

4.1.2.1 Osteoporosis patients

Seven patients (50%) were receiving antiresorptive treatment for osteoporosis, of which four were receiving subcutaneous Prolia, one received yearly intravenous infusions of Zoledronic acid, and two were on oral bisphosphonates (Table 21). Patient 1 had previously been on oral Alendronic acid 70mg/week for 72 months; however, her disease was classed as Denosumab related osteonecrosis as she had an 18-month history of Denosumab. The mean duration of total antiresorptive treatment in the osteoporotic group was 33.2 months (SD +/-10.0). The mean duration of bisphosphonates in the osteoporosis group was 37 months (SD +/-11.7). The mean duration for Denosumab was 21.25 (SD +/-6.04). The mean duration of antiresorptives in the female group was 34.8 months (SD +/-11.69) and the duration of antiresorptives for the single male patient was 24 months.

4.1.2.2 Oncology patients

Seven patients (50%) were on antiresorptives for an oncological diagnosis. Three patients were being treated for metastatic prostate cancer, two for metastatic breast cancer, and two for multiple myeloma. Six of these patients were on monthly Zoledronic acid IV infusions (Table 21). Patient 13 was on monthly Denosumab injections for 12 months, and prior to this he had been on monthly IV Zoledronic acid infusions for 27 months; his disease was classed as Denosumab induced. The mean duration of total antiresorptive treatment was 26.2 months (SD+/-6.7). The mean duration of antiresorptives in the females was 27.75 months (SD +/- 8.2) and for the males 24.3 (SD +/- 13.3). The mean duration of antiresorptive treatment in the subjects with breast cancer was 33.5 months (SD +/-18.5), prostate cancer was 24.3 months (SD +/-13.3) and multiple myeloma 22 months (SD +/- 2).

Patient	Primary disease	Drug	Dose (mg)	Time (month)	History of 2nd antiresorptive
1	Osteo	Deno	60mg/month SC	18	Alendron 70mg/wk for 72 months
2	Breast Ca	Zoledron	4mg/month IV	15	
3	Osteo	Deno	60mg/6 months SC	7	
4	Osteo	Alendron	70mg/week PO	28	
5	Prostate Ca	Zoledron	4mg/month IV	11	
6	Osteo	Zoledron	5mg/annually IV	24	
7	Prostate Ca	Zoledron	4mg/monthly IV	11	
8	Osteopo	Deno	60mg/6 months SC	24	
9	Multiple myeloma	Zoledron	4mg/month IV	24	
10	Osteo	Ibandron	150mg/month PO	24	
11	Multiple myeloma	Zoledron	4mg/month IV	20	
12	Osteo	Deno	60mg/6 months SC	36	
13	Prostate Ca	Deno	120mg/month IV	12	Zoledron 4mg/month for 27 months
14	Breast Ca	Zoledron	4mg/month IV	52	No

Table 21: Antiresorptive drug history

Abbreviations: Ca cancer; Osteo osteoporosis, Deno Denosumab, Zoledron zoledronic acid, Ibandron Ibandronic acid, Alendron Alendronic acid

4.1.3 Precipitating factor

Twelve cases (85.7%) reported a history of a dental extraction preceding the development of MRONJ (Table 22). Four of these cases had extractions in keeping with JIDA guidelines with preoperative and postoperative systemic antimicrobials.[12, 30] Patient 1 had an ill-fitting denture with a severely atrophic mandible, Class V flattened ridge as per the Cawood and Howell Classification (1988) precipitating bilateral sites

of MRONJ in the premolar region.[143] Patient 5 reported MRONJ following exfoliation of a tooth, with subsequent failure to heal.

4.1.4 Site

Nine cases arose in the mandible (64%), five in the posterior mandible, three in the premolar region, and one in the anterior segment. Five cases occurred in the maxilla (36%), with four in the anterior segment, and one in the posterior maxilla.

4.1.5 Stage and symptoms

The most common presenting complaint was pain, swelling, and foul taste. One patients presented with Stage 1 disease, nine patients were classed with Stage 2 disease, and three with Stage 3. Patient 8 presented with Stage 0 disease, previously he had an extraction, and was diagnosed with Stage 2 disease 14 months later at this site. This healed with mucosal coverage, however he described constant pain at the site 11 months on. None of these cases had clinical symptoms of antral or nasal involvement.

4.1.6 Radiographic signs

Radiographically, five patients showed evidence of a sequestra, three showed evidence of a non-healing socket, 3 had osteosclerosis, one had a bony defect, and 2 patients had no radiographic evidence of MRONJ. None of these cases had radiographic signs of antral, nor nasal communication, nor osteolysis extending to the lower border of the mandible.

Patient	MRONJ site	Exposed bone (mm)	Luxating factor	IDA protocol followed	Symptom	Stage AAOMS[1]
1	Mand 2 sites	Probable (both sites)	Denture trauma		Pain	2 (both sites)
2	Max	Probable	XLA	Unknown	Pain	2
3	Mand	6 x 5	XLA	No	Pain	2
4	Max	6 x 5	XLA	No	Swelling	3
5	Mand	2 x 2	Exfoliated tooth		Pain	3
6	Max	45 x 10	XLA	No	Swelling	3
7	Mand	20 x 12	XLA	Yes	None	2
8	Mand	7 x 1	XLA	No	Pain	2
9	Mand	10 x 9	XLA	Unknown	Foul taste	2
10	Max	Not applicable	XLA	Yes	Pain	0
11	Max	5 x 6	XLA	Yes	Pain	2
12	Mand	5 x 3	XLA	Unknown	None	1
13	Mand	5 x 4	XLA	Yes	Pain	2
14	Mand	4 x 3	XLA	Unknown	Swelling	2

Table 22: Clinical features of MRONJ within the patient sample

Abbreviations: Max maxilla; Mand mandible; XLA extraction

4.1.7 Smokers

Five patients had an every-day smoking habit (Table 23). Patient 1, 7, and 8 were moderate smokers, smoking on average <20/day. Patient 5, and 12 were heavy smokers, smoking ≥20 cigarettes/day. Patient 8 was the only person to abstain from smoking in the first 10 days after the surgical intervention, although the others reported a reduced intake.

4.1.8 Co-medication and immunosuppression

Two patients were on anti-angiogenic medications that interfere with wound healing, and one had also previously had a stem cell transplant, which was associated with long-term immunosuppression. One patient was on Methotrexate for the treatment of rheumatoid arthritis. Two patients were on systemic glucocorticoids, which affects the host defence mechanisms, and one was concurrently on radium chemotherapy injections. Additionally, one had Type II diabetes mellitus which affects wound healing capacity, and host defence mechanisms.

Patient	**Relevant co-morbidity**	**Relevant co-medication**	**Smoker (pack years)**	**MRONJ site adjacent to teeth**
1			2	Edentulous
2	Oncology			Edentulous
3				
4				Edentulous
5	Oncology	Radium injections, glucocorticoids	30	Yes
6		Methotrexate		No
7	Oncology		15	Yes
8			8	Yes
9	Oncology, stem cell transplant	Lenalidomide (anti-angiogenic)		Yes
10		Glucocorticoids		No
11	Oncology			Yes
12			45	Edentulous
13	Oncology, Type 2 diabetes	Metformin, Gliclazide		Yes
14	Oncology	Trastuzumab (anti-angiogenic)		Yes

Table 23: Medical and social co-factors for MRONJ

4.1.9 Previous treatment

All patients had been managed with conservative treatment by improving oral hygiene, antimicrobial mouthwash, saline mouth rinses, and at least one course of systemic antimicrobials prior to treatment in this study. Patient 2 had refractory disease following failed conservative surgical debridement 11 months prior to being recruited to this study. The mean duration of conservative treatment from the time of diagnosis to the surgical intervention was 22.57 weeks (SD+/-5.07), with a range of 3-64 weeks.

4.1.10 Changes in antiresorptive drug regime

All patients had finished their antiresorptive treatment or discontinued treatment upon diagnosis of MRONJ. This decision was made by the patient's prescribing medical practitioner/ oncologist. The mean duration since the last dose of Denosumab was 3.7 months (SD +/- 1.06). The mean duration since the last dose of bisphosphonate therapy was 11.8 months (SD +/- 3.37).

4.1.11 Surgical intervention

Nine patients (64%) underwent the treatment with local anaesthetic alone, and five patients (36%) had intravenous conscious sedation with midazolam titrated, according to their response. All 14 patients were anaesthetised with Scandonest 3% plain local anaesthetic as per JIDA guidance (2010, 2017).[12, 30] Supplemental top-up Scandonest local anaesthetic was administered to 11 of the 14 patients due to failed anaesthesia. However, nine of the 14 patients required further supplemental 2% lidocaine 1:80,000 with epinephrine to enable completion of treatment (Figure 30). The difference in healing outcomes between patients who had Scandonest only, compared with those

who had supplemental local anaesthetic with adrenaline was not significant, although the sample size is too small to draw conclusions. (Fishers Exact p=1).

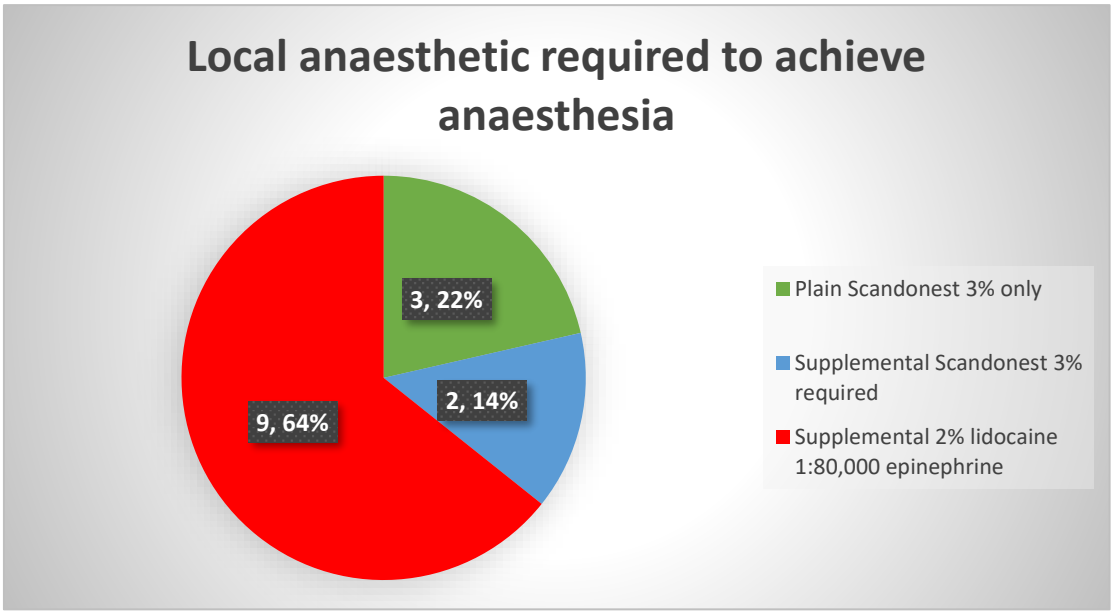


Figure 30: Proportion of patients who required no additional local anaesthetic, and proportion requiring top-up anaesthetic.

Six of the fourteen patients had mobile sequestra present. Eight of the patients underwent conservative ostectomy to remove necrotic non-viable bone to fresh bleeding margins with rotary instruments or Rongeurs (Table 24). Three patients did not undergo ostectomy due to the risks to adjacent vital structures. Patients 4 and 6, were due to proximity to the floor of the nose. Patient 9 was due to proximity of the lingual nerve. Patients 5 and 7 had a limited ostectomy due to proximity to the mental nerve, and patient 11 had an ostectomy which was limited by proximity to the floor of the nose.

Primary closure was achieved in 11 of the 15 disease sites (73%). Six disease sites (40%) required additional relieving incisions to achieve primary closure. Of the four disease sites (27%) in which we failed to achieve primary closure, efforts were made in two patients to get closure with additional relieving incisions.

Patient	Ostectomy	Limiting structure	1°closure	Additional relieving incisions
1	Yes		Yes, at site 1 & 2	No
2	Yes		Yes	No
3	Yes		No	No
4	No	Floor of the nose	Yes	No
5	Limited	Mental nerve	Yes	No
6	No	Floor of the nose	No	Yes
7	Limited	Mental nerve	No	Yes
8	Yes		No	No
9	No	Lingual nerve	Yes	Yes
10	Yes		Yes	No
11	Limited	Floor of the nose	Yes	Yes
12	Yes		Yes	Yes
13	Yes		Yes	Yes
14	Yes		Yes	No

Table 24: Factors relating to surgical intervention

4.1.12 Response to treatment

Sites which had fully epithelialised at 1 month post-operatively remained healed throughout the study until the end-point (73.3% n=11 sites) (Table 25). Three of the sites which had exposed/ probable bone at one month post-operatively remained refractory for the duration of the study. Patient 9 had probable bone at 1 month with suppuration, which was treated with oral antibiotics, and at the 3-month review the site was asymptomatic and epithelialized, however reverted to Stage 2 disease at the end-point of the study.

Patient	Hb	Healed 1 months	Healed 3 months	Healed 6 months	Radiographic signs MRONJ 6 months	Clinical symptoms 6months
1	Normal	Yes	Yes	Yes (Site 1&2)	None	Resolved
2	Normal	Yes	Yes	Yes	None	Resolved
3	Normal	Yes	Yes	Yes	None	Resolved
4	Normal	Yes	Yes	Yes	None	Resolved
5	Normal	No	No	No	Good bone infill	Stage 1
6	Low	Yes	Yes	Yes	None	Resolved
7	Low	No	No	No	Yes, extension of lesion	Stage 1
8	Normal	Yes	Yes	Yes	None	Resolved
9	Normal	No	Yes	No	None	Improved Stage 2
10	Low	Yes	Yes	Yes	None	Resolved
11	Low	No	No	No	Necrotic sequestra	Unchanged Stage 2
12	Normal	Yes	Yes	Yes	None	Resolved
13	Low	Yes	Yes	Yes	None	Resolved
14	Normal	Yes	Yes	Yes	None	Resolved

Table 25: Clinical and radiographic outcome of surgical intervention

Abbreviation: Hb haemoglobin

Nine sites in the female group healed, and two failed to heal. Two of the sites in the males group healed and two failed to heal. A Fisher Exact test was performed to examine the relation between gender and surgical healing. The relation between these variables was not significant, although the numbers within this study were too small to draw conclusions from this result ($p = 0.5165$).

Five of the sites in patients on bisphosphonates healed, four failed to heal. All the disease sites in the patients on Denosumab healed following surgery (n=6). A Fisher Exact test was performed to examine the relation between the type of medication,

and surgical healing. The relation between these variables was not significant, p = 0.1033. However, the numbers within the study are too small to draw conclusions from this result.

All disease sites in the osteoporosis patients healed (n=8). Three of the sites in the oncology patients healed and four failed to heal. A Fisher Exact test was performed to examine the relation between indication for antiresorptive medications and surgical healing. The relation between these variables was significant, p = 0.0256.

The two patients on oral bisphosphonates healed. Three of the seven patients on intravenous bisphosphonates healed, and four failed to heal. A Fisher Exact test was performed to examine the relation between the route of administrating bisphosphonates (oral/IV) and surgical healing. The relation between these variables was not significant, however there were insufficient numbers within each group to fully investigate this (p = 0.4444).

The outcome of surgery by site in the oral cavity was assessed. Four of the disease sites in the maxilla healed, and one failed to heal. Seven sites in the mandible healed, and three failed to heal. A Fisher Exact test was performed to examine the relation between the location of MRONJ and surgical healing. The relation between these variables was not significant, however the small numbers within the study may have contributed to the result (p = 1).

The relationship between the level of haemoglobin, and healing was assessed. Eight of the sites healed and two failed to heal in patients with a normal haemoglobin. Patients with a low haemoglobin had healing in three cases and no healing in two cases. A Fisher Exact test was performed, and the relation between these variables was not significant, however the numbers within the study were too small to fully investigate this (p = 0.5604).

Healing was assessed in relation to Stage of disease. Both patients with stage 0 disease and Stage 1 disease had curation. Seven of the sites graded as Stage 2 disease healed and three failed to heal. Of the sites graded as Stage 3, two sites healed and one remained refractory to treatment. A Fisher Exact test was performed to examine the relation between stage 2 and 3 and response to surgery. The relation between these

variables was not significant, p = 1. However, the numbers within the study were again too small.

The impact of smoking on healing was also assessed. Seven of the non-smokers healed and two failed to heal. Four of the smokers healed, and two failed to heal. A Fisher Exact test was performed to examine the relation between smoking and surgical healing. The relation between these variables was not significant, however the numbers within the study are too small to draw conclusions (p = 1).

The impact of achieving primary closure on healing was assessed. Eight of the sites which were closed primarily healed, and three failed to heal. Of the four sites that healed by secondary intention, three healed and 1 failed to heal. A Fisher Exact test showed the relationship was not significant, p=1. The number of patients within the study are too small to draw conclusions from this result.

The presence of a necrotic sequestra of bone in relation to curation was examined. Six disease sites had a mobile sequestra present, of which one failed to heal. Nine disease sites did not have a sequestra present, of which 3 failed to heal. A Fisher exact test found no statistical relationship between the presence of a sequestrum and healing, however the patient numbers within the study are small to fully explore this factor (p=0.6044).

4.1.13 Symptoms at 6 months

Ten of the patients reported resolution of symptoms at 11 disease sites at the end-point of the study. Three of the patients report improved symptoms since the surgical intervention. Patient 7 reported no change in his symptoms since the surgical intervention (he was asymptomatic pre-operatively also). No patient reported worsened symptoms.

4.1.14 Radiographic review at 6 months

Radiographically, there was no evidence of MRONJ pathology in the 11 disease sites that had healed at the end-point of the study. Patient 9 had no radiographic signs of pathology despite failure to heal. Patient 5 had a persistent defect at 6 months, however there is evidence of bony infill (Figure 31-33).

Fig: 31

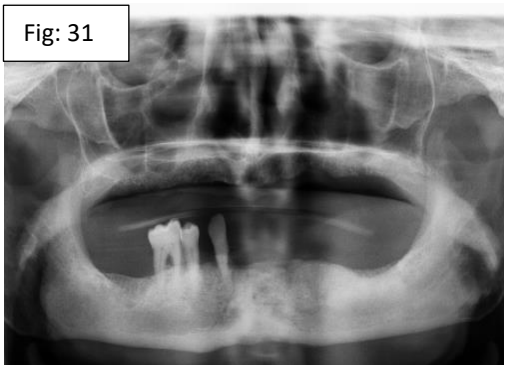


Figure 31: Patient 5 OPG pre-operatively

Fig: 32

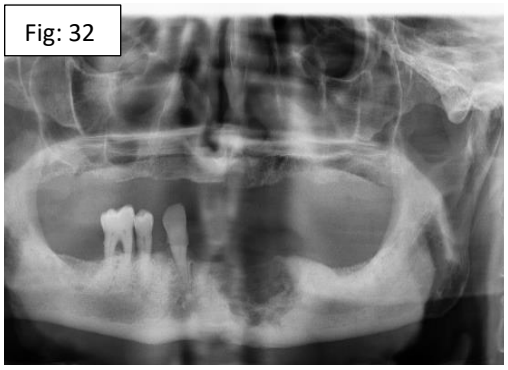


Figure 32: Patient 5 OPG 10-days post-operatively

Fig:33

Figure 33: Patient 5 OPG 6-months post-operatively

There was radiographic evidence of disease progression evident in Patient 7's 6-month radiograph (Figure 34-36). The lower right canine remained positive to vitality testing throughout.

Fig:34

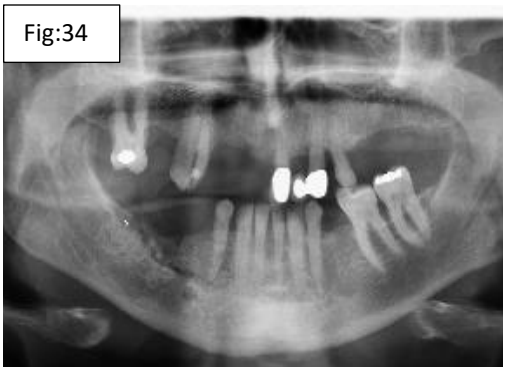


Figure 34: Patient 7 OPG pre-operatively

Fig:35

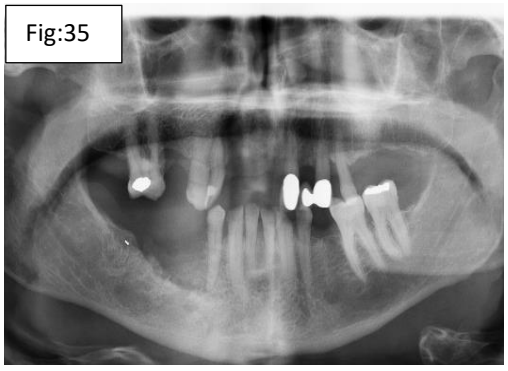


Figure 35: Patient 7 OPG 10-days post-operatively

Fig:36

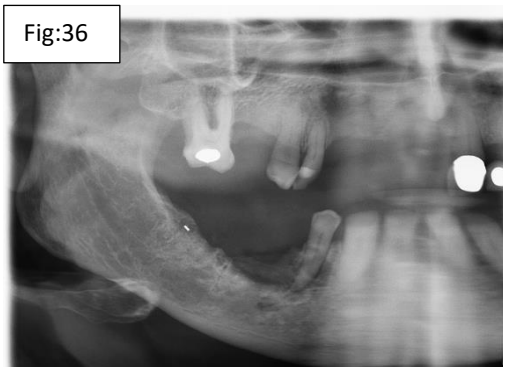


Figure 36: Patient 7 OPG 6-months post-operatively

Initial radiographic assessment of Patient 11 at day 10 post surgery showed possible persistence of necrotic bone, where further bone could have been removed, but intraoperatively the ostectomy was limited by the possibility of perforating the floor of the nose (Figure 37 and 38). At the 6-month radiographic review, the bone is visibly forming a sequestrum (Figure 39).

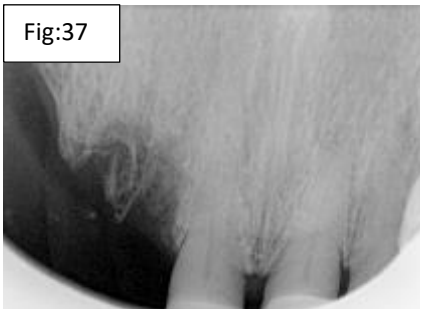


Figure 37: Patient 11 Maxillary occlusal pre-operatively

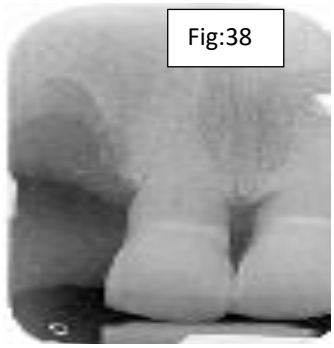


Figure 38: Patient 11 periapical 10-days post-operatively

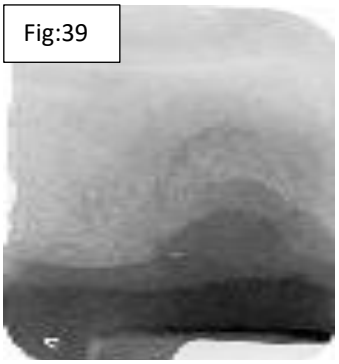


Figure 39: Patent 11 periapical 6-months post-operatively

4.1.15 Early intervention

Five patients who proceeded from initial diagnosis to surgery within ≤3 months, showed healing in 80% (n=4) and 20% failed to heal (n=1). This may indicate early intervention is favourable, with delayed treatment resulting in progression of disease and larger defects requiring more extensive surgery. However, the numbers within the study are too small to confirm this.

4.1.16 Visual analogue scale for surgical intervention

Visual analogue scores were recorded at day 10 to assess the patients experience, and attitude towards the surgical experience. The mean VAS score for the surgical intervention in the local anaesthetic group was 3.1 (SD +/- 3.48) (n=9), and in the intravenous conscious sedation group 1.8 (SD +/- 1.09) (n=5).

4.1.17 Topical and systemic antimicrobials

Post-operatively, 13 patients were prescribed a 7-day course of Co-amoxiclav 625mg to be taken three times daily. One patient experienced nausea. The patient with a Penicillin allergy was prescribed Clindamycin 300mg twice daily for 7 days with a probiotic. She described symptoms of diarrhoea. All patients reported compliance with local measures and maintained good oral hygiene.

4.1.18 Histopathology

All 14 samples sent for histological examination reported necrotic bone consistent with sequestra. There was no evidence of malignancy in any of the samples.

4.1.19 Microbiology

On a microbiological level, samples sent for culture and sensitivity from 15 disease sites showed Gram +ve cocci (40%), Gram +ve bacilli (33%), Gram -ve bacilli (27%), candida species (20%), and pus cells in 27% of the samples (Figure 40).

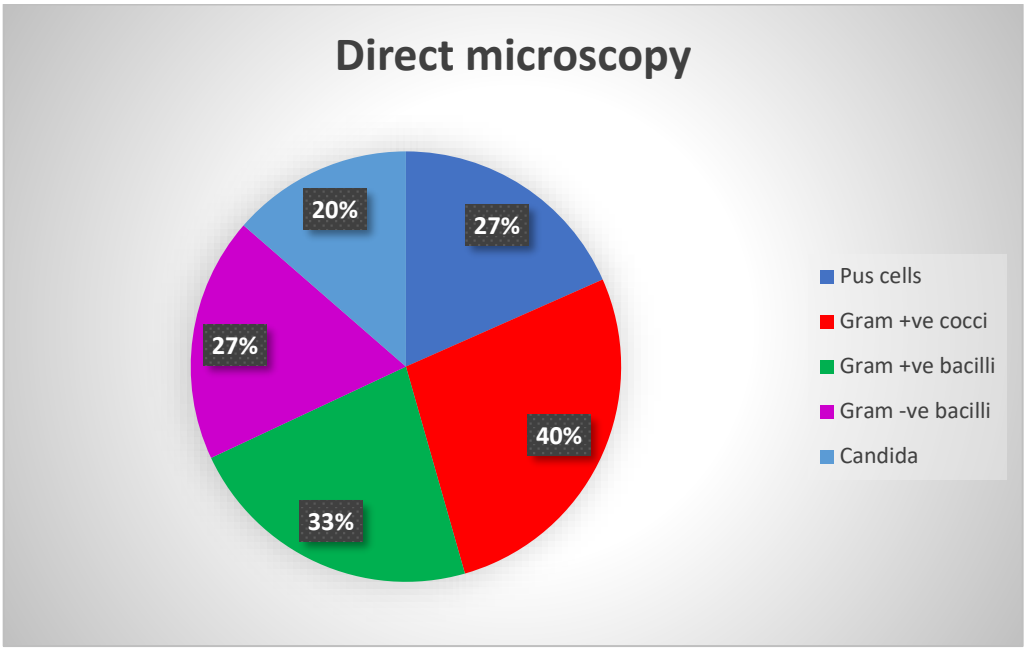


Figure 40: Proportion of isolates identified macroscopically from 15 samples

There appeared to be no gross differences on a macroscopic level between patients on bisphosphonates or Denosumab, or when oncology patients were compared with osteoporotic patients (Table 26).

Direct microscopy	Bisphosphonate group	Denosumab group	Osteoporosis	Oncology
Pus cells	20%	6.6%	13.3%	13.3%
Gram +ve cocci	33.3%	6.6%	6.6%	33.3%
Gram +ve bacilli	20%	13.3%	13.3%	20%
Gram -ve bacilli	13.3%	13.3%	6.6%	20%
Candida	13.3%	6.6%	13.3%	6.6%

Table 26: Macroscopic results of culture and sensitivity testing

Of the 15 samples sent for culture and sensitivity, the following microbes were isolated from samples; Strep constellatus (33%), Strep. anginosus (27%), Strep. mitis (20%), Staph. epidermidis (7%), Proprionbacterium acnes (7%), Staph. haemolyticus (7%), Actinomyces odontolyticus (7%), Borrelia. Vincenti (7%). Forty seven percent of the samples isolated anaerobes, two with heavy growth, 4 with few, and 1 with scanty presence of anaerobes. Forty-seven percent of samples reported normal oral flora (Figure 41).

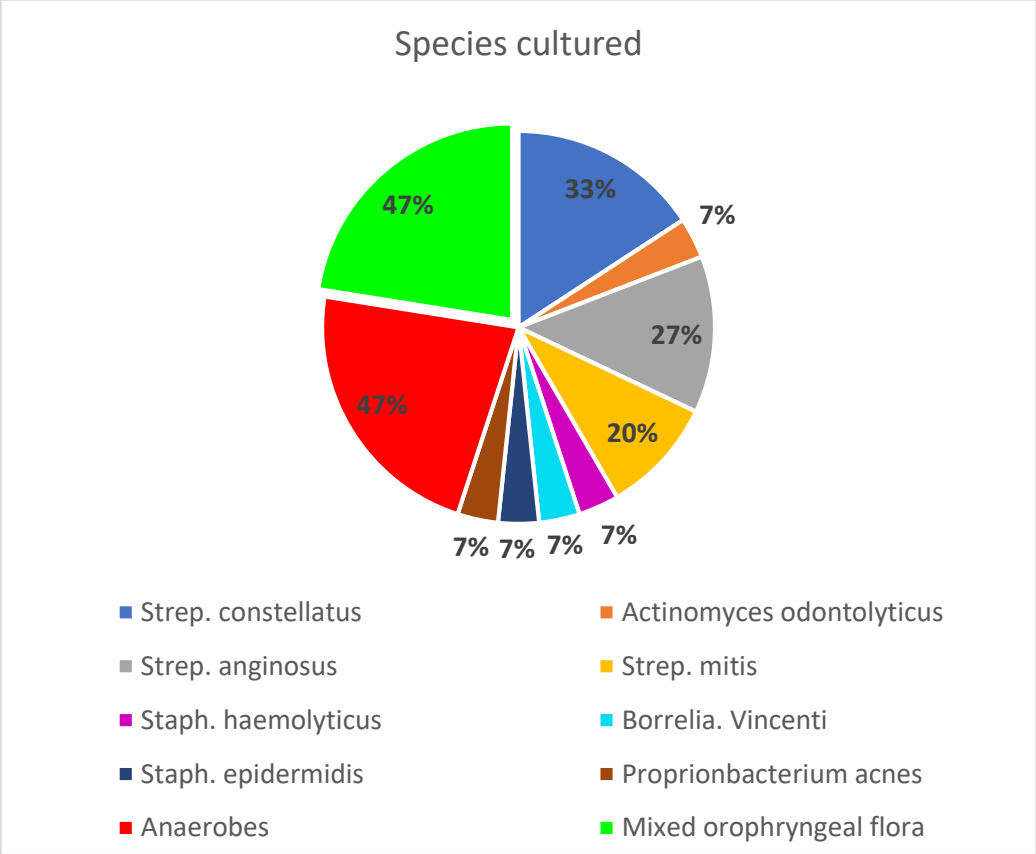


Figure 41: Proportion of microbial species cultured from 15 samples

When the species cultured from the patients on bisphosphonates were compared with those on Denosumab, there would appear to be little difference in the species cultures (Table 27). Similarly, when the oncology patients were compared with osteoporotic, there was no gross difference in the species isolated. However, samples from the oncology group returned more positive results for anaerobes, and pure growth of Strep. constellatus and Strep. mitis species. Eight of the 15 samples provided results on sensitivity, all of which were sensitive to Penicillin. Two samples reported

resistance of Strep. anginosus to Erythromycin, Clarythromycin, and probably Clindamycin.

Species cultured	Bp	Deno	Osteo	Oncology	Sensitivity	Resistance
Strep. constellatus	20%	13.3%	6.6%	26.6%	Penicillin	
Actinomyces odontolyticus	6.6%		6.6%		Penicillin	
Strep. anginosus	13%	13.3%	6.6%	20%	Penicillin	Erythromycin Clarythromycin Clindamycin
Strep.mitis	13.3%	6.6%	13.3%	6.6%	Penicillin Amoxicillin	
Staph.haemolyticus	6.6%			6.6%		
Borrelia vincenti	6.6%		6.6%			
Staph.epidermidis	6.6%			6.6%		
Proprionbacterium acnes	6.6%			6.6%		
Anaerobes	26.6%	20%	13.3%	33.3%	Amoxicillin Penicillin Ceftriaxone	
Mixed oropharyngeal flora	46.6%	20%	40%	26.6%		

Table 27: Microscopic results of culture and sensitivity testing

4.2 Part 2: Results from the questionnaire

4.2.1 Number of respondents

A total of 70 blank questionnaires were sent out, and 41 (59%) completed questionnaires were returned. A representative sample from each level of hierarchy was achieved (Figure 42).

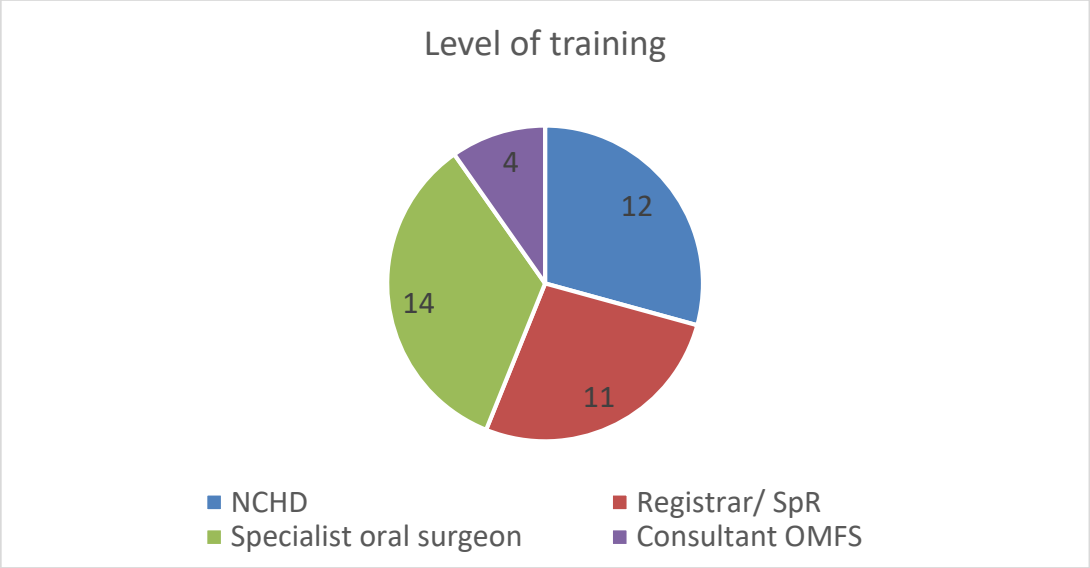


Figure 42:The breakdown of respondents by role

4.2.2 Knowledge of International or local guidance resources

The respondents were aware of various International, and local guidance resources available for the management of MRONJ (Figure 43). The AAOMS (2014) position paper was referenced by most respondents (78%). Interestingly, 36% mentioned the SDCEP (2017) guidance, however this guidance paper is a risk assessment tool for the development of MRONJ and does not advise on the management of established disease. A chi-square test was performed to examine the relation between staff (Consultant OMFS and Specialist Oral Surgeon) with trainees (Registrar, SPR and NCHDs) and their awareness of the AAOMS, SDCEP, and JIDA guidelines. The relation between these variables was not significant, chi-square = 1.32, p = 0.517.

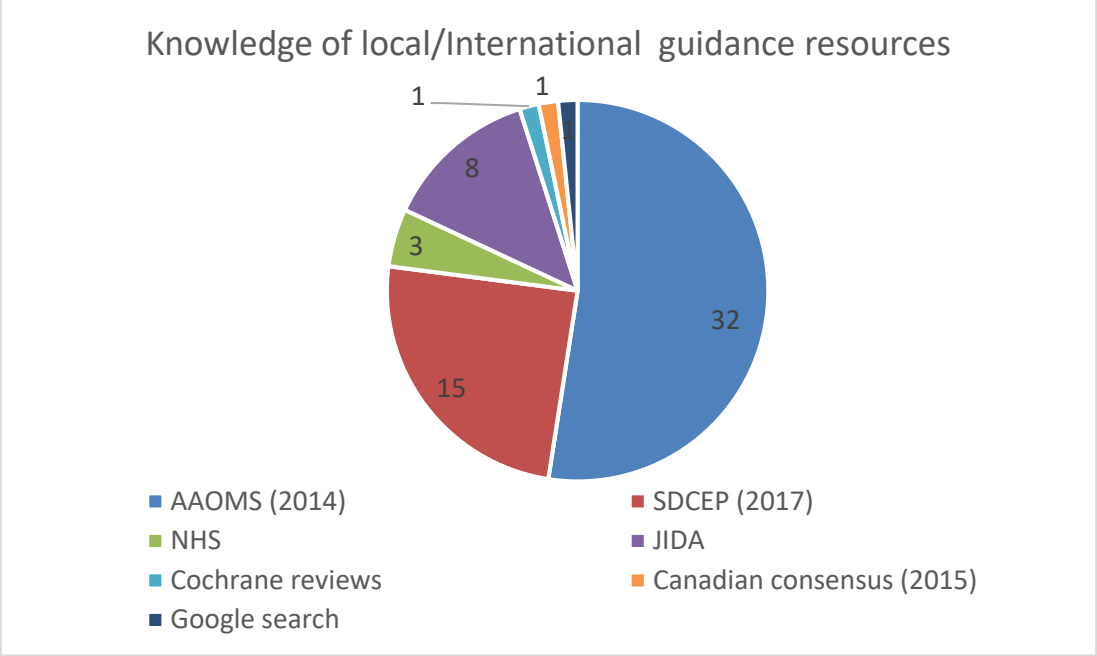


Figure 43: Awareness of various International, and local guidance resources available for the management of MRONJ

4.2.3 Guidance used by clinicians

Respondents were asked if they followed a specific guideline (n=40). Seventy percent followed a guideline, whereas 30% followed no guidance (Figure 44). A chi-square test was performed to examine the relation between staff (Consultant OMFS and Specialist Oral Surgeon) with trainee (Registrar, SPR and NCHD), and use of any guideline. The relation between these variables was not significant, chi-square = 0.394, p = 0.530.

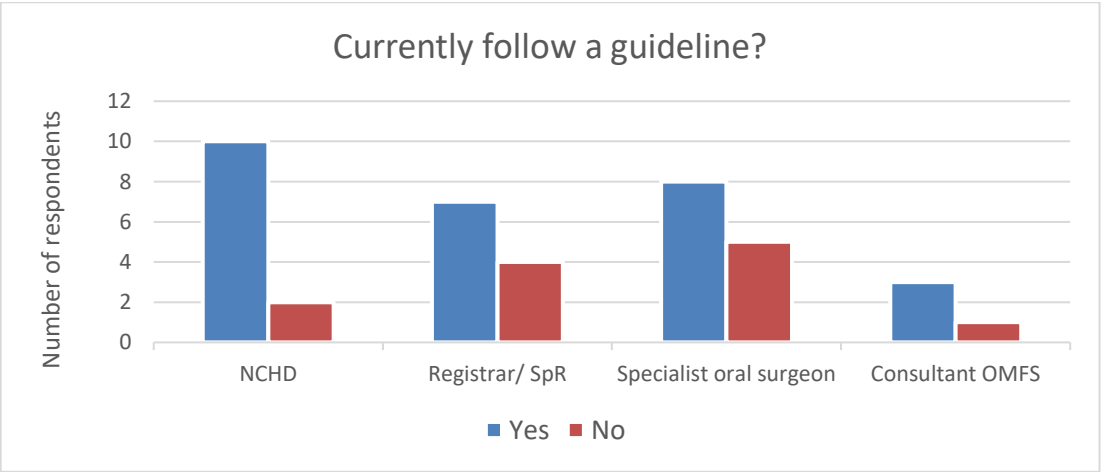


Figure 44: Number of respondents that follow a guideline, and number who do not

4.2.4 Which guidance is used by clinicians?

Respondents who followed a guideline were asked which guidance specifically they apply to their practice (n=28) (Figure 45). Sixty-eight percent used the AAOMS (2014) guidance only, and was mostly used by all staff levels. Eighteen percent used a combination of the AAOMS (2014) guidance with local JIDA or SDCEP (2017) guidance. Eleven percent used local JIDA guidance, and 3% used SDCEP (2017) guidance. A chi-square test was performed to examine the relation between staff and trainees, and the use of 1, or more than 1 guideline. The relation between these variables was not significant, chi-square = 1.095, p = 0.259.

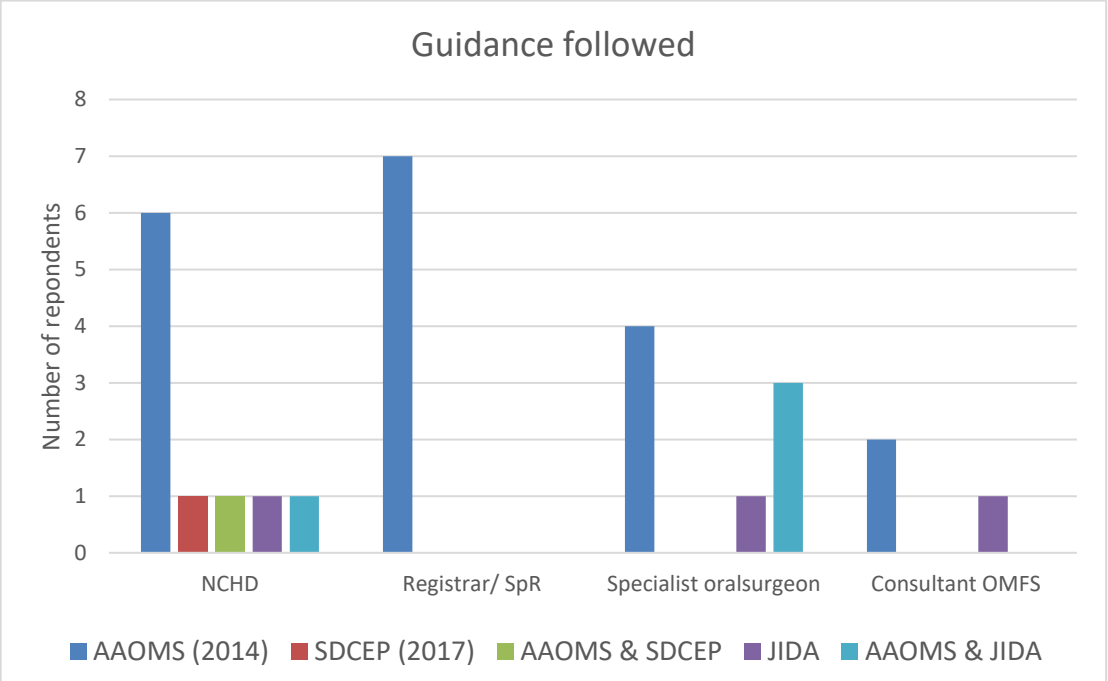


Figure 45: Number of respondents who follow a specific guidance

4.2.5 Independent treatment strategies

Of the 12 respondents who do not follow local or International guidance, they managed their cases with a variety of options mostly governed by patient symptoms, conservative treatment, and on a case by case basis (Figure 46).

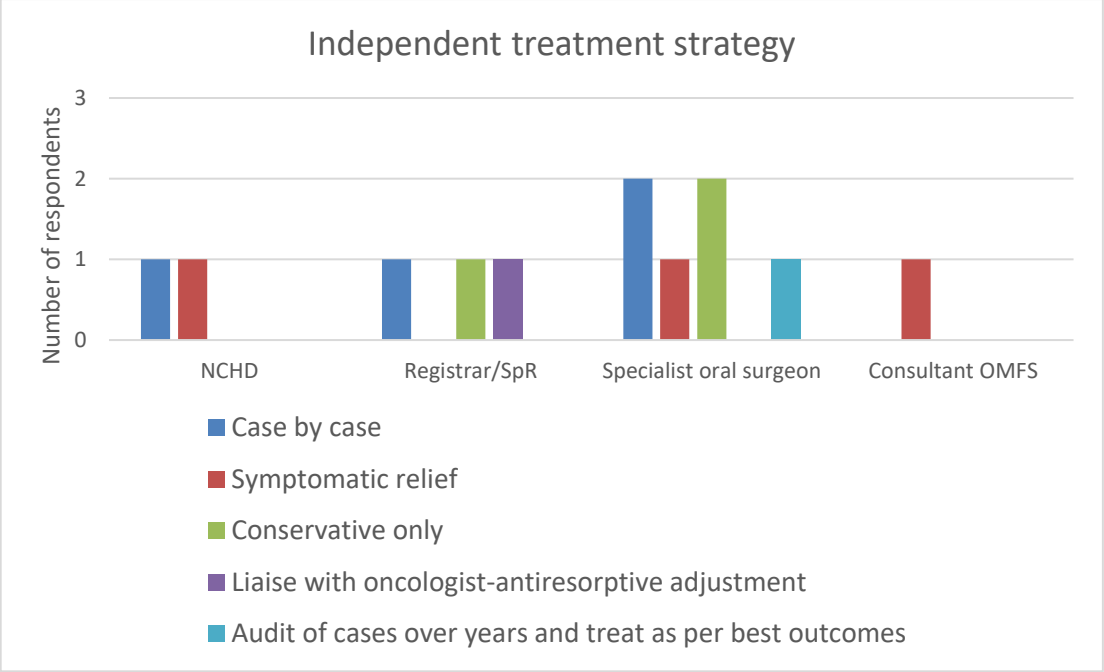


Figure 46: Strategies employed for the treatment of MRONJ by respondents who do not follow guidance

4.2.6 Current guidance relevant and comprehensive?

Most respondents found the current guidance resources comprehensive (65% strongly agreed/agree), however 35% disagreed or remained neutral (Figure 47). None strongly disagreed with the statement that the resources were comprehensive. Total number of respondents n=37. A chi-square test was performed to examine the relation between staff and trainee opinion of guidelines being relevant and comprehensive. The relation between these variables was not significant, chi-square = 0.0692, p = 0.792.

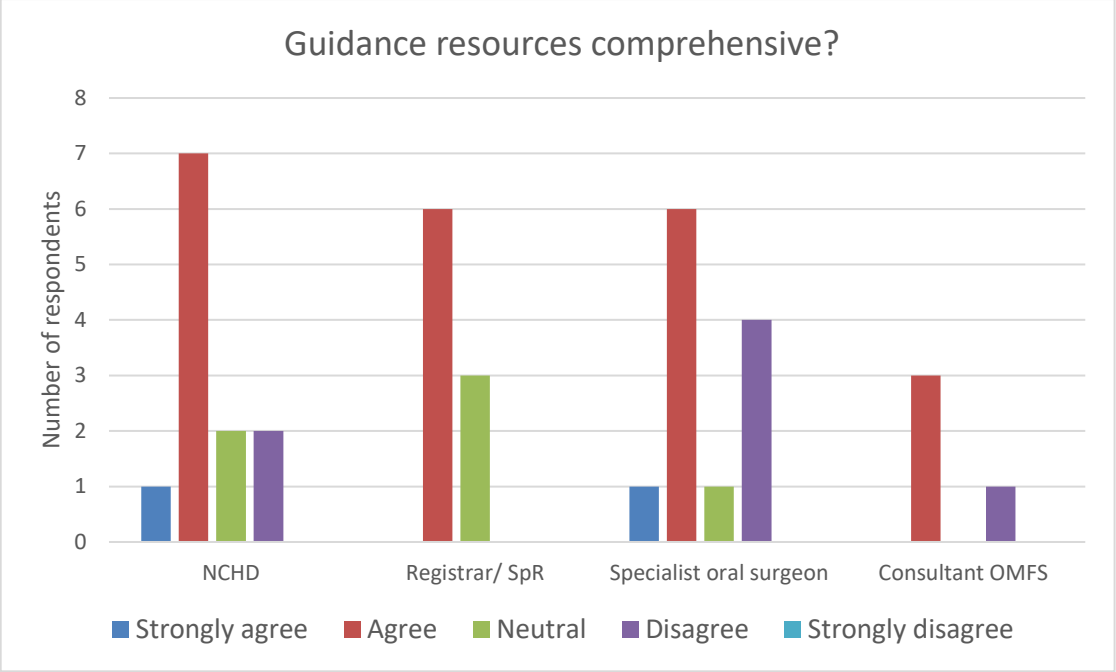


Figure 47: Attitude of respondents to current guidance resources available

4.2.7 Problems with current guidance resources

Respondents were asked what the key issues with the current guidance resources were (Figure 48). Lack of evidence base was the main barrier (n=6).

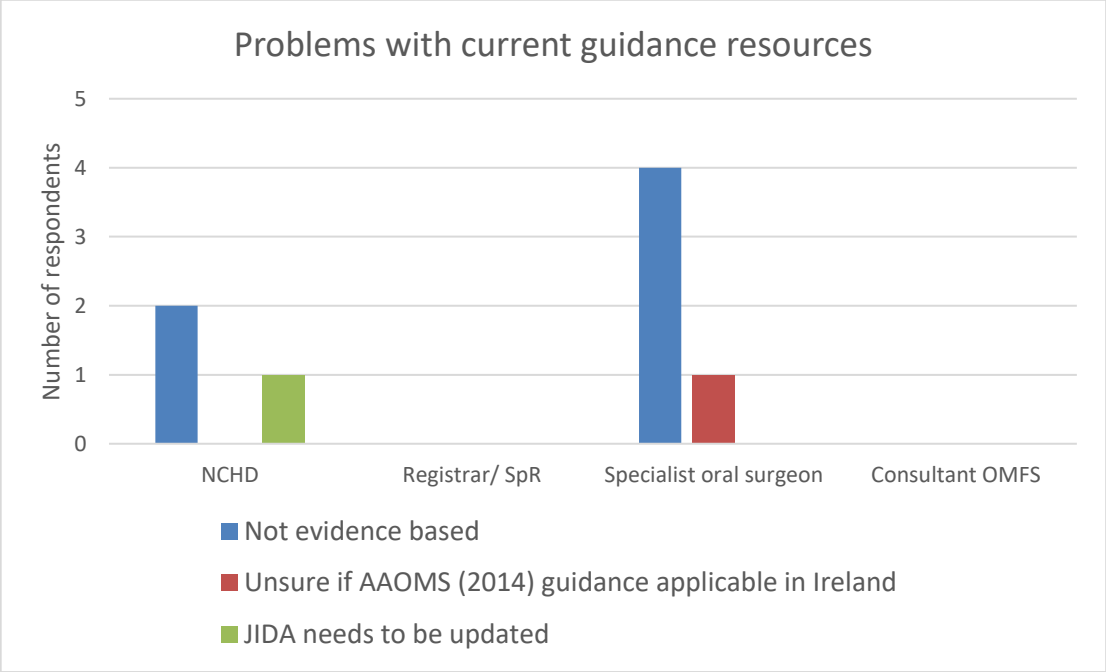


Figure 48: Problems with current guidance resources cited by respondents

4.2.8 Comfortable managing MRONJ?

Clinicians were asked if they were comfortable managing patients with MRONJ (n=41) (Figure 49). Forty-nine percent strongly agreed or agreed with this statement. Fifty-one percent disagreed, strongly disagreed, or remained neutral. A chi-square test was performed to examine the relation between staff and trainees, and the level of confidence managing MRONJ. The relation between these variables was not significant, chi-square = 0.5895, p = 0.443. This may indicate that the lack of confidence in treating MRONJ is not due to level of training and skills of the operator.

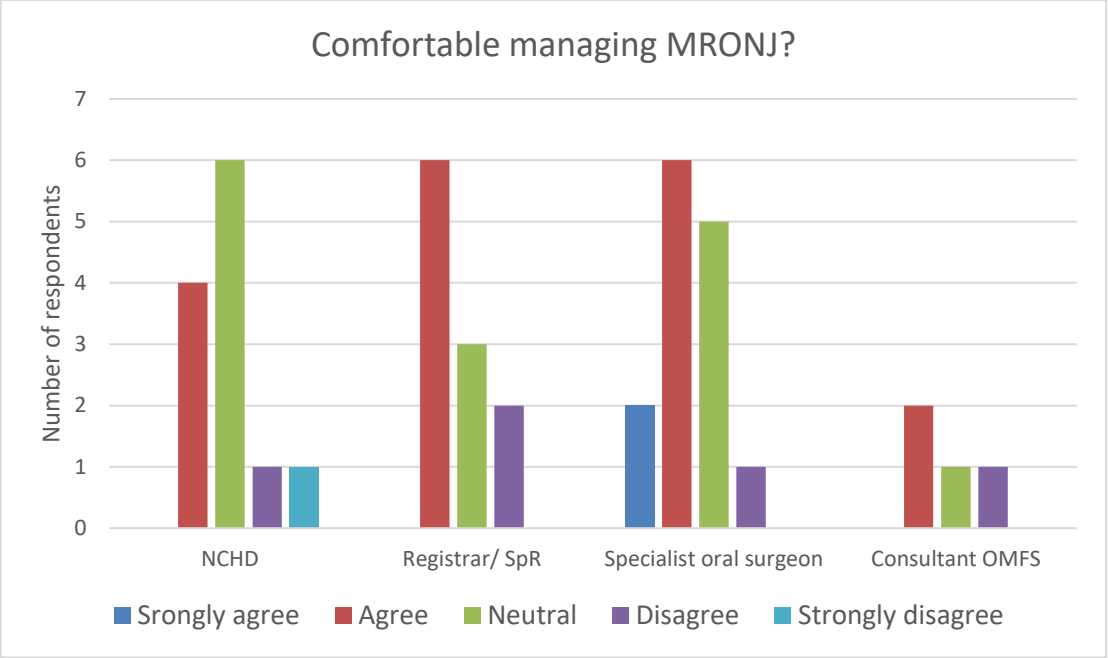


Figure 49: Attitude of respondents to managing patients with established MRONJ

4.2.9 Barriers to managing MRONJ

Respondents felt that the main barriers were poor evidence base, unpredictable outcomes, minimal exposure to MRONJ, and difficulties managing Stage 3 disease (n=13) (Figure 50).

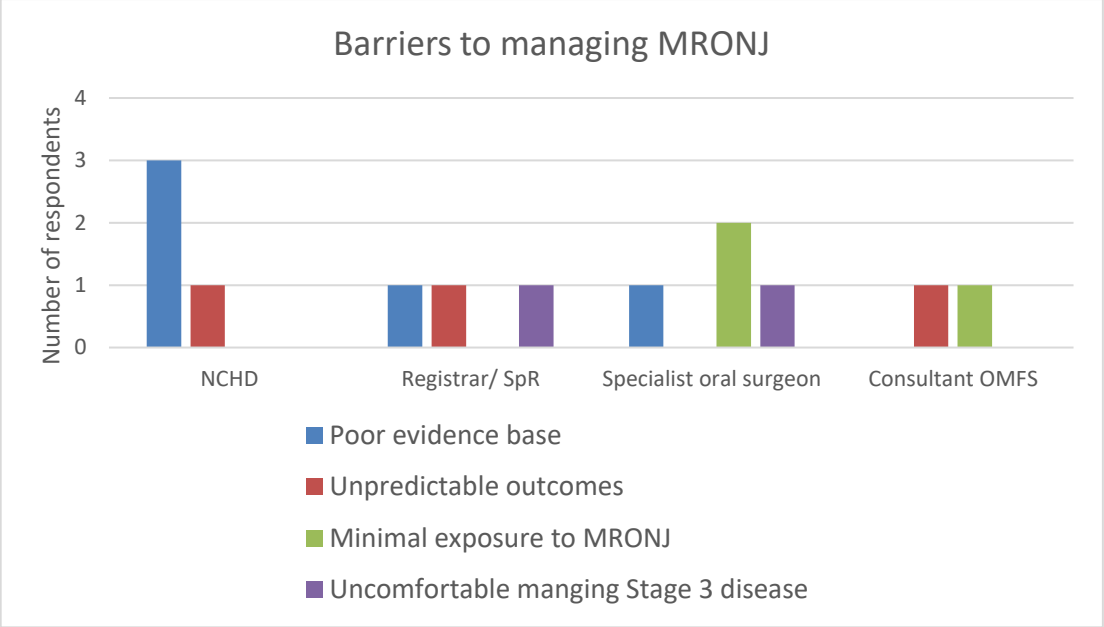


Figure 50: Barriers identified by respondents to managing patients with MRONJ

4.2.10 Case 1 Management

Case one was diagnosed with Stage 2 MRONJ. Seventy-six percent of respondents would treat this case with a non-surgical conservative approach (n=31), and twenty-four percent would treat the case with surgery (n=10) (Figure 51). In keeping with the AAOMS (2014) guidance this should be treated with local measures, with topical and systemic antimicrobials, however this case was treated successfully within this study. A Fisher Exact test was performed to examine the relation between staff and trainees choosing conservative treatment with topical and systemic antimicrobials or surgical intervention. The relation between these variables was not significant, $p = 0.0823$

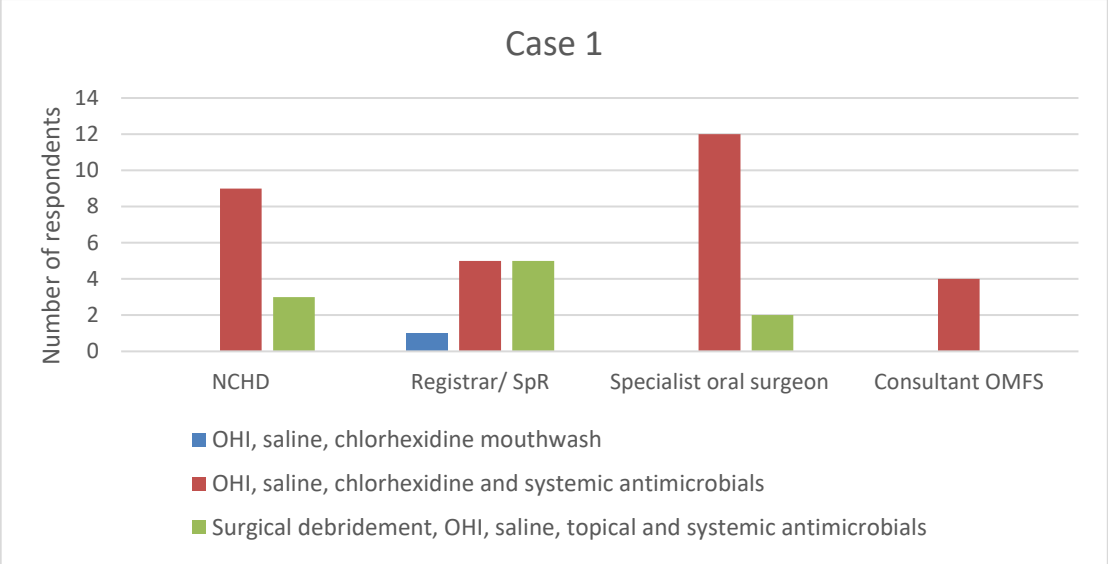


Figure 51: Case 1 treatment strategy adopted by respondents

4.2.10.1 Case 1 non-surgical prescribing

Of the 30 respondents that would prescribe antimicrobials (no surgery); 23% prescribed Amoxicillin, 30% Clindamycin, 10% Doxycycline, 23% Co-amoxiclav, 7% a combination of Amoxicillin and Metronidazole, and 7% a combination of Co-amoxiclav and Metronidazole (Figure 52). There was a wide variation in the duration of prescribing ranging from 5 days to over 3 months, with a median duration of 7 days.

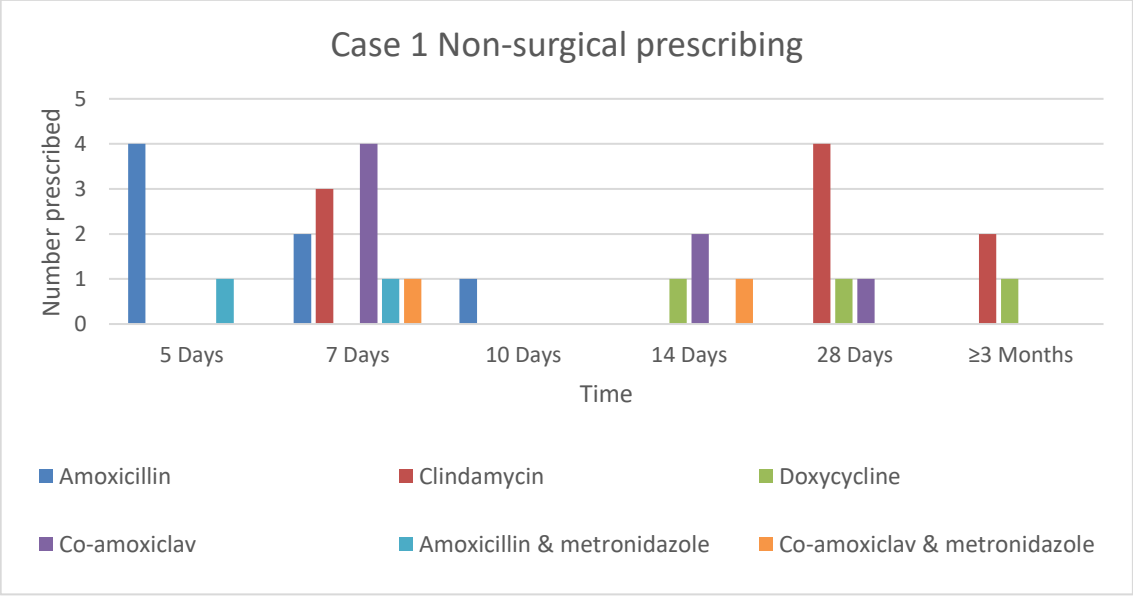


Figure 52: Case 1 Non-surgical antibiotic prescribed by respondents

4.2.10.2 Case 1 Non-surgical dose

The dose of non-surgical antibiotic largely adhered to the recommended oral dose to treat oral infection as per HSE guidance (2016) and SDCEP (2016) for Amoxicillin, Co-amoxiclav and when combining Amoxicillin or Co-amoxiclav with Metronidazole (Figure 53). However, variation was observed in the doses of Clindamycin and Doxycycline prescribed.

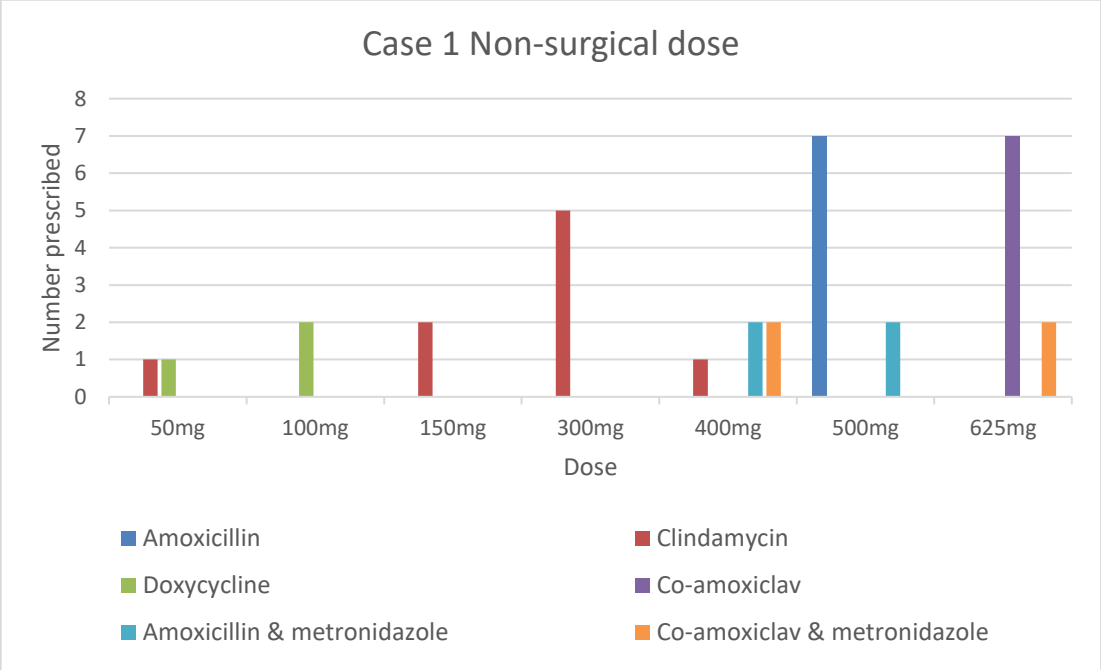


Figure 53: Case 1 Non-surgical antibiotic dose prescribed by respondents

4.2.10.3 Case 1 Pre-surgical antibiotic

Of the 10 clinicians that would treat Case 1 surgically, 9 would give an oral loading dose, while 1 opted parenterally. There was a wide variation in antibiotic choice and dose (Figure 54). The JIDA (2010, 2017) guidance advocated Amoxicillin 3g prior to surgical treatment, however only 3 respondents adhered to this guidance.[12, 30] Other antibiotic choices included; Doxycycline, Co-amoxiclav, Flucloxacillin with Benzylpenicillin or Co-amoxiclav with Metronidazole.

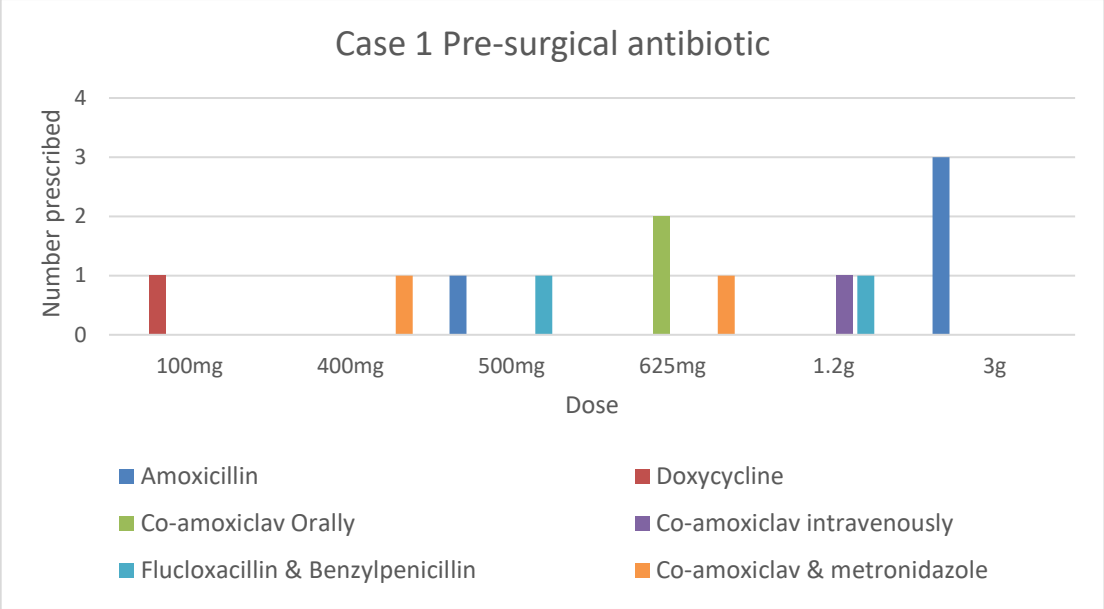


Figure 54: Case 1 Pre-surgical antibiotic prescribed by clinicians

4.2.10.4 Case 1 Post-surgical antibiotic

Post-operatively, the duration post-surgical antibiotics varied widely from 5 days to 6 weeks with a median duration of 7 days (Figure 55). Clinicians prescribed Amoxicillin, Clindamycin, Co-amoxiclav, or a combination of Flucloxacillin with Penicillin V, or Metronidazole with Amoxicillin/Co-amoxiclav. Novel combinations of Co-amoxiclav and Metronidazole were prescribed by one operator for 7 days with a further 28 days of Lymecycline.

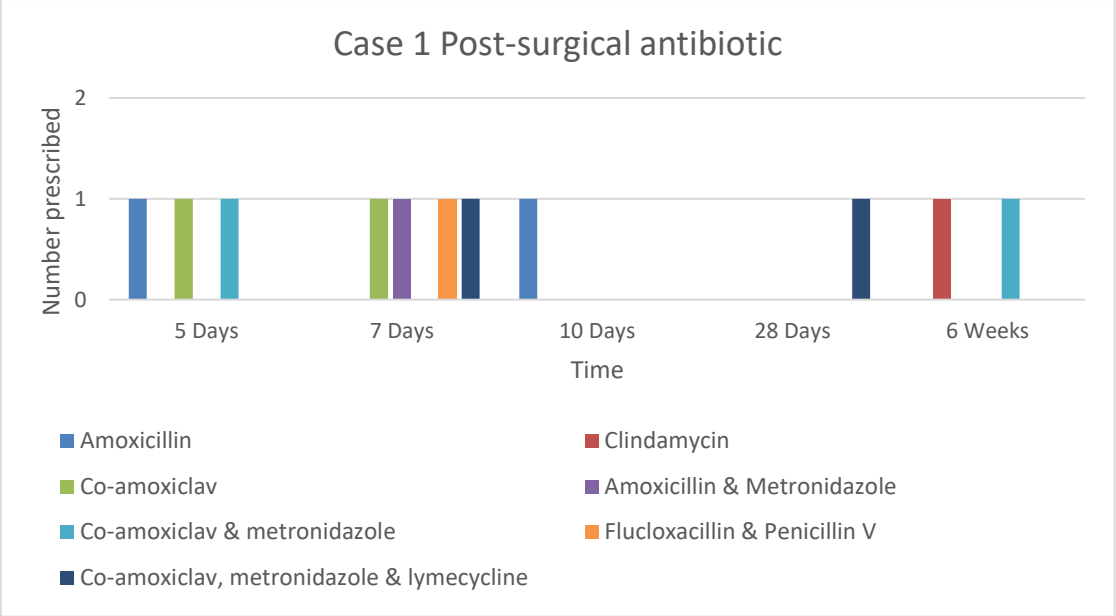


Figure 55: Case 1 post-surgical antibiotic prescribed by respondents

4.2.10.5 Case 1 Post-surgical antibiotic dose

The post-operative surgical antibiotic dose was in keeping with normal prescribed dosing regimens (Figure 56).

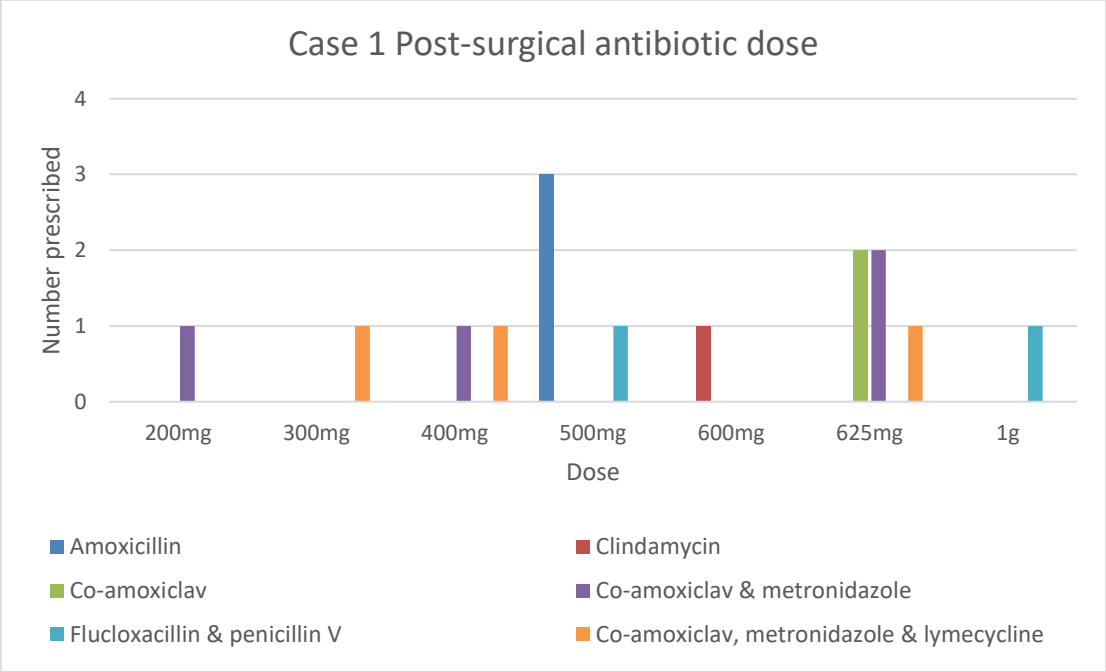


Figure 56: Case 1 post-operative surgical antibiotic dose prescribed by respondents

4.2.11 Case 2 Management

Case 2 was diagnosed with Stage 3 MRONJ as it extended beyond alveolar bone. Seventy-three percent of respondents would treat this case with surgery (n=30) (Figure 57). Fifteen percent would treat the case with saline, topical antimicrobial mouthwash (n=6). Ten percent would treat with topical and systemic antimicrobials (n=4) and 2% would refer to OMFS (n=1). In keeping with the AAOMS (2014) guidance this should be treated with surgery, and this case was treated successfully within this study. A Fisher Exact test was performed to examine the relation between staff and trainees choosing conservative treatment with topical antimicrobials, or local measures and systemic antimicrobials. The relation between these variables was not significant, p = 0.5714. A Fisher Exact test was performed to examine the relation between staff and trainees choosing conservative treatment with topical antimicrobials or surgery. The relation between these variables was not significant, p = 0.6582. A Fisher Exact test was performed to examine the relation between staff and trainees choosing conservative treatment with topical and systemic antimicrobials, or surgery. The relation between these variables was not significant, p = 0.283.

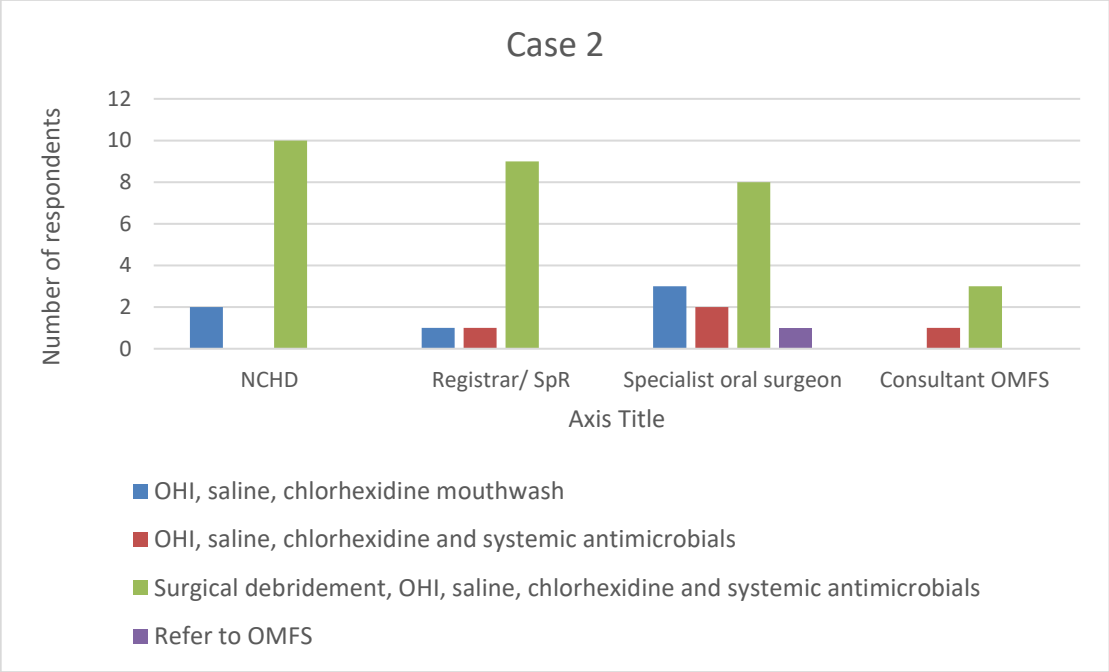


Figure 57: Case 2 treatment strategy adopted by respondents

4.2.11.1 Case 2 Non-surgical prescribing

Of the four patients who were prescribed conservative management with systemic antimicrobials, all were in-keeping with commonly prescribed antibiotics and durations for the management of MRONJ, except Lymecycline (Figure 58).[87] The median duration of prescribing was 28 days, with a range of 7 days to over three months.

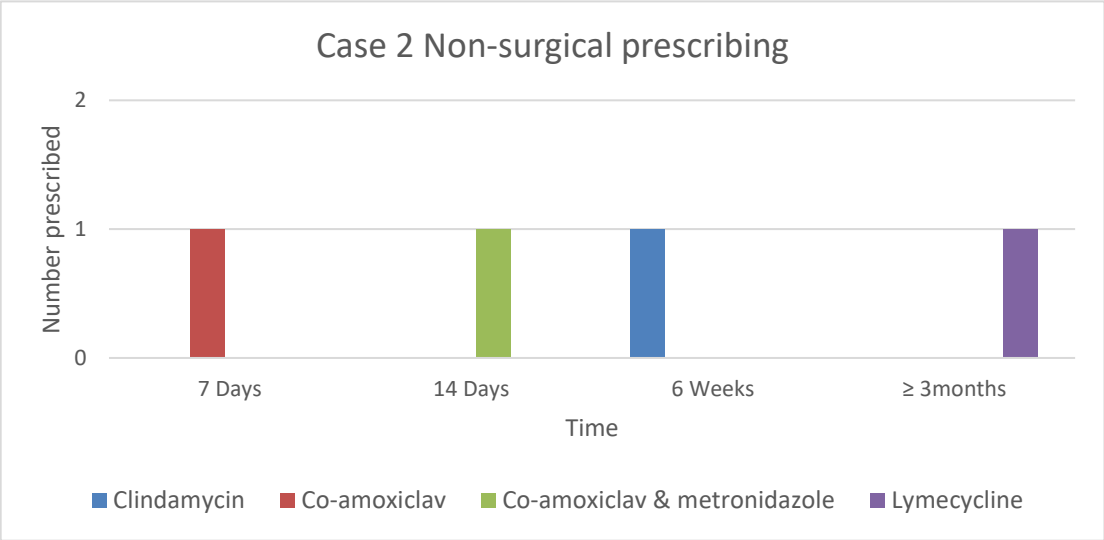


Figure 58: Case 2 Non-surgical antibiotic prescribed by respondents

4.2.11.2 Case 2 Non-surgical dose

The dosages prescribed were in accordance with normal dosing regimens (Figure 59).

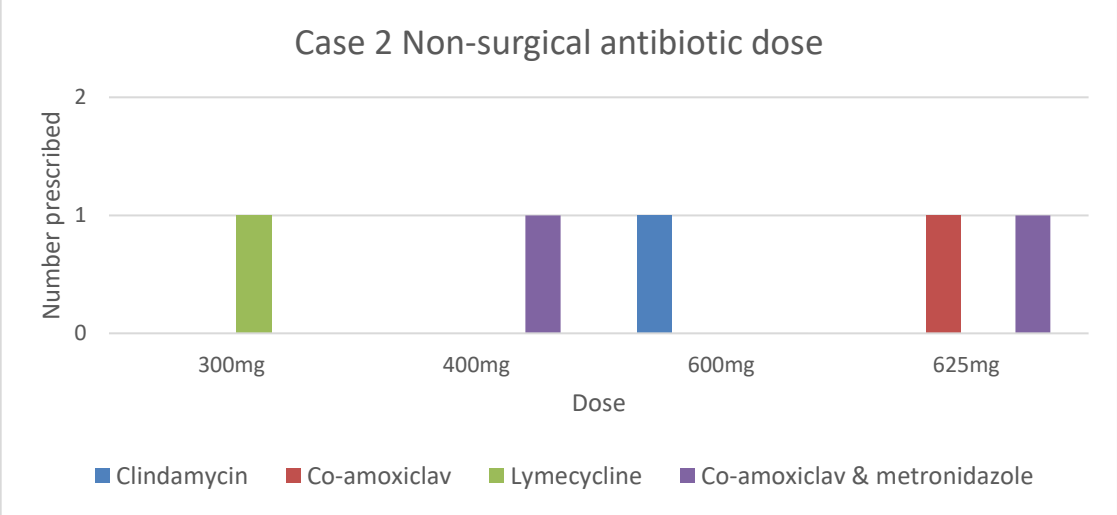


Figure 59: Case 2 Non-surgical antibiotic dose prescribed by respondents

4.2.11.3 Case 2 Pre-surgical antibiotic

Of the 30 respondents that managed the case with surgery, 47% adhered to JIDA (2010, 2017) guidance prescribing a 3g or 2g loading dose of Amoxicillin (n=14), and 2 prescribed a 500mg preoperative dose (Figure 60).[12, 30] Twenty-three percent prescribed Co-amoxiclav (n=7), and 7% Clindamycin (n=2). Other antibiotic choices included Doxycycline, or a combination of Benzylpenicillin and Flucloxacillin, Amoxicillin and Metronidazole, or Co-amoxiclav and Clindamycin. Twenty percent opted for parenteral, and 80% oral formulations.

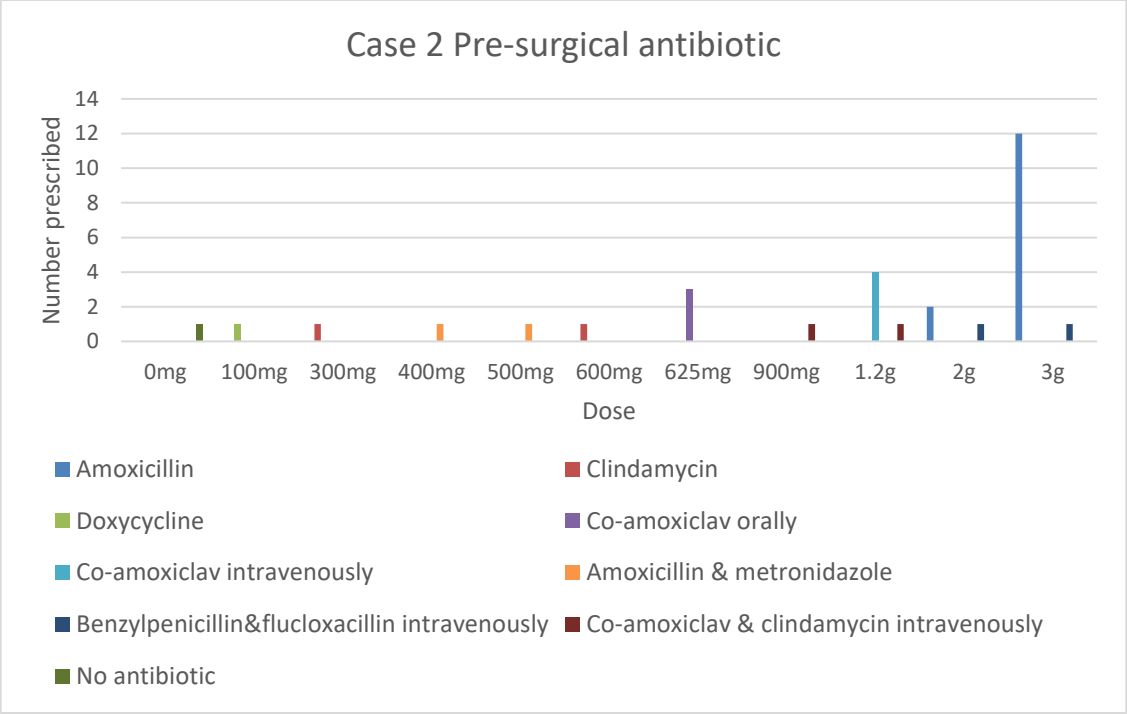


Figure 60: Case 2 Pre-surgical antibiotic prescribed by respondents

4.2.11.4 Case 2 Post-surgical antibiotic

Post-operatively 47% prescribed Amoxicillin, 20% Co-amoxiclav, and 13% Clindamycin (Figure 61). Combinations of antibiotics included Amoxicillin (7%) or Co-amoxiclav (7%) with Metronidazole, Co-amoxiclav with Clindamycin (3%) or Doxycycline (3%). All respondents opted for the oral formulation. Duration of prescribing varied widely from 5 days to over 3 months, with a median duration of 7 days.

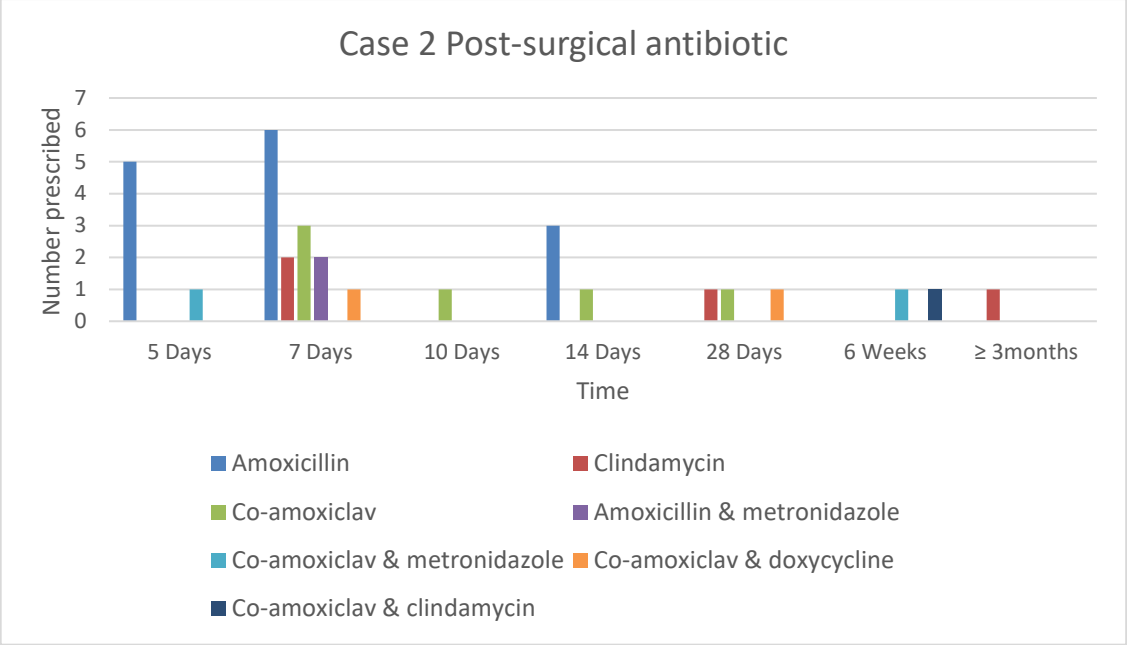


Figure 61: Case 2 post-surgical antibiotic prescribed by respondents

4.2.11.5 Case 2 Post-surgical antibiotic dose

The dosages prescribed were in accordance with normal dosing regimens (Figure 62).

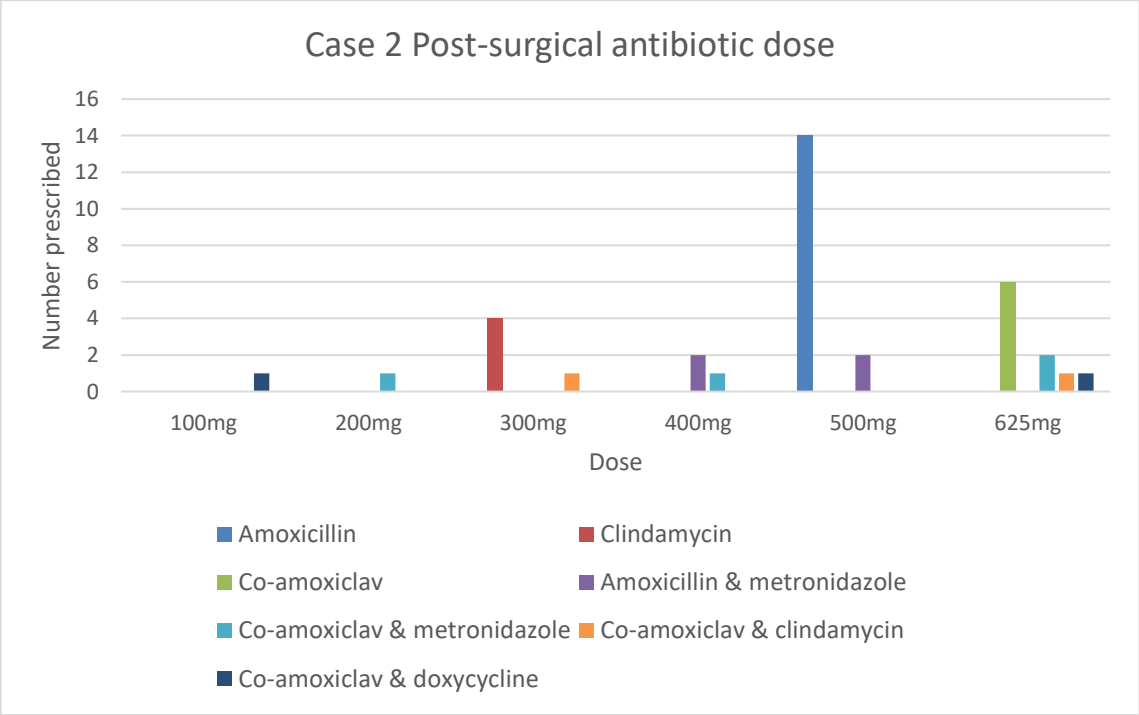


Figure 62: Case 2 post-surgical antibiotic dose prescribed by respondents

4.2.12 Case 3 Management

Case 3 was diagnosed with Stage 3 MRONJ, as it extended beyond alveolar bone into basal bone. Five percent of respondents would treat this case with conservative local measures and smoking cessation advice (n=2) (Figure 63). Sixty-three percent would treat the case with conservative local measures and systemic antimicrobials (n=26), and 43% would treat the case with surgery, local measures, and systemic antimicrobials (n=13). In keeping with the AAOMS (2014) guidance this should be treated with surgery, topical, and systemic antimicrobials. This case failed to heal within this study, however it reverted to asymptomatic Stage 1 disease and showed good bony infill at the end-point. A Fisher Exact test was performed to examine the relation between staff, and trainees choosing conservative non-surgical treatment with topical and systemic antimicrobials or surgical intervention. The relationship between these variables was not significant, p = 0.307

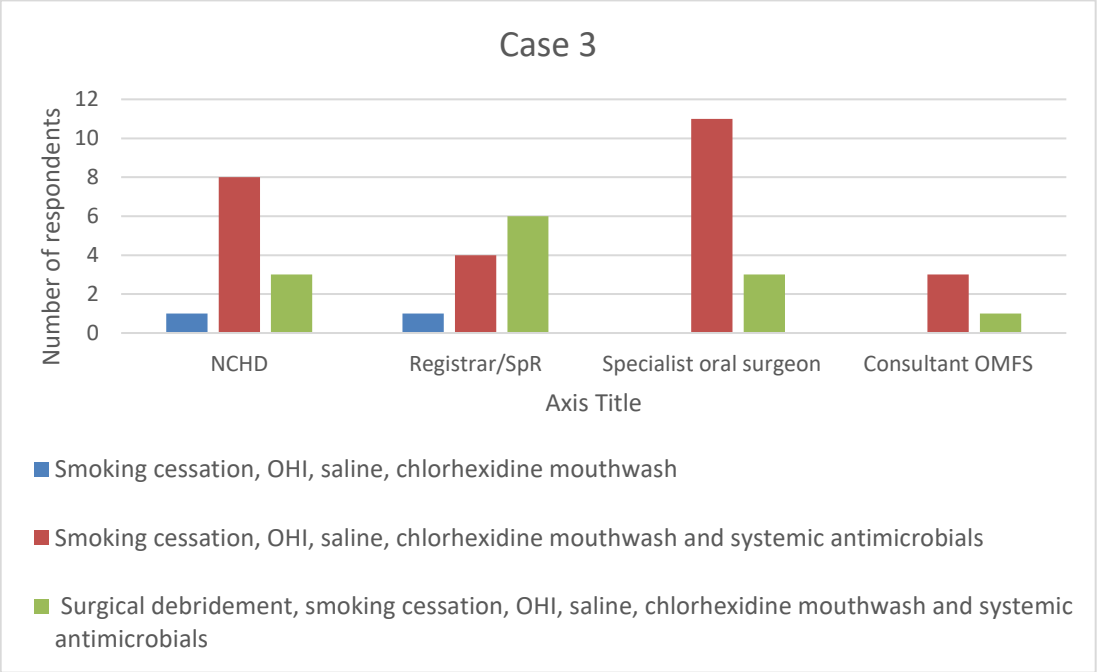


Figure 63: Case 3 treatment strategy adopted by respondents

4.2.12.1 Case 3 Non-surgical prescribing

A wide variation in antibiotic choice for non-surgical medical management was recorded (Figure 64). Co-amoxiclav was prescribed by 31% of respondents, Clindamycin 26%, Doxycycline 15%, and Amoxicillin 8%. Combinations of Amoxicillin (8%) or Co-amoxiclav (8%) with Metronidazole, and Flucloxacillin and Penicillin V (4%) were prescribed. A wide variation of duration of antibiotic ranged from 5 days to ≥3 months with a median duration of 14 days.

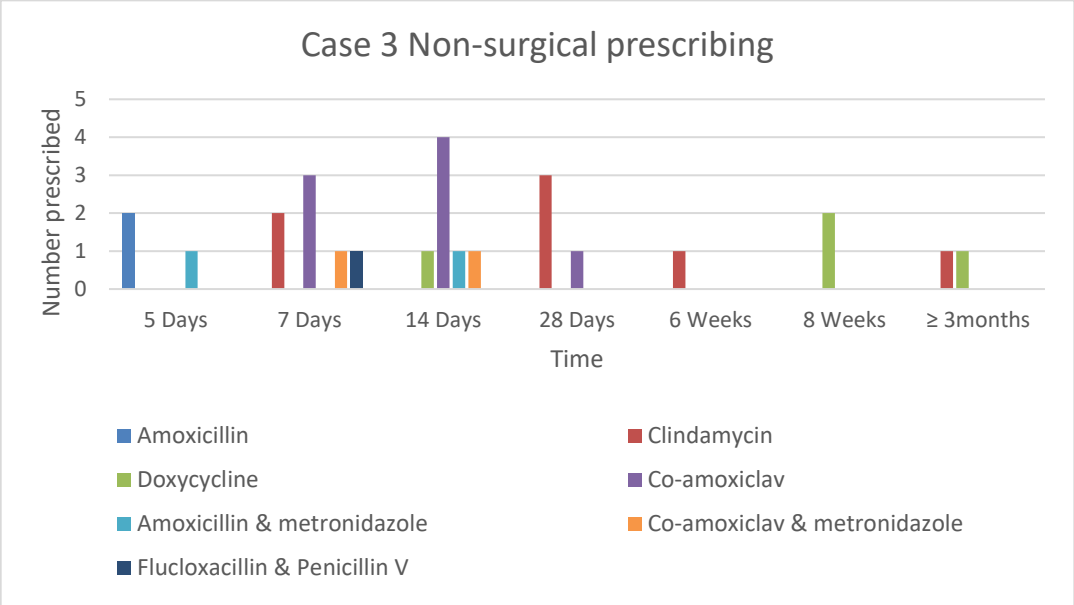


Figure 64: Case 3 non-surgical antibiotic prescribed by respondents

4.2.12.2 Case 3 Non-surgical antibiotic dose

The dosages prescribed were in accordance with normal dosing regimens (Figure 65).

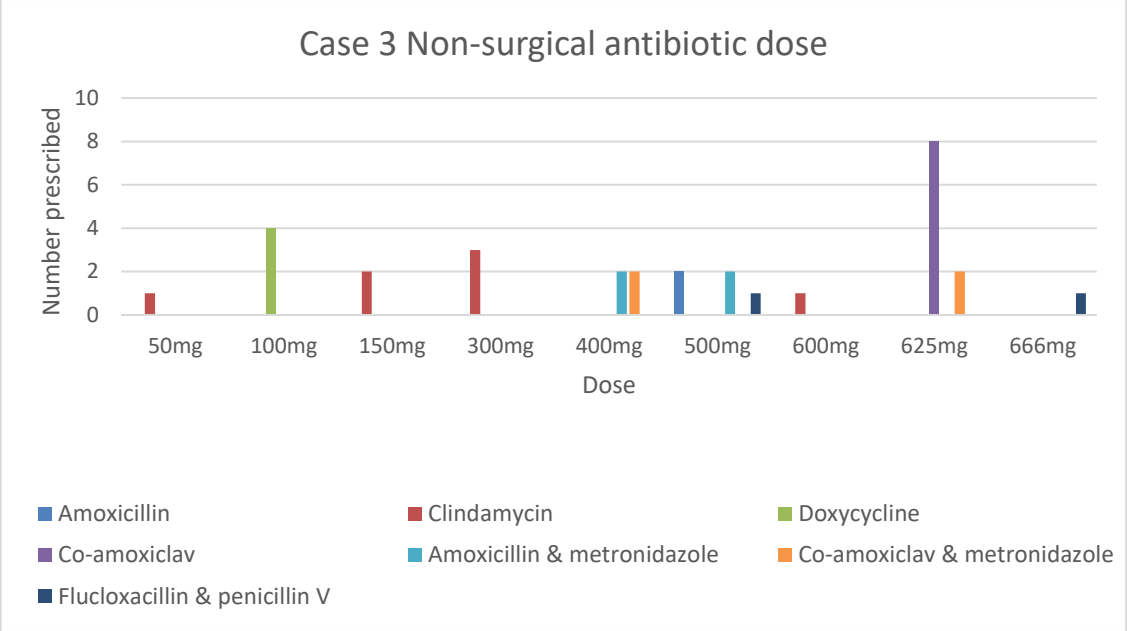


Figure 65: Case 3 non-surgical antibiotic dose prescribed by respondents

4.2.12.3 Case 3 Pre-surgical antibiotic

Of the 13 respondents that managed this case with surgery, 54% adhered to JIDA (2010, 2017) guidance prescribing a 2g or 3g loading dose of Amoxicillin (n=7) (Figure 66).[12 , 30] Twenty-three percent prescribed Co-amoxiclav (n=3), 8% Doxycycline (n=1) and 8% Benzylpenicillin and Flucloxacillin (n=1). Fifteen percent opted for parenteral, and 75% for oral formulations.

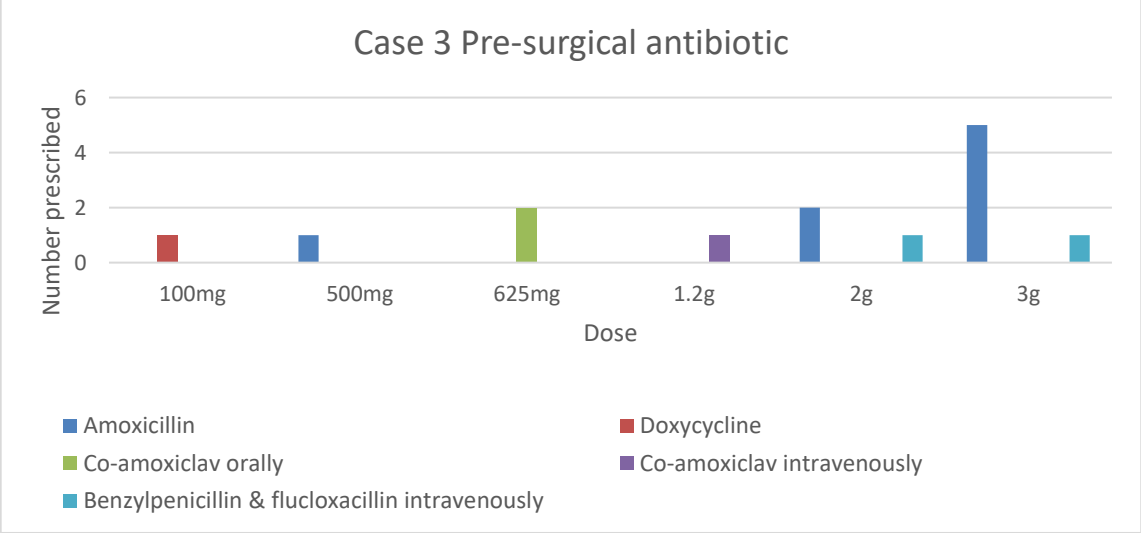


Figure 66: Case 3 pre-surgical antibiotic prescribed by clinicians

4.2.12.4 Case 3 Post-surgical antibiotic

Post-operatively 38% prescribed Amoxicillin, 15% Co-amoxiclav, 15% Clindamycin and 8% Doxycycline. Combinations of antibiotics included Amoxicillin (8%) or Co-amoxiclav (8%) with Metronidazole, and Co-amoxiclav with Doxycycline (8%) (Figure 67). All respondents opted for the oral formulation. Duration of prescribing varied widely from 5 days to over 3 months, with a median duration of 7 days.

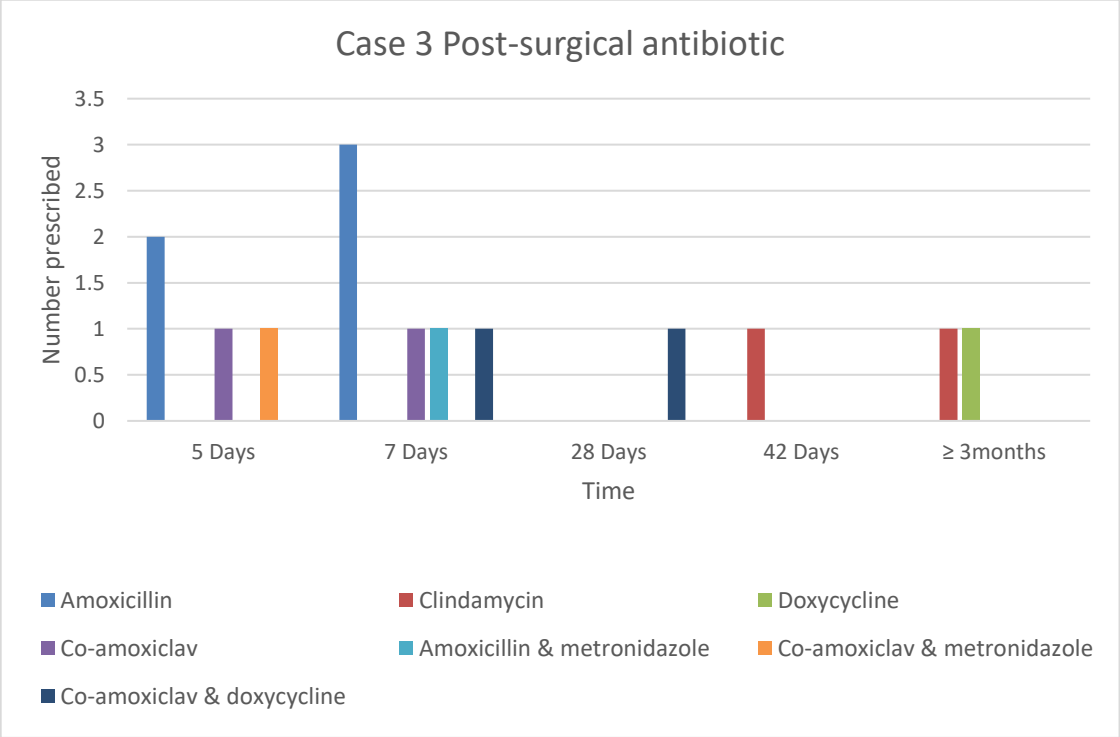


Figure 67: Case 3 post-surgical antibiotic prescribed by respondents

4.2.12.5 Case 3 Post-surgical antibiotic dose

The dosages prescribed were in accordance with normal dosing regimens (Figure 68).

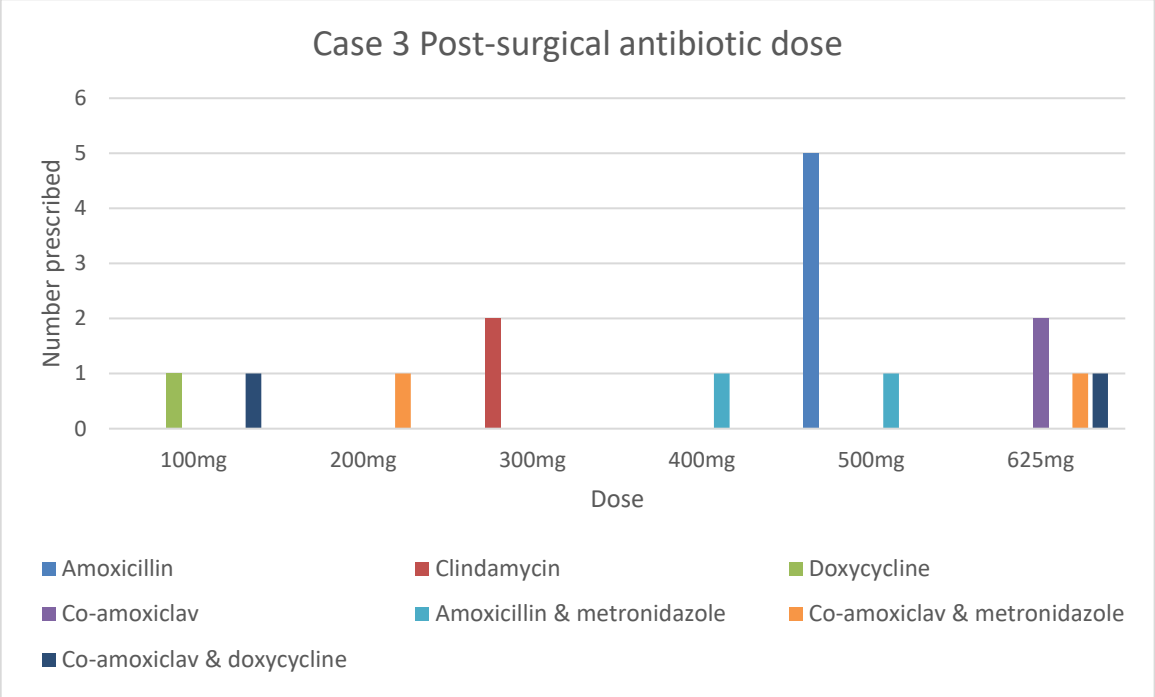


Figure 68: Case 3 post-surgical antibiotic dose prescribed by respondents

4.2.13 Case 4 Management

Case 4 was diagnosed with Stage 3 MRONJ, as it extended beyond alveolar bone. Two percent would manage this case with local measures alone, forty-four percent of respondents would treat this case with local measures, and systemic antimicrobials (n=18) (Figure 69). Fifty-four percent would treat the case with surgery (n=22). In keeping with the AAOMS (2014) guidance this should be treated with surgery, and this case was treated successfully within this study.[1] A chi-square test was performed to examine the relation between staff and trainees choosing conservative non-surgical treatment with topical and systemic antimicrobials, or surgical intervention. The relation between these variables was not significant, chi-square = 1.473, p = 0.229.

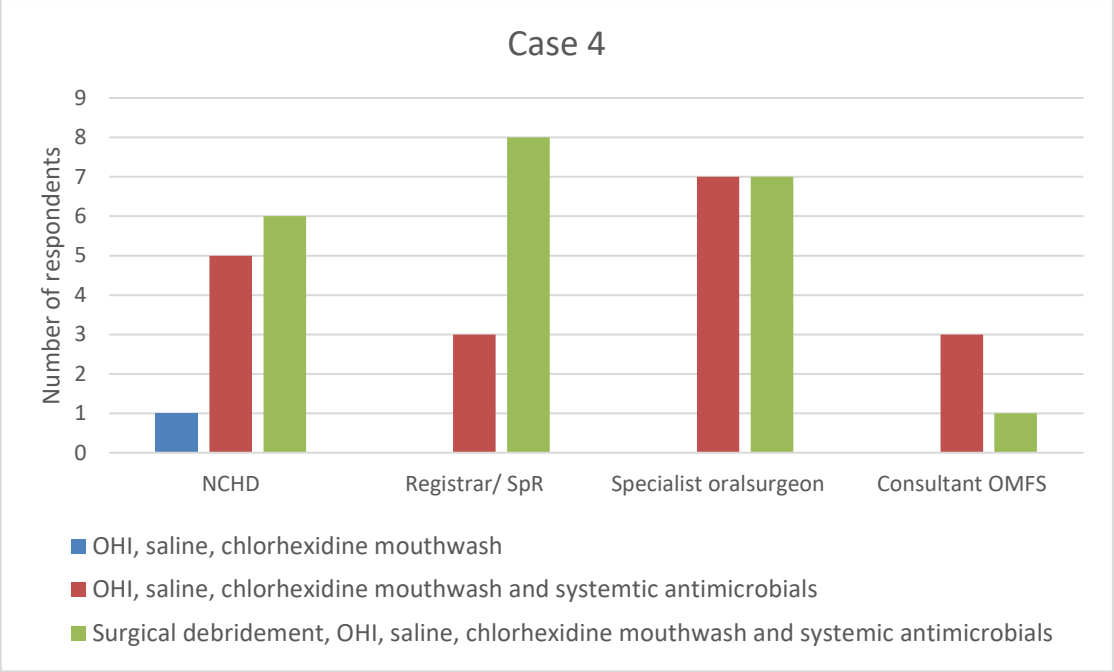


Figure 69: Case 4 treatment strategy adopted by respondents

4.2.13.1 Case 4 Non-surgical prescribing

A total of 18 respondents prescribed systemic antibiotics for the conservative management of case 4 (Figure 70). There were seven different antibiotics/combinations of medication prescribed; Co-amoxiclav (28.5%), Clindamycin (28.5%), Amoxicillin (17%), Doxycycline (11%), and combinations of Amoxicillin (5%) or Co-amoxiclav (5%) with Metronidazole, and Flucloxacillin and Penicillin V (5%). The median duration of prescribing was 10.5 days, with a range of 5 days to 8 weeks.

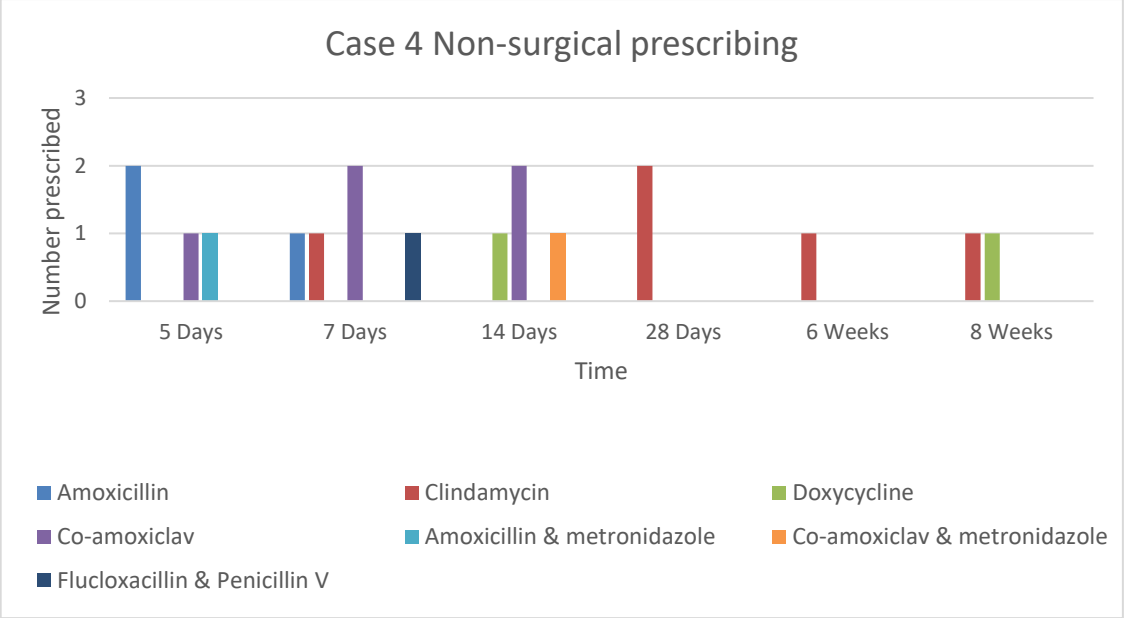


Figure 70: Case 4 non-surgical antibiotic prescribed by respondents

4.2.13.2 Case 4 Non-surgical antibiotic dose

The dosages prescribed were in accordance with normal therapeutic dosing regimens (Figure 71).

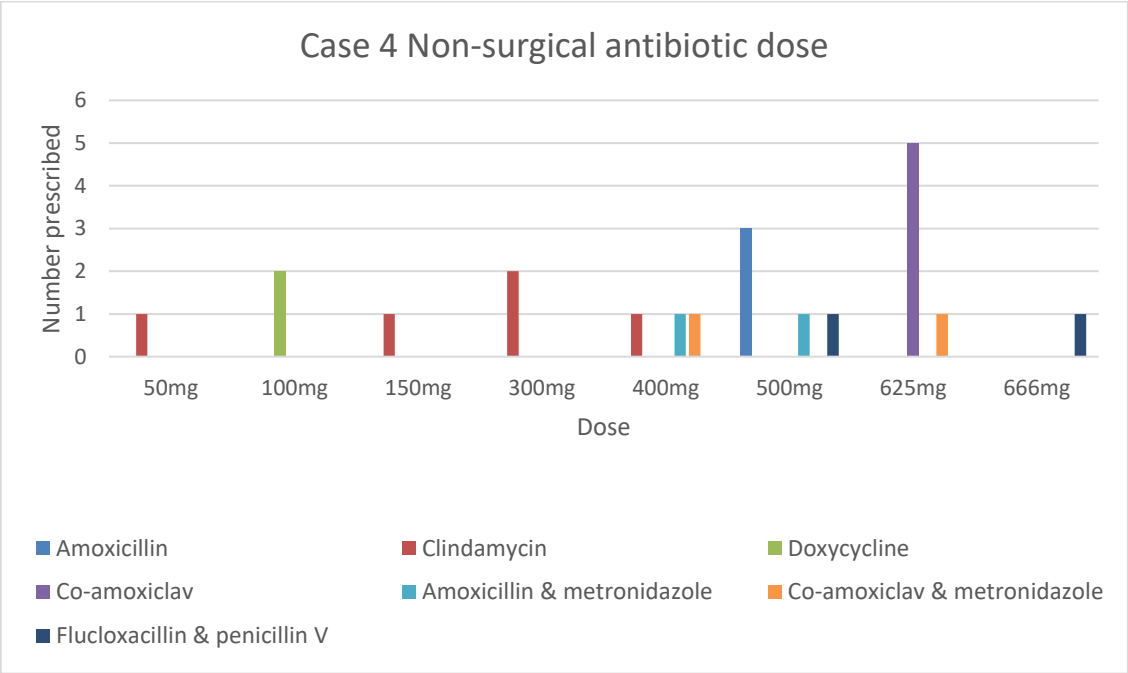


Figure 71: Case 4 non-surgical antibiotic dose prescribed by respondents

4.2.13.3 Case 4 Pre-surgical antibiotic

Of the 22 respondents that managed this case with surgery, 50% adhered to JIDA (2010, 2017) guidance prescribing a 2g, or 3g loading dose of Amoxicillin (n=7) (Figure 72).[12, 30] Twenty-three percent prescribed Co-amoxiclav (n=5), 14% prescribed Clindamycin (n=3), and 5% prescribed either Doxycycline, Amoxicillin and Metronidazole or Benzylpenicillin and Flucloxacillin (n=1). Eighteen percent opted for parenteral, and 82% for oral formulations.

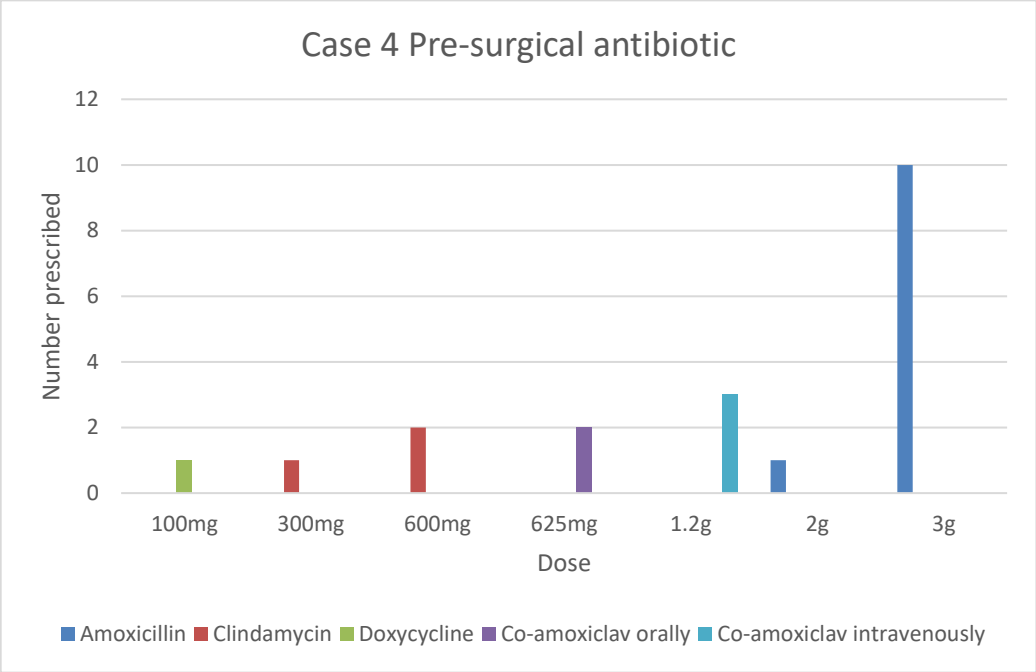


Figure 72: Case 4 pre-surgical antibiotic prescribed by respondents

4.2.13.4 Case 4 Post-surgical antibiotic

Post-operatively 32% prescribed Amoxicillin, 22% Clindamycin, and 18% Co-amoxiclav. Combinations of antibiotics included Amoxicillin and Metronidazole (5%), Co-amoxiclav with Metronidazole (9%) or Doxycycline (9%) or Metronidazole and Lymecycline (5%) (Figure 73). All respondents opted for the oral formulation. The median duration of prescribing was 7 days, with a range of 5 days to over 3 months.

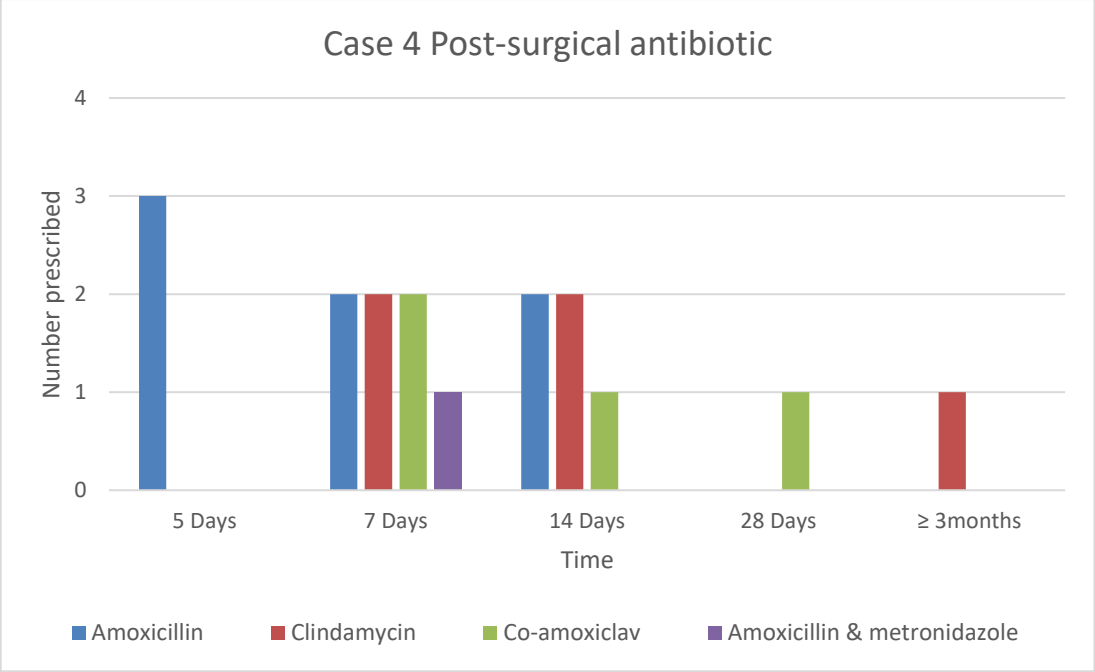


Figure 73: Case 4 post-surgical antibiotic prescribed by respondents

4.2.13.5 Case 4 Post-surgical antibiotic dose

The dosages prescribed were in accordance with normal therapeutic dosing regimens (Figure 74).

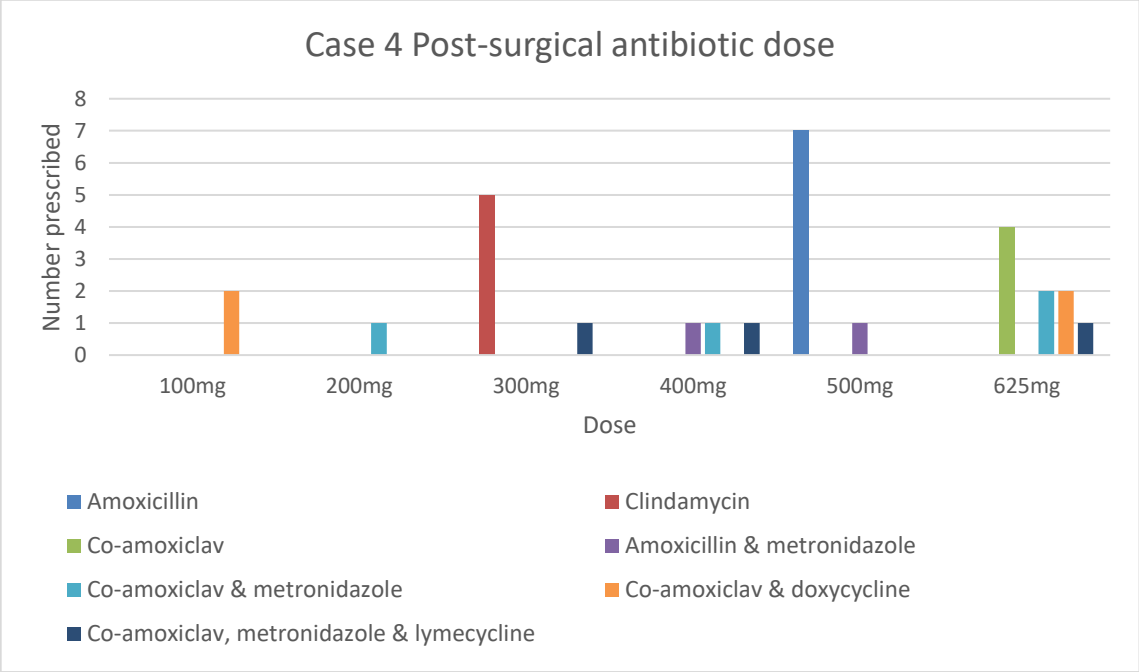


Figure 74: Case 4 post-surgical antibiotic dose prescribed by respondents

4.2.14 Case 1: Choice of antibiotic

Respondents were asked to explain their reasoning for antibiotic choice. The results from Case 1 were only included as this question was poorly answered from thereon, and with considerable repetition (Figure 75). The results of non-surgical and surgical management were collapsed together. Nine prescribed Amoxicillin for its broad-spectrum coverage, and one because it is a first-line antibiotic. Clindamycin was chosen because it has good bone penetration (n=8), broad spectrum (n=1), best evidence-based outcomes (n=1), and because it can be taken long-term. Doxycycline was prescribed because it is broad spectrum (n=2), good bone penetration (n=4), and patients are compliant with taking it once daily (n=2). Augmentin was prescribed for its broad-spectrum properties (n=13), and because it is well tolerated (n=1). Metronidazole was chosen for its anaerobic coverage (n=4), and Lymecycline as it is broad-spectrum (n=1).

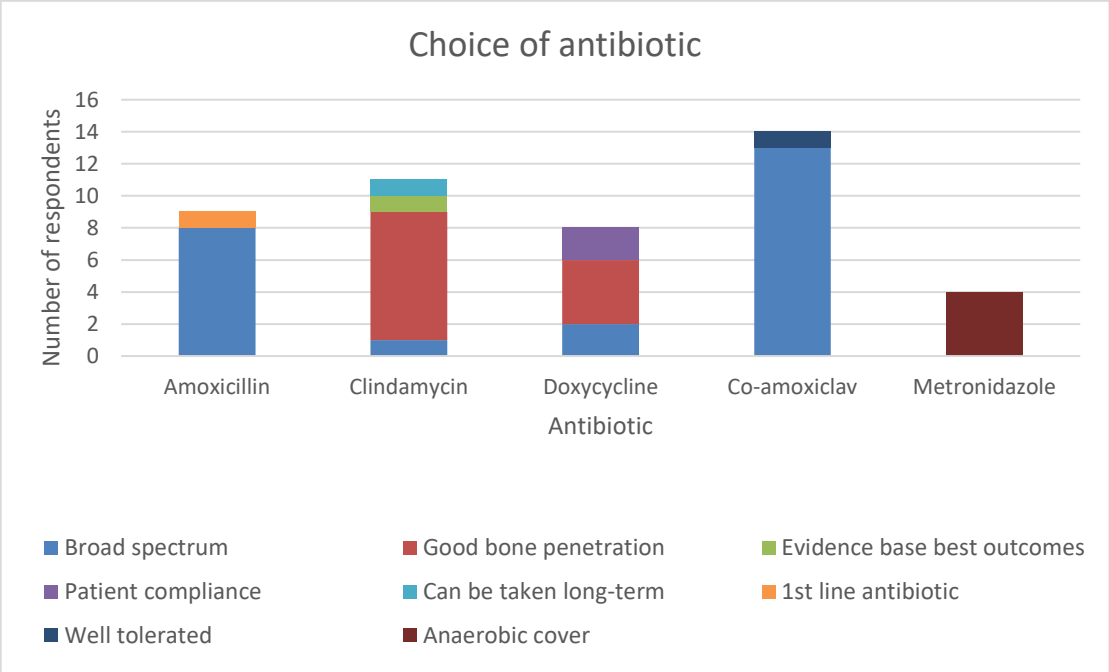


Figure 75: Case 1 justification for choice of antibiotic prescribed non-surgical and surgical

4.2.15 Case 1: Duration of antibiotic prescribing

Respondents were asked to explain the reasoning for the duration of antibiotic prescribing. The results from Case 1 were only included as this question was poorly answered from thereon, and with considerable repetition (Figure 76). The results of non-surgical and post-operative surgical management were collapsed together. Antibiotics were prescribed for 14 days or less to manage the acute phase (n=13), until culture and sensitivity results return (n=3), or because that is the standard recommended duration for that drug (n=5). Antibiotics prescribed for over 14 days were used for management of bone involvement (n=3), due to its chronic nature (n=3), to manage the acute phase (n=2), and because senior colleagues advocated this regime (n=1).

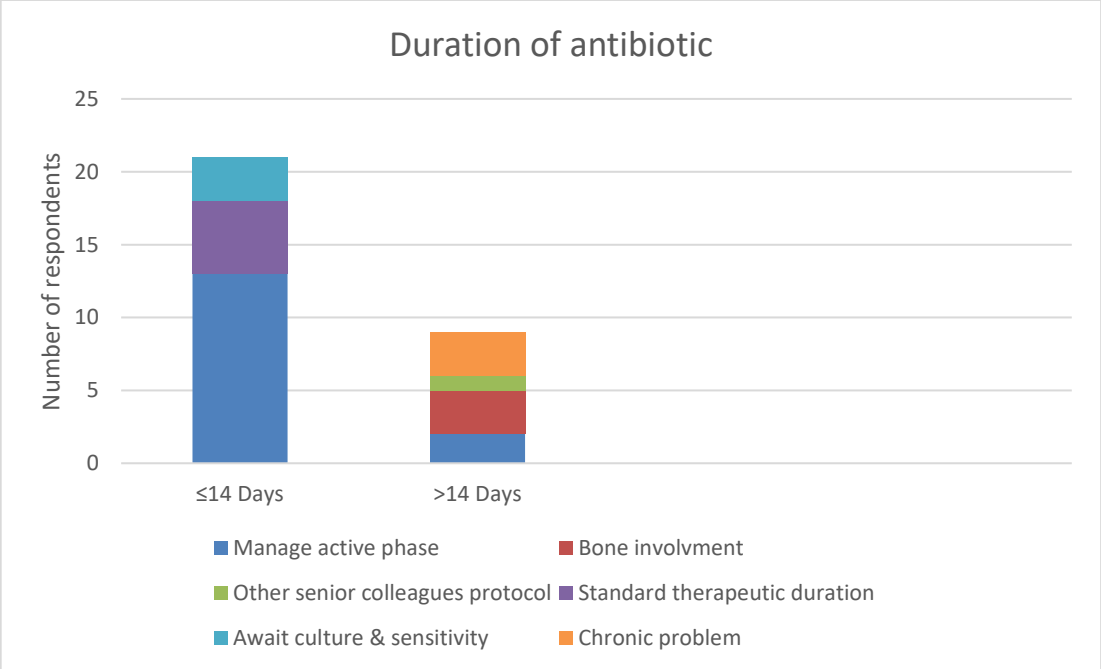


Figure 76: Case 1 justification for duration of antibiotic prescribing for non-surgical and post-operative antibiotic prescribing

5 Discussion

In this study, fourteen patients diagnosed with MRONJ, were treated with conservative surgical debridement, L-PRF, and prospectively analysed. The patients were characterised by investigating age, sex, medication use, medical and dental history, co-medications, smoking status, previous treatment, and disease presentation, specifically looking at common risk factors for the development, and presentation of MRONJ. Secondary outcomes included:

a) Investigate the pathogens isolated from MRONJ sites, with culture and sensitivity tests
b) Examine the current trends in treatment strategies adopted by clinicians in Ireland for the management of MRONJ.

5.1 Epidemiology

Patient details of race, age, and gender were comparable to previously published studies.[113, 144] There was a ratio of 2.5:1 female. All but one patient was >60 years of age, which is in accordance with previous studies.[113, 144] The higher incidence of MRONJ amongst women is possibly a reflection of the underlying conditions being treated such as breast cancer and osteoporosis, which are more common in females. MRONJ occurs in a more aged population, and this may be due to improved healing capacity in adolescents, differences in bone composition, fewer concomitant social/medical risk factors, or due to less invasive dental treatment in younger people. The mean age at presentation was 69.7 (range 58-82 years), which closely corresponds to the findings of an 8-year epidemiological study by Jacobsen *et al.*, (2012), with a mean age of 67.[113] Generally oncology patients present with MRONJ at a slightly younger age than patients with osteoporosis, with the mean age of osteoporotic patients being 70.2, and 69.1 in the oncology group.

5.2 Risk factors and precipitating factors for MRONJ

The patho-aetiological process of MRONJ is not fully understood, and would appear to be a multifactorial process. However, several major risk factors have been identified in the literature, and these appear to be applicable to bisphosphonates, and Denosumab related cases of osteonecrosis of the jaw observed in this study.[1]

5.2.1 Drug-related factors

The type of antiresorptive (potency), cumulative dose, and duration of treatment were correlated with the risk of developing MRONJ.[1,2,42] In this study 7 patients were treated with high doses of potent antiresorptives as part of their oncology treatment, and seven were treated with lower cumulative doses of antiresorptives for osteoporosis. It is generally accepted that oncology patients carry a higher risk of developing MRONJ than osteoporotic patients.[2,10,40,42] However, 50% of the patients in this study were on antiresorptive treatment for osteoporosis, of which four were on Denosumab. This unusually high incidence may be due to the small sample size. Anecdotally clinicians are reporting increased cases in osteoporotic patients treated with Denosumab. The observation of osteonecrosis of the jaws occurring following treatment with Denosumab, despite having different pharmacokinetics to bisphosphonates, indicates that it is the degree of osteoclastic inhibition, and resultant reduction in bone turnover that is central in the pathogenesis of MRONJ.

5.2.2 Duration of antiresorptive medication

Several authors have shown a direct correlation with MRONJ incidence, and increasing duration of antiresorptive treatment, although this risk appears to plateau at 2 years for Denosumab.[46-48] This was most likely due to the cumulative effect of bisphosphonates, exhibiting a half-life of 1-10 years within bone, whereas Denosumab is not incorporated into the bone.[1,29] The mean duration of bisphosphonate

treatment for oncology patients was 22.1 months, with two patients with metastatic prostate disease presenting with disease at 11 months. Patients on bisphosphonates for osteoporosis presented later with a mean duration of 37 months. In the literature there is no defined minimum duration of antiresorptive treatment that must be satisfied before MRONJ can be diagnosed, for osteoporotic or oncology patients on bisphosphonates. The AAOMS report that the risk for developing MRONJ increases when oral bisphosphonate treatment exceeds 36 months, and the SDCEP suggested 5 years.[6, 14] Other studies have reported a requisite of at least 12 months for oncology, and 24 months of oral bisphosphonate therapy to satisfy a diagnosis of MRONJ.[18] There is no published literature on the minimum time of onset for Denosumab related osteonecrosis in osteoporotic, or oncology patients, and other studies have observed patients presenting with disease as early as 7 months of treatment.[8] The mean duration of Denosumab treatment observed was 21.25 months, with one osteoporosis patient presenting with disease after 7 months. This may indicate that the onset of DRONJ appears earlier than in patients on bisphosphonates, especially when osteoporotic patients are considered.

5.2.3 Patient risk factors

States of immunosuppression have been implicated as a risk factor for developing MRONJ and may very well compromise healing in surgically treated cases, regardless of whether it is due to the underlying disease, co-morbidities or co-medications. In this study 9 of the 14 patients had a degree of immunosuppression. The immune status of oncology patients is often severely compromised by the condition itself, or the therapy that they are on, which may contribute to the development of MRONJ.

Cancer type is variably reported as a risk factor, although there is currently no consensus on which type of malignancy poses the greatest risk.[42] A large meta-analysis by Qi *et al.*, (2014) reported that metastatic prostate cancer posed the highest risk, whereas a longitudinal cohort study by Vahtsevanos *et al.*, (2009) reported that multiple myeloma posed the highest risk although this did not reach statistical significance.[45, 46] Different drug regimes, and different follow-up times act as

confounding factors, making it difficult to draw conclusions. Within this study, two of the oncology patients were treated for multiple myeloma, two for metastatic breast cancer, and three for metastatic prostate cancer. Other co-morbidities, such as diabetes mellitus, and low haemoglobin, have also been inconsistently reported as risk factors.[42] In this study one patient had diabetes Type II. Co-medications including immunosuppressants, corticosteroids, and concurrent anti-angiogenics have been identified as risk factors.[14, 42] Two of the patients were on systemic steroids, one on Methotrexate, and two patients were treated with Zoledronic acid, and anti-angiogenics concomitantly, which may have increased their risk of developing MRONJ.

Tobacco use is variably reported as a risk factor for developing MRONJ.[42] A study by Borromeo *et al.*, (2014) reported a significant increased risk of MRONJ in smokers, however Vahtsevanos *et al.*, (2009) reported no association with smoking.[46, 59] It is unclear whether tobacco use was implicated in the pathogenesis, and a literature review by Dodson (2015) reported there was insufficient evidence to support or refute its role.[42] In this study, 5 of the 14 patients were everyday smokers, three moderate smokers, and two heavy smokers. The rarity of MRONJ, and the heterogeneity across patient groups makes it difficult to answer research questions, such as such as causality, incidence, and risk factors.

5.2.4 Local factors

The literature suggests dentoalveolar surgery is the most common risk factor, with exodontia being the most common precipitating risk factor in 52%-77% of cases.[1, 35, 42] In this study, dental extraction was the luxating factor in twelve of the sites, and denture trauma at two sites. This indicated a dental focus in 93.3% of the cases, which was in accordance with the results of Pichardo and Van Merkesteyn (2013) who found 97.5% of cases had a dental origin.[145] Previously spontaneous cases of MRONJ were reported, but most of the literature now supports a dental origin.[73, 145] Four of the patients who underwent a dental extraction, did so in accordance with the prophylactic measures outlined by the Journal of Irish Dental Association[12], however this did not eliminate the risk of developing MRONJ.[12, 14, 30]

A Cochrane review (2016) has shown that preventative measures reduce the risk of developing MRONJ, but they do not eliminate the risk, and the healing process following an extraction does not always follow a predictable course.[10] Interestingly, two of the osteoporotic patients on Denosumab subsequently underwent extractions, adhering to JIDA guidance (2010), and placement of L-PRF in the socket. The sites healed with no postoperative complications. It is impossible to say whether this was due to adhering to JIDA (2010) guidance, and or the L-PRF intervention, or whether the pharmacokinetic half-life of Denosumab meant it was eliminated at the time of extraction (>4 months since last dose).[1, 12]

Denture wearing has also been implicated as a risk factor for MRONJ with a 2-fold increased risk in incidence, and was observed in one patient in this study with bilateral disease sites in the mandibular premolar region, secondary to an ill-fitting poorly retentive denture.[46] Interestingly, this patient had a prior 72 month history of Alendronic acid but only developed MRONJ after 18 months of Denosumab, which may indicate Denosumab carries a higher risk of osteonecrosis despite not having a cumulative dose effect at therapeutic levels treating osteoporosis.[37]

MRONJ is diagnosed more often in the mandible (73%), than the maxilla (22.5%), and can appear in both jaws (4.5%).[48] In this study more disease sites were observed in the mandible (n=10) than in the maxilla (n=5). This is in keeping with the published literature, which proposed the mandible had a higher content of cortical bone, which was associated with reduced vascularisation, and hence reduced healing potential.[48] Furthermore, significantly higher rates of bone turnover have been observed in the maxilla when compared to the mandible, in patients on bisphosphonates and Denosumab by Ristow *et al.*, (2014) using bone scintigraphic imaging.[64] In this study, four cases occurred in the anterior maxilla, one in the posterior maxilla, two in the anterior mandible, four in the mandibular premolar region, and three in the posterior mandible. Other studies report that most sites are localised to the mandibular premolar and molar regions.[113]

5.3 Treatment of MRONJ

In this study fourteen patients with a total of 15 MRONJ sites were treated with conservative surgical debridement and L-PRF, and prospectively analysed. Of the 15 sites, 11 (73.3%) showed complete mucosal coverage, absence of pathological symptoms, and radiographically no evidence of pathology at the 6-month end-point. Four patients (26.7%) had persistent exposed/ probable bone at the end-point.

Overall, there is a lack of consensus on how to manage MRONJ. Cochrane reviews, by Rollason *et al.*, (2016), and Beth-Tasdogan *et al.*, (2017) concluded there was a lack of evidence from well-designed randomised controlled trials to guide treatment of MRONJ.[10, 11] Systematic reviews by Fliefel *et al.*, (2015) and Spanou *et al.*, (2015) concluded there was insufficient evidence to support evidence-based guidance on treatment options for MRONJ.[81, 92] However, systematic reviews by Silva *et al.,* (2016) Rupel *et al.*, (2014) and El-Rabbany *et al.*, (2017) all reported improved outcomes with surgery, albeit based on evidence graded as medium to high risk of bias.[17, 20, 146] Khan *et al.*, (2015) adopted a pragmatic approach, recommending each case should be considered by stage and size of the disease, and the presence of contributing drug therapy and comorbidities, although a non-surgical approach was the mainstay of their recommendations.[2] The AAOMS Guidelines (2014) suggest conservative treatment for Stages 0, 1, and 2 disease and to reserve surgery for advanced Stage 3 disease only or refractory cases.[1] While there was anecdotal and some clinical evidence to support a non-surgical approach for stabilising, and palliating MRONJ lesions, there was little evidence to support it as a successful definitive treatment option.[17, 20, 81] Rupel *et al.*, (2014) reported consistently superior outcomes with surgical treatment when compared to a non-surgical approach, independent of disease stage.[20] Most studies reporting surgical outcomes did not comment on the individual patient status but only considered the surgical technique.[146]

The literature reports complete healing ranging from 36% to 50% with conservative medical management alone.[20, 81, 146] There is little evidence to support adjuvant conservative treatments including hyperbaric oxygen, Pentoxifylline, low level laser therapy, Teriparatide, and drug holidays.[10] This is in contrast to studies from various

research centres reporting mucosal healing ranging from 49% to 93.2%, following surgical treatment (conservative and extensive surgery), with the majority consistently above 80%, with follow-up ranging from 3 months to 7 years post-operatively.[16, 18, 19, 107, 110-113] Although the level of evidence was often poor with small sample numbers, heterogeneity amongst patients, and different surgical protocols, it cannot be overlooked that recent studies are treating MRONJ with superior success than the initial protocol of avoiding surgery.

Systematic reviews by Rupel *et al.*, (2014), El-Rabbany *et al.*, (2017) and Fliefel *et al.*, (2015) reported better outcomes with extensive surgery when compared to conservative surgical debridement.[17, 20, 81] Prospective studies, specifically comparing extensive surgery with conservative surgical debridement by Mucke *et al.*, (2011) and Graziani *et al.*, (2012), both concluded extensive surgery was associated with better outcomes, and lower rates of recurrence of MRONJ.[106, 107] However difficulties are encountered when interpreting the literature as it is not always clear whether cases were treated with extensive, or conservative surgery. Furthermore, the lack of definite methods to delineate necrotic bone from vital bone, and operator variability make it difficult to standardise the surgical procedure, and the classification of the debridement.[146] Extensive surgical resections are associated with increased surgical morbidity, including the risk of intra- or postoperative mandibular fracture, sensory nerve deficits, donor site morbidity when vascularised free flaps are used, and intraoral defects which may be difficult to restore with a prosthesis. Issues with lack of access to general anaesthetic services, and in-patient facilities may preclude some units from providing this service, and the associated cost burden associated with accessing these services.

The conceptual benefit of using L-PRF with conservative debridement is to maintain a balance between improving healing at the surgery site, while negating the risks of surgical morbidity associated with extensive surgery. The level of evidence, supporting the use of L-PRF to enhance wound healing in cases of MRONJ, is low, and limited to small studies with multiple sources of bias. When the results of the larger studies >15 patients are considered, using an extensive surgical protocol with L-PRF as an adjuvant treatment, it has a curative rate of 83.7% in 74 patients,[22-24] which is similar to the

results of extensive surgery alone (84%) reported by Rupel *et al.,* (2014).[20] Only one study used a conservative surgical approach with L-PRF, which treated two cases, both of which failed to heal,[116] and it was impossible to draw conclusions from that study. A systematic review of the literature by Rupel *et al.*, (2014) reported overall healing in 75% of cases treated with conservative surgery (without L-PRF), while Fliefel *et al.*, (2015) reported healing as low as 39.2% for conservative surgery alone.[20, 81] The results of this study are consistent with the higher outcomes achieved for conservative surgical debridement at 73.3%. Due to the small sample size in this study and lack of a control group it is impossible to draw conclusions on the critical benefits of L-PRF, but it would appear L-PRF certainly does not worsen outcomes. Well-designed randomised controlled trials with large sample numbers are required to answer these questions. A systematic review by Lopez-Jornet *et al.*, (2016), reported there is little evidence to support the use of autologous platelet concentrates in the treatment of MRONJ.[147] However, of the 8 studies included in the analysis, only one used L-PRF, while the other studies used PRP. Studies comparing L-PRF with PRP have shown L-PRF provides superior quality clots, improved platelet, leucocyte, and cytokine trapping, remains active for longer than PRP, and therefore provides a superior slow release of growth-factors over time.[121, 125, 126, 130] Most of the literature, purporting CPRP's benefits, have been published by the company that developed the system, and therefore may have some bias. Studies investigating PRP and L-PRF should be treated as separate entities.[119]

Achieving primary closure is associated with favourable outcomes.[18] There is little evidence in the literature to confirm this, but it is based on a basic surgical algorithm. However, it is also associated with raising large flaps, scoring of the periosteum, reduction of bone height, and can pose a risk of damage to adjacent structures. Primary closure is not always feasible with conservative surgical debridement, and therefore the L-PRF fibrin matrix acts as a medical dressing, which may facilitate wound coverage by affecting the metabolism of epithelial and fibroblast cells. Epithelial cells at a wound edge lose their basal and apical polarity, which enables lateral extension that project towards the wound edge to cover the defect.[136] Stem cells trapped within the fibrin matrix provide a source of undifferentiated cells, that

have the potential to develop into different cell types.[136] L-PRF membranes have been shown to retain their shape and release growth factors for at least 7 days. [124, 130] Of the 4 cases that we were unable to achieve primary closure, only 1 site failed to heal. This healing may be attributable to the L-PRF acting as a protective membrane, facilitating epithelial migration in the initial stages of wound healing. However, despite the purported benefits of L-PRF at a cellular level most of these studies are *in-vitro*. Although, the IntraSpin- Intra-Lock system is the only CE/FDA approved system and appears to be the most standardised reproducible system, further *in-vivo* studies are required to measure its growth factor release over time, its benefit as an "immune-organising node" and its clinical effects on tissue healing and cells of the body.

5.3.1 Treatment in an out-patient setting

This study has demonstrated that conservative surgical debridement as a procedure was well tolerated by patients with a mean VAS score of 1.8 for sedated patients, and 3.1 for local anaesthetic only. No significant post-operative complications, such as sensory nerve deficits were encountered. No patient complained of worsened symptoms or a deterioration in Stage of disease. This study shows that 73.3% of cases can be treated successfully with a conservative surgical debridement protocol, using the oral form of antibiotics, in an outpatient facility under local anaesthetic (+/- intravenous conscious sedation). This is contrary to a lot of the literature, reporting extensive surgical protocols performed under general anaesthetic, requiring admission for preoperative, and postoperative intravenous antibiotics. Local access to general anaesthesia in Ireland is very limited due to constraints within the health system and waiting lists for procedures are in a dire situation for all medical and dental specialities. In the context of MRONJ, there is a high probability that patients would wait over a year to gain access to services under general anaesthesia, thus facilitating extension of disease and deterioration in the Stage of disease. Although treatment under general anaesthesia enables more thorough removal of necrotic bone and a higher success rate, working within the constraints of our health service providing early intervention at an early Stage in outpatient facilities under local anaesthesia or

intravenous sedation enables patients to gain timely access to care. There is of course a place for extensive surgical protocols, when conservative surgical debridement has failed, or in the extensive cases excluded from this study such as oronasal, oroantral communications, and osteolysis extending to the lower border of the mandible with a pathological fracture. This highlights the argument for early surgical intervention at initial presentation, which may reduce amount of osteolysis, that occurs over time, and minimises the need for extensive surgical resections, reducing surgical morbidity.[18] In addition, actively observing the soft tissues overlying necrotic bone breakdown, means that there is less soft tissues to achieve primary closure when surgery is performed. Five patients who proceeded from initial diagnosis to surgery within ≤3 months, showed healing in 80% (n=4), and 20% failed to heal (n=1). This may indicate than an early intervention protocol is favourable.

5.3.2 Surgical outcomes depending on underlying disease

Most studies reported better surgical outcomes for patients with osteoporosis, than those with an underlying diagnosis of malignancy.[110] Conservative surgery in osteoporotic patients on oral bisphosphonates achieved successful healing in 91.8% of cases.[109] Similar results were observed by Jacobsen *et al.*, (2012) who treated 64 cases with conservative surgical debridement, 18 (95%) on oral bisphosphonates healed, while only 32 (71%) on IV bisphosphonates healed.[113] This is largely due to the increased cumulative dose, and potency of the antiresorptives. Furthermore, oncology patients have additional comorbidities that may compromise their healing potential such as immunosuppression secondary to concomitant chemotherapy, steroids, low haemoglobin levels, and some are treated with additional anti-angiogenic medications which can affect healing.[40] Seven patients with 8 disease sites were on antiresoptives for the treatment of osteoporosis and all of these patients responded to treatment, and had complete mucosal coverage at the 6-month point (100%). Seven of the patients were on antiresorptives for oncological reasons, of these only 3 (42.8%) resulted in complete mucosal coverage at the end-point. The relation between indication for antiresorptive medications, and surgical healing was

significant, p = 0.0256, meaning surgical intervention in osteoporotic patients is more predictable, and usually has better surgical outcomes than surgical intervention for MRONJ in oncology patients.

In contrast, extensive surgery consistently produced good outcomes for oncology patients. Carlson and Basile, (2009) treated 95 sites of MRONJ. Of the 27 sites treated in the oral bisphosphonates group 26 fully healed (96.3%), whereas in the IV bisphosphonate group 61 of the 68 sites healed (89.7%).[110] It would appear oncology patients may respond better to extensive surgery than conservative debridement. It is difficult to quantify this, as most of the literature reports the total healing and not the healing outcomes specific to oral, intravenous or subcutaneous antiresorptives.

5.3.3 Surgical outcomes based on stage of disease

Considering the outcomes in relation to stage of disease, one patient had Stage 0 disease which resulted in resolution, one patient had Stage 1 disease which resolved. In nine patients with ten Stage 2 disease sites, 7 resolved. Three patients with Stage 3 disease of which 2 resulted in healing. The relation between stage 2 and 3 disease, and response to surgery was not significant, p = 1, although this is not a true representation as some Stage 3 disease criteria were excluded from this study. However other studies with larger samples have shown that Stage of disease has been identified as a predictive factor for successful outcomes. Vescovi *et al.*, (2014) reported success rates of 92.6% in Stage 1 disease treated with conservative surgical debridement.[86] Rupel *et al.*, (2014) reported successful outcomes for 72% of Stage 1 disease, and 79% with Stage 2 disease treated with conservative surgery.[20] The outcomes markedly diminish to 27% for Stage 3 disease, when treated with conservative surgical debridement. Conservative surgical debridement is unlikely to result in healing where the disease has extended into the antrum, nose or to the lower border of the mandible, and hence these cases were excluded from this study. However, Stage 3 disease which has extended beyond the limits of alveolar bone may still benefit from conservative surgical debridement in the first instance, and this surgery is associated with less surgical morbidity. If this fails, then extensive surgery

may be employed. The presence of mobile sequestra has been shown to significantly improve the curation of disease sites. [109] This may be because of soft tissue formation beneath the sequestra as it is exteriorized from the disease site, however this was not found to be significant within this study, p=0.6044. However, the sample size is too small to evaluate this.

5.3.4 Outcomes in relation to maxillary or mandibular disease

Of the 10 sites of mandibular disease treated, 70% resolved and 80% of maxillary sites healed. The relation between the location of MRONJ, and surgical healing was not found to be significant, p = 1. However, the literature suggests that superior outcomes are observed in the maxilla when compared to the mandible, and the lack of significance found in this study may be due to the small sample size. Carlson and Basile (2009) observed healing in 100% of maxillary sites, compared to 87.8% of mandibular sites treated with extensive surgical debridement.[110] Factors identified in the literature, which may explain this are a richer blood supply, and less dense cortical bone in the maxilla, and increased bone turnover in the maxilla irrespective of antiresorptive treatment.[64] Limiting factors that may affect the outcomes for mandibular surgery include debridement limited by vital neurosensory structures, and the increased difficulty to achieve primary closure in the mandible compared to the maxilla.

5.3.5 Outcomes in relation to medical co-factors, and smoking habits

Co-medication such as steroids, immunosuppressants, and medical co-factors may have a negative impact on surgical healing.[2, 109] It is hard to draw conclusions from the small numbers in this study, but failure to heal was likely due to antiresorptive drug related factors in the oncology patients, and not medication co-factors, as other studies have shown.[18] Two patients were receiving anti-angiogenic medications; one healed, and one who previously had a stem cell transplant failed to heal. Complete

healing was observed in the patients with other immunosuppressive co-factors, including steroids, methotrexate and diabetes Type II. Interestingly both patients with previous breast cancer healed, however all the patients with previous multiple myeloma (n=2), and metastatic prostate disease (n=2) failed to heal. Different studies have reported that both metastatic prostate[45] and multiple myeloma[46] patients are at a higher risk of developing MRONJ than other cancers, and this may also have an effect on the surgical outcomes, although this relationship has not been investigated in the literature.

Anaemia is also associated with impaired wound healing. Five patients had a low haemoglobin; 3 oncology patients and 2 osteoporotic patients. Three patients achieved resolution of their MRONJ lesion and 2 failed to heal. The relation between a low haemoglobin and surgical outcome was not significant, however the sample size was too small to make conclusion from this study ($p = 0.5604$).

5.3.6 Outcome in relation to tobacco habits

Smoking has been reported to increase the overall time to heal for surgically managed cases of MRONJ.[55] The three cases which showed resolution were on antiresorptives for osteoporosis, and healed regardless of smoking heavily, and within the first 10 days following surgery (only 1 abstained). However, two of the patients with metastatic prostate cancer smoked within the first 10 days, and both failed to heal with one patient's disease progressing. There is some evidence to suggest that smoking delays healing in cases treated with surgery, however the relation between smoking and surgical healing was found not to be significant, $p = 1$.[55] There were too many variables to fully investigate the implications of tobacco use.

5.4 Denosumab

Of the 14 patients included in the study, 5 patients were on Denosumab, and all these cases showed full mucosal healing, and absence of pathological symptoms at the end-point of the study. Four patients were being treated for osteoporosis, and one oncology patient. There is relatively sparse literature on the aetiology, pathogenesis, and management of DRONJ, and it has largely been treated as a similar entity to BRONJ, whether this is right or wrong.[1,2,9] This is likely for convenience reasons, and because it is a relatively new rare disease entity, with similarities in the aetiological risk factors and clinical presentation to BRONJ. A systematic review by Boquete-Castro *et al.*, (2016) reviewed 35 articles, identifying 7 which reported on 144/9,847 cases of DRONJ with an overall incidence of 0%-2%.[35] They identified risk factors for developing DRONJ including dental extraction (66-77%), chemotherapy (64%-75%), poor oral hygiene (55% and 77%), dental appliances (48% and 77%), and concomitant anti-angiogenic therapy (20%). These are all risk factors that have been implicated in the aetiology of BRONJ.[1,2] Interestingly, they reported six of the seven articles noted more severe cases arising in the DRONJ cohort compared to Zoledronic acid, this is similar to the aggressive, and progressive nature of DRONJ reported by Badr *et al.*, (2017).[148]

Boquete-Castro *et al.*, (2016) reported longer average times taken for DRONJ cases to present when compared to Zoledronic acid, ranging from 13 -21.4 months, which are similar to the findings of this study, with a mean time of 22.3 months (excluding patients with a history of bisphosphonates). Interestingly, one of the osteoporosis patients in this study presented with MRONJ following a dental extraction with a 7-month history of Denosumab. Similar findings were reported in the study by Pichardo *et al.*, (2016).[8] Difficulties also arise when characterising patients on Denosumab and assessing the outcomes of treatment interventions as many of these patients have a history of bisphosphonate treatment in the past. A study by Pichardo *et al.*, (2016) treated eleven cases of Denosumab related MRONJ with extensive surgery, of which five also had a history of bisphosphonates.[8] Nine of the patients had complete healing, two failed to heal of which had no prior history of bisphosphonates. Within this study, the relationship between surgical outcome comparing bisphosphonates with

Denosumab was not found to be significant, p = 0.1033, although all the 6 DRONJ sites healed. This is likely due to the small sample size within this study.

DRONJ appears to respond well to surgical intervention, however large controlled trials are required to investigate this. Boquete-Castro *et al.*, (2016) reviewed the literature, and concluded there was no consensus for the treatment of DRONJ at present.[35] Another issue of contention is whether a drug holiday was beneficial in a prophylactic setting when planning invasive dental treatment, or prior to surgery for the treatment of DRONJ. The AAOMS (2014) report the effects of Denosumab should mostly be depleted at 6 months after interruption of the medication.[1] Unlike bisphosphonates, Denosumab is not incorporated into the bone matrix, and hence its influence on bone resorption may be negligible after 6 months following cessation of treatment with Denosumab.[1, 36] The European Medicines Agency report the half-life serum level for Xgeva is 28 days, and 26 days for Prolia with undetectable levels in 53% of patients on Prolia at 6 months.[37, 38] Although the serum levels may be undetectable, the effect on bone suppression may persist longer than this, as shown by Bekker, *et al.*, (2004).[39] Sclerotic bone with minimal bleeding was noted intra-operatively in the study by Pichardo *et al.*, (2016) and they postulated that the effects on the bone itself may take longer than 6 months to remodel.[8] In this study, the mean duration since the last dose of Denosumab was 3.7 months. As a prophylactic measure, the Cochrane Study Group (2017), advised that cessation of Denosumab therapy would be required for at least two months to significantly reduce the risk of MRONJ during invasive dental procedures.[10] Although the half-life of Denosumab is considerably shorter than bisphosphonates, and a drug holiday would seem reasonable, there are no studies to support or refute the strategy of a drug holiday in the treatment of Denosumab related MRONJ.[1]

5.5 Local anaesthetic

The JIDA (2010, 2017) advocated plain local anaesthetic without adrenaline to be used in patients undergoing a surgical procedure at risk of MRONJ, and it has been the Dublin Dental University Hospital's protocol to apply this to patients with established

MRONJ undergoing a surgical procedure.[12, 30] Vasoconstriction may also compromise the identification of fresh bleeding margins during ostectomy when delineating necrotic bone from vital bone, resulting in more extensive bone removal. However, supplemental top-up local anaesthetic with 2% lidocaine 1:80,000 with epinephrine was required in 9 of the 14 patients (65%) to enable completion of treatment. It is unclear why Scandonest has poor efficacy in this clinical scenario. It may be due to its low potency, absence of adrenaline, inability to permeate necrotic bone, or the low tissue pH due to infection inhibits conversion to its active lipophilic form. Of the 9 patients that required supplemental local anaesthetic with adrenaline, 6 had complete healing, and 3 had failed to heal at the end-point. The difference in healing outcomes between patients who had Scandonest only, compared with those who had supplemental local anaesthetic with adrenaline was not significant, although the sample size is too small to draw conclusions. (p=1). Although it is unclear whether the temporary vasoconstriction affects the initial stages of clot formation and wound healing, it can be concluded that it does not inhibit healing.

5.6 Microbiology

Samples from each patient were sent for culture and sensitivity testing. Macroscopically Gram-positive cocci (40%), Gram-positive bacilli (33%), Gram-negative bacilli (27%), pus cells (27%), and candida (20%) were identified from the samples. The microbes isolated were largely in keeping with the normal oral flora, and no overt pathogens were isolated apart from Staph. epidermidis, which has been implicated in states of pathology, forming thick biofilms which may exhibit bacterial resistance. Similar microbes were isolated in the oncology, and osteoporosis groups, however the oncology group reported pure growth of Step constellatus and Strep mitis more frequently on the plates, and anaerobic species more frequently. Similar pathogens were also noted in the Denosumab and bisphosphonate groups. Only one case identified Actinomyces, which contrasted with most of the literature. Sedghizadeh *et al.*, (2008) identified Actinomyces as a common isolate in MRONJ, and Mucke *et al.*, (2011) isolated Actinomyces in 46.7% of samples.[68, 106] A systematic

review by Hinson *et al.*, (2014) identified Actinomyces in 68.8% of isolates, and Streptococcus species as the second most common isolate identified.[78] This may have been due to the patients in this study previously being treated with topical, and systemic antimicrobials. However, Actinomyces species are part of the normal oral flora, and therefore isolation of this pathogen is not always indicative of an opportunistic Actinomycosis infection. Anaerobes were isolated in 7 of the samples, however they were identified mostly in "few" numbers indicating that they are unlikely to be driving the pathological process. Streptococcus species were the most common microbes isolated. Chlorhexidine exerts a strong cytotoxic effect on Streptococci species, highlighting the importance of topical antimicrobials in the peri- and post-operative phase of surgical management. Most microbes within the oral flora are sensitive to Penicillin, and therefore broad-spectrum Penicillin was recommended as a first line antibiotic in all 8 of the samples which returned positive sensitivity tests. This is in line with the recommendations of the AAOMS (2014).[1] Sensitivity tests also highlighted Streptococcus anginosus presented resistance to Erythromycin, Clarithromycin, and probably Clindamycin. Clindamycin is cited as one of the antibiotics commonly chosen to treat MRONJ by clinicians, who completed the questionnaire, due to its potential for bone penetration. However, this study shows one of the common isolates in MRONJ may show resistance to Clindamycin. Furthermore, Clindamycin is strongly associated with causing pseudomembranous colitis, a serious inflammatory condition of the gut due to overgrowth of Clostridium difficile.

5.7 Questionnaire results

A total of 70 blank questionnaires were sent out, of which 41 (59%) completed questionnaires were returned. The respondents of the questionnaire represent clinicians who are working in hospital settings, and specialised practitioners who are active in the diagnosis, and management of patients with MRONJ in secondary and tertiary care settings. Sixty-eight percent of clinicians followed local, or International guidance published by the AAOMS position paper, the SDCEP, the Journal of the Irish

Dental Association or a combination of the latter, and surprisingly 32% follow no guidance.[1, 12, 14, 30] Strictly speaking the SDCEP (2017) guidance offered an assessment tool for assessing a patient's risk of developing MRONJ but does not discuss the management of MRONJ.[14] Only 61% of respondents felt the available guidance resources were comprehensive. Clinicians who found the current guidance resources poor, mostly felt that they were not based on sound evidence. The respondents who did not follow official guidance mostly treat cases conservatively only, cases by case or symptomatically. Only 48.7% of respondents felt comfortable managing MRONJ with 51.3% feeling neutral or negative about managing cases. Reasons included lack of evidence base, unpredictable outcomes, poor exposure to MRONJ, and not being comfortable managing Stage 3 disease. Irrespective of the level of experience that the respondent had, there was no statistical significance when the responses of Consultants in OMFS and Specialist Oral Surgeons were compared with those of SpRs, Registrars, and NCHDs with regard awareness of guidance resources, choice of guidance, use of more than one guidance, and whether they feel the current guidance resources available are comprehensive. In addition, there was no significant difference between the level of confidence treating MRONJ between senior staff and junior trainees, meaning only 48.7% of clinicians felt comfortable managing MRONJ despite having superior academic and surgical training. These results reflect the consensus within the literature that there is insufficient evidence base to provide guidance on best practice.[10, 11]

5.7.1 Case based management

Case 1 was managed conservatively by 75.6% of respondents, and 24.4% reported they would treat with surgery. In accordance with the AAOMS (2014) guidance this was Stage 2 disease and should be managed conservatively with systemic and topical antimicrobials, however this case resolved with surgery. There was no significant difference between staff or trainees opting to treat this case with conservative treatment (topical and systemic antimicrobials) or surgical intervention ($p = 0.0823$).

However, the small numbers within this study may have resulted in a failure to reach significance.

Case 2 was treated conservatively by 26.8% of clinicians, and surgically by 73.1%. This Stage 3 case of MRONJ was treated with surgery in accordance with AAOMS (2014) guidance and resolved.[1] Such large areas of necrotic bone have a profoundly negative impact on the patient quality of life due to halitosis, difficulty eating, and the appearance. Surgery resulted in a rapid improvement in her circumstances. Antibiotics have little place in the conservative management of this case, with 10% opting for conservative treatment with systemic antibiotics There was no evidence of suppuration, and inappropriate prescribing leads to selection, and dominance of resistant microbial strains.[84] There was no statistical significance between staff and trainee members of staff opting to choose the option of local topical antimicrobials over systemic antimicrobials ($p = 0.5714$), local topical antimicrobials or surgery ($p = 0.6582$), or systemic antibiotics over surgery ($p=0.283$), however this may be as a result of small numbers within the study.

The third case was managed conservatively by 68.2% of clinicians and surgically by 31.7%. The necrotic sequestra extended beyond the alveolar bone radiographically, and in accordance with the AAOMS (2014) Stage 3 disease should be managed surgically. This case failed to heal by the end-point, however it has reverted to Stage 1 disease, and the patient is asymptomatic and has not required subsequent antibiotics, and is able to maintain better hygiene at the site with topical antimicrobials. Radiographically there was evidence of good bony infill. Clinicians are generally cautious when patients have a history of intravenous bisphosphonates for fear of worsening the situation. There was no significant relation between staff and trainees opting for conservative treatment (topical and systemic antimicrobials) over surgical intervention ($p = 0.307$), meaning it was not due to lack of surgical training and competence, although the small numbers within the study may have resulted in the lack of significance observed.

Case 4 was managed conservatively by 46.3%, and surgically by 53.6% of clinicians. Again, the necrotic bone extended beyond alveolar bone radiographically to the floor of the nose. Surgical intervention resulted in complete healing at the site in

accordance with AAOMS (2014) guidance.[1] Persistent chronic disease may well have perforated the floor of the nose if this case continued to be managed conservatively, and an earlier intervention is likely to have benefited this patient. There was no significant relation between staff, and trainees choosing conservative treatment with topical and systemic antimicrobials over surgical intervention, however this may be due to the small sample numbers (chi-square = 1.473, p = 0.229).

It is apparent from the results of this questionnaire that there is there is no clear consensus in the management of MRONJ, in general terms or in accordance with the AAOMS guidance, and largely the treatment decision is operator dependent, and case dependent. It would also appear that the level of academic, and surgical training attained does not influence clinician's treatment strategies, with some OMFS consultants, and Specialist Oral Surgeon groups consistently choosing conservative options for all four cases. Clinicians were more likely to avoid surgery in oncology patients with a history of intravenous bisphosphonates, than osteoporotic patients, for fear of worsening the situation, despite the literature reporting successful treatment in 89.7% of IV bisphosphonate cases.[110] The initial management strategy when BRONJ first appeared in 2003 was a non-surgical blanket approach to treatment, however 15 years on, there is mounting evidence that early surgical intervention provides improved outcomes, and earlier resolution for these cases.[17, 20] Local and International guidance should be updated accordingly to reflect these results.

5.7.2 Antibiotic prescribing

The consensus on antibiotic prescribing showed even greater disparity amongst clinicians, which is unsurprising with a further lack of evidence-based guidance on appropriate prescribing available.

5.7.2.1 Conservative medical management prescribing

The non-surgical antibiotics most often prescribed by clinicians included Amoxicillin, Co-amoxiclav, Clindamycin, Doxycycline, or a combination of Metronidazole with Amoxicillin, or Co-amoxiclav. Less frequently prescribed antibiotics included Lymecycline and combinations of Flucloxacillin and Penicillin V. Lymecycline was recurrently prescribed by one clinician, which is a tetracycline usually prescribed for acne vulgaris, and a literature search shows no evidence for it being used in cases of MRONJ. The AAOMS (2014) report sensitivity to Penicillin, quinolones, Metronidazole, Clindamycin, Doxycycline and Erythromycin, however the most recent HSE (2016) guidance report resistance to Erythromycin and is therefore not recommended for treating infection.[1, 149] The oral form was prescribed in all cases of conservatively managed cases. Dosing largely corresponded to normal recommended prescribing protocols, although variations were observed in the doses of Clindamycin prescribed varying from 50mg/od, 150mg/bd, 300mg bd or tds. The duration of antibiotic prescribing varied widely from 5 days to over 3 months for nearly all cases of non-surgically managed cases. The median duration of conservative non-surgical antibiotic prescribing for case, 1, 2, 3, 4 was 7, 28, 14, and 10.5 days respectively. A systematic review by Bermudez-Bejarano *et al.*, (2017) looked at antibiotic prescribing for the conservative management of MRONJ in patients on oral or IV antiresorptives.[87] There was no difference in the choice of antibiotic for oral or IV induced MRONJ, and cases were treated with Penicillin, Amoxicillin, Co-amoxiclav, Metronidazole, and/or a combination of thereof. Other combinations included Penicillin G and IV Metronidazole, Levofloxacin and Metronidazole, Piperacillin and Tazobactam, or Imipenem and Cilastatin. Clindamycin was chosen in the event of allergy. The duration of antibiotics was variable with studies prescribing for one week, 10 days, 15 days, 3 or 4 weeks or until complete healing had occurred. They concluded there was no consensus on the choice of antibiotic, the route of administration (oral/IV), the dosage, temporal patterns or duration for conservative management antibiotic prescribing.[87] These are consistent with the finding of this study.

5.7.2.2 **Pre**-surgical antibiotic prescribing

Amoxicillin was the most common pre-surgical loading dose antibiotic, followed by Co-amoxiclav. Others included; Clindamycin, Doxycycline, and combinations of Metronidazole with Amoxicillin or Co-amoxiclav, Co-amoxiclav and Clindamycin, Benzylpenicillin and Penicillin V, Flucloxacillin and Penicillin V. The choice of antibiotic was largely in keeping with those advocated by the AAOMS (2014) based on sensitivity tests, although they do not mention Co-amoxiclav.[1] A retrospective study by Zirk *et al.*, (2017) looked at 143 surgically treated cases of MRONJ over a 9-year period.[150] They found β-lactams, cephalosporins and tetracyclines showed the highest sensitivity rates. Nearly half of all isolates tested for Clindamycin, Erythromycin and Co-trimoxazol revealed resistances towards these antibiotics. They found sensitivity rates to Clindamycin (44.9%), in contrast to ampicillin/sulbactam which presented significantly better sensitivity rates ($p < 0.05$, 79.1%), and patients treated with Ampicillin/Sulbactam required significantly less re-operation surgeries.

The oral form was mostly prescribed pre-operatively for all four cases, with clinicians opting for parenteral route in 10%, 20%, 15% and 18% respectively. The dose varied widely from large loading dose to lower prophylactic doses. The JIDA (2010,2017)[12] guidance advocate a 3g loading dose of Amoxicillin pre-operatively (HSE prophylactic dose changed to 2g in 2016[149]), and clinicians adhered to this guidance in 30%, 47%, 54% and 50% for cases 1, 2 , 3, and 4 respectively. [1, 87] Studies treating MRONJ with surgery, especially extensive surgery often admit patients for pre-operative, and post-operative parenteral antibiotics, based on an antibiotic regime used to treat osteomyelitis, which is primarily an infective process.[18, 151] However, none of the patients in this study were acutely unwell, and their white cell counts were all within normal range or very slightly below. A Cochrane review (2013) reported there is evidence that the route of administration does not affect the remission of chronic osteomyelitis.[152] It is unclear if intravenous antibiotics offer a superior bactericidal effect than the oral form in the treatment of MRONJ, and there are no trials to date investigating this important question. The AAOMS offer no guidance on whether parenteral antibiotics are preferable to oral, and the systematic review by Bermudez-

Bejarano *et al.*, (2017) failed to answer this question too in cases of conservative management.[1,87]

The samples sent for culture and sensitivity from 15 disease sites showed gram +ve cocci (40%), gram +ve bacilli (33%), Gram -ve bacilli (27%), candida species (20%), and pus cells in 27% of the samples. These are similar to the results of the study by Zirk *et al.,* (2017) who isolated gram +ve microbes in 38.2% of samples, and gram -ve in 19.1%.[150] Co-amoxiclav provides bactericidal cover for Gram +ve, staphylococcus coverage, and superior anaerobic, and Gram –ve coverage than Penicillin, and hence provides good antibiotic cover in MRONJ cases. However, there is no consensus on pre-surgical antibiotic prescribing amongst the respondents, or the literature.

5.7.2.3 Post-surgical antibiotic prescribing

The post-surgical antibiotics most often prescribed by clinicians included Amoxicillin, Co-amoxiclav, Clindamycin, Doxycycline, or a combination of Metronidazole with Amoxicillin, or Co-amoxiclav. Less frequently prescribed antibiotics included combinations of Flucloxacillin and Penicillin V, Co-amoxiclav with Metronidazole and Lymecycline, Co-amoxiclav and Clindamycin, and Co-amoxiclav and Doxycycline. Duration of antibiotic prescribing varied widely from 5 days to over 3 months for post-operative surgical antibiotic prescribing. The median duration for post-surgical prescribing was 7 days (all four cases). One respondent prescribed a combination of Metronidazole for 5 days, and Co-amoxiclav for 6 weeks. Co-amoxiclav has been implicated in hepatobiliary reactions, ranging from hepatitis and cholestatic jaundice reported rarely to moderately, and asymptomatic increases in liver enzymes occurring occasionally.[84] This would be more likely to occur with extended durations of treatment. All cases were treated with the oral form of antibiotic post-operatively. There is little evidence in the literature regarding the most appropriate antibiotic choice after surgery, however it should be tailored as indicated from culture and sensitivity tests as per AAOMS (2014) guidance.[1]

Overall the findings show a lack of consensus surrounding the choice of antibiotic, dose, temporal patterns, route of administration, and total time of treatment for conservative medical management, and surgical management. These are consistent with the findings of a systematic review by Bermudez-Bejarano *et al.*, (2017) and the AAOMS (2014) who concluded there was no consensus surrounding an antibiotic protocol due to sparse clinical data, and lack of randomised controlled trials.[1, 87]

5.8 Limitations

There were several limitations to this study. The most significant was the lack of a control group, to investigate the benefit of L-PRF as an adjuvant to surgery. Initially the study had been designed as a randomised controlled trial. However it soon became apparent that this would not be feasible because MRONJ was relatively uncommon. This was compounded by the issue of clinicians who have always treated MRONJ conservatively were slow to refer their patients for inclusion in the study. This affirms the consensus of a non-surgical approach that has been engrained, and it is difficult to change people's perception. In addition, time constraints of the course, seeking ethical approval, and follow-up for 6 months meant the time to recruit and treat the patients was limited to 2 years. These factors resulted in a small sample number, which diminished the power of the study, introduces bias, and made it difficult to statistically analyse interventions.

Ideally, there would have been only one operating surgeon to minimise operator variability (not two), as there is potentially a high degree of variability in delineating necrotic bone margins. However, the lead surgeon operated on 14 of the 15 cases which helped to reduce the risk of this type of bias.

Within the population of patients, there is naturally a high degree of heterogeneity regarding underlying disease, drug factors, medical and medication co-factors, which can introduce confounding factors especially with small samples, and makes it difficult to draw meaningful conclusions. We tried to address this with an inclusion and exclusion criteria, but it can be difficult to control for all the various factors.

Preoperative radiographic assessment with CT or CBCT provides accurate detail on disease extension when compared to conventional plain film radiographs. However strict radiation guidelines are implemented by the Irish Dental Council in relation to research studies. Furthermore, access to medical CT scanning can be associated with long waiting times within the Irish Health System, and CBCT imaging carries cost implications for the patient which is not always feasible.

The follow-up period was limited to 6 months, ideally the patients would be followed up for at least 1 year.

Finally, the feedback from the clinical questionnaire was that it was time consuming, and difficult to answer. Closed format questions were used where appropriate and are generally easier for people to answer, however this was not feasible when exploring antibiotic prescribing. Using open-format questions enables respondents freedom with their answers but explaining the choice and duration of the antibiotic prescribed can be difficult to answer. This resulted in respondents omitting some of these questions. Open-format questions can also make it difficult to interpret the extracted data. Furthermore, collapsing the groups of respondents into staff (Consultant OMFS and Specialist Oral Surgeons) and trainees (NCHDs/ SpR/Registrar) was due to the small numbers. Collapsing of groups can result in information being lost from individual groups.

5.9 Future work

Prior to investigating the adjunctive role of L-PRF in surgery, a more important research question must be answered; is surgical management for all Stages of MRONJ in oncology and osteoporosis patients superior to a non-surgical conservative approach. There are numerous studies which indicate that surgery yields excellent outcomes, but they often have no control arm, small sample numbers, poor study design with a lack of reproducibility, and vast heterogeneity across samples.

The low-quality evidence makes it very difficult to undertake a systematic review the literature and draw meaningful conclusions. Clinicians who have treated MRONJ conservatively since the emergence of this entity are understandably slow to convert their protocol to the exact antithesis of what was originally advocated. This problem can only be overcome by strong evidence-based studies with multi-centric collaboration, or referral to single designated specialised unit.

Further studies are required to investigate the aetiology, risk factors, pathogenesis and treatment of Denosumab related osteonecrosis of the jaws. Although it appears to behave in the same way as bisphosphonate related ONJ, this has not been confirmed with evidence-based research.

Additionally, a multinational database for MRONJ would help to study the epidemiology, and the role of various risk factors in the pathogenesis. It would also give a better indication of disease incidence regarding specific medications in osteoporotic and oncology patients, as these figures are likely underestimated due to lack of reporting.

6 Conclusions

Medication related osteonecrosis of the jaws is a significant debilitating condition which affects a small percentage but an ever increasing number of patients. The emergence of this disease entity in 2003 was met with some scepticism from the medical world, but with increased awareness, and reporting of cases by the OMFS and dental profession, pharmacovigilance amongst prescribing clinicians has led to effective preventative strategies being implemented.

This is of increasing importance with further new medications such as Denosumab and anti-angiogenics being implicated as predisposing factors, it is likely that we will encounter more patients suffering from MRONJ.

However, the consensus on treatment strategies for MRONJ is largely divided between conservative non-surgical management, and that of adopting a surgical approach. Recent emerging studies appear to yield improved outcomes for patients undergoing surgical intervention. This approach is contrary to the initial non-surgical management guidance which was advocated, and the concept of surgery has been met with caution, and scepticism. The literature review also highlights a paucity of high quality research to formulate evidence-based guidance for best practice.

From this study, the following conclusions can be made:

1. Dental extraction was the major precipitating factor in the pathogenesis of MRONJ.
2. Treatment was feasible in an outpatient facility, under local anaesthetic +/- intravenous conscious dental sedation, and is tolerated well by patients.
3. Conservative surgical debridement with L-PRF was successful treatment option for patients afflicted by Stage 0, 1, 2, and selected cases of Stage 3 MRONJ with 73.3% curation rates.
4. It is unclear whether L-PRF was a useful adjuvant to conservative surgical debridement as the numbers within the study were too small.

5. Patients with an underlying diagnosis of osteoporosis appeared to respond better to treatment than oncology patients
6. Denosumab induced osteonecrosis of the jaws appeared to respond well to surgery, with all cases healing
7. Refractory cases, and Stage 3 disease with oro-antral communication, oro-nasal communication, or osteolysis extending to the inferior border of the mandible or sinus floor should be treated with an extensive surgical protocol.
8. The oral form of Co-amoxiclav antibiotic appears to be successful choice and route of administration for MRONJ cases.
9. The bacterial pathogens isolated from MRONJ sites are largely normal oral pathogens, which raises doubt on the pathogenic role of infection in disease progression, and it is likely down to opportunistic colonisation of disease sites.
10. There appears to be little difference in the pathogens isolated between oncology patients and osteoporotic patients.
11. There appears to be little difference in the pathogens isolated in bisphosphonate induced MRONJ compared with Denosumab induced disease.
12. Most bacterial pathogens isolated revealed sensitivity to Penicillin.
13. Most clinicians in Ireland apply the AAOMS, SDCEP and JIDA guidance to their practice and management of MRONJ patients.[1, 12-14, 30]
14. There appears to be no consensus on the management of MRONJ amongst clinicians, considering the AAOMS Stage dependent guidance or the level of training and education.[1]
15. There appears to be no consensus on the choice of antibiotic, the route of administration (oral/IV), the dosage, temporal patterns or duration for conservative management antibiotic prescribing, pre-surgical prescribing and post-surgical prescribing amongst prescribing clinicians in Ireland.

7 Appendices

7.1.1 Appendix: 1 Patient information leaflet

PARTICIPANT INFORMATION LEAFLET

Medication related osteonecrosis of the jaws: Prospective cohort study analysing the aetiology, microbiology, and response to surgical debridement with adjunctive leucocyte and platelet rich fibrin

You are being invited to participate in a research study:

In order to make an informed judgement on whether you wish to be part of this research study or not, you should understand any associated potential risks and benefits. This is called informed consent. This leaflet gives you information about the research study which will be discussed with you fully. You are invited to ask if there is anything that is not clear or if you would like further information. Once you understand the nature of the study and you wish to participate, you will be asked to sign a consent form. Should you decide not to participate in the study, simply do not return the consent form.

Description of study:

This study that we have proposed you participate in is an attempt to further our understanding and treatment strategy for medication related osteonecrosis of the jaws. Medication related osteonecrosis of the jaws is a condition whereby people develop areas of exposed dead bone within the mouth following dental treatment or in other cases spontaneously, as a side effect of taking medications that cause poor healing in the mouth (bisphosphonates or monoclonal antibodies). This study proposes a new method of treatment whereby the sites of exposed bone are treated with a membrane made from your own blood cells to aid healing.

Background information:

Each tooth sits within a bony socket of the jaw bone. Gum tissues cover and protect the jaw bones within the mouth. When a tooth is extracted the gum heals over the extraction site as a normal part of the healing process.

Medications used in the treatment of osteoporosis or cancer treatments can affect the normal sequence of gum and bone healing within the mouth. This can result in failure to heal, leading to exposed sites of dead bone in the mouth. People may experience pain and infection at these sites which has a negative impact on a person's quality of life. The problem can persist for many months or even years and treatment and resolution of this condition can be challenging. New research into methods that promote the healing process by using membranes from a patient's own blood cells may offer a solution to this problem. The standard procedure used at the Dublin Dental University Hospital to treat medication related osteonecrosis of the jaws, is with antibacterial mouthwashes, antibiotics and surgery. Surgical treatment involves numbing the site with local anaesthetic, reflecting the gum back at the site of disease, removing any sharp edges of dead bone and replacing the gum over the site with stiches.

Study Overview:

The study aims to collect information on whether leucocyte platelet rich fibrin (L-PRF) which is made from your own blood cells, helps to heal sites affected with medication related osteonecrosis of the jaws.

Description of treatment for:

Under local anaesthetic (area will be numbed) you will have a simple surgical procedure performed, whereby the gum at the site of disease will be peeled back, any dead bone will be removed, and rinsed thoroughly with salty water. Samples of dead bone will be sent to St James's Pathology Laboratory to be analysed, as part of normal

treatment protocol to confirm a diagnosis of medication related osteonecrosis of the jaws. You will have a blood sample taken on the day of the procedure (4-8 tablespoons of blood) which will be used to make the L-PRF membrane and also a full blood count screen. To form the L-PRF membrane a special machine is used to spin the blood which produces the membrane. The L-PRF membrane will be placed under the gum and closed over with stiches. Antibiotics will be given immediately before the procedure on the day, and for 1 week after. Advice on how to look after the area will be explained to you. You will have 4 review appointments following surgery to monitor healing.

Are you suitable for the study?

To be included in the study you must fulfil the criteria below:

- You must be over 18 years of age.
- You must have a diagnosis of medication related osteonecrosis of the jaws
- Be in good general health and able to tolerate minor dental surgery
- Be willing to co-operate with the requirements of the study protocol and be able to attend review appointments described below
- Be able to give consent to participate in this study and sign a consent form approved by The Research Ethics Committee of the Faculty of Health Sciences, Trinity College Dublin

If you are initially included in the study but you fail to complete all stages of the treatment due to poor attendance, you will be excluded from the study.

Study Appointments:

Screening assessment: You will be assessed, and if you fit the inclusion criteria for the study you will be invited to participate. You will be given a detailed description of the study and some written information. You will receive a phone call two weeks later to enquire whether you wish to participate in the study.

Study visit 1: Surgery An informed consent form will be signed to show you understand and agree to participate in the study. The operative surgical procedure will be completed at this appointment. In addition, we will record the necessary bone measurements and take some clinical photographs to document the appearance of the bone and gum tissues.

Study visit 2: Review After 10 days we will see you, and as part of your normal care we will rinse the sites, remove the stiches, take some clinical photographs of the site, and take a low dose x-ray. We will also ask you to fill in a quick questionnaire.

Study visit 3: Review Healing will be monitored at 1 month. The site will be examined, and clinical photographs taken.

Study visit 4: Review Healing will be monitored at 3 months. The site will be examined, and a clinical photograph taken.

Study visit 5: Review At 6 months we will review you for the final time. The site will be examined, clinical photographs will be taken, and a low dose x-ray to check for bone healing and persistence of disease.

**Should any further appointments be required this will be arranged on your normal clinic, but details will not be recorded for the purpose of the study.

Treatment is associated with risk. The risk associated with this type of treatment include:

- Pain following treatment
- Bleeding following surgery
- Swelling/ bruising after the surgery
- Infection at the site of surgery
- Damage to adjacent teeth
- Altered sensation to surgical soft tissue sites which may be permanent or temporary
- Failure to heal at the site of medication related osteonecrosis of the jaw
- Following a blood sample being taken from the arm you may experience pain, bleeding bruising or mild swelling.

There are no anticipated risks or harm associated with L-PRF. There are no risks of disease transmission using a blood sample from your own body.

Other information of relevance to the study:

- Your identity will remain confidential and your name will not be disclosed or published
- Investigators will have access to your Dublin Dental Hospital file which includes clinical notes, x-rays, clinical photographs, blood results and histopathology results
- If you decide to participate in this study, you may withdraw at any stage. If you decide not to participate, or withdraw during the study, your decision will be respected and your treatment in Dublin Dental University Hospital will continue as normal
- There will be no consequences to the standard of care you receive, should you decide not to participate in the study.
- You understand that the investigators may withdraw your participation in the study at any time without your consent

- If you are initially included in the study, but fail to complete all stages you will be excluded from the study

Contact details:

If you have any further questions please contact the principal researcher,

Dr Daphne Halley Email: daphne.halley@dental.tcd.ie Tel: 01-6127314

7.1.2 Appendix: 2 Consent form for participation in the study

Consent form for participation in study

Title of the research study: Medication related osteonecrosis of the jaws: Prospective cohort study analysing the aetiology, microbiology, and response to surgical debridement with adjunctive leucocyte and platelet rich fibrin (L-PRF)

Names of researchers:

Ms Daphne Halley (Dublin Dental University Hospital)

Professor Leo F.S Stassen (Dublin Dental University Hospital)

I ……..confirm that this study and the consent form have been explained to me. The investigator has answered all questions to my satisfaction. I believe that I understand what will happen if I agree to be part of this study. I have read, or had read to me this consent form and have had the opportunity to ask questions and all of my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study. I have received a copy of this agreement.

I have been assured that my identity will remain confidential and my name will not be disclosed or published. Any results from my histopathological test or full blood screen analysis will be added to my Dublin Dental University Hospital file as per normal hospital policy, and not used in future studies. Clinical photographs, x-rays and details from the completed questionnaire will be added to my Dublin Dental University Hospital file as per normal hospital policy, and not used in future studies. No specimen samples will be retained for the purpose of this study. I understand that I may withdraw from participating in the study at any time. I have also been assured that there will be no consequences to the standard of care I receive, should I decide not to participate in the study.

Name of participant …… Date…… Signature……..

Statement of investigator's responsibility:

I have explained the nature, purpose, procedures, benefits, risks of, or alternatives, to this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given consent.

Name of researcher …… Date…… Signature……

7.1.3 Appendix: 3 Ethical approval

THIS NOTEPAPER MUST NOT BE USED FOR PRESCRIPTIONS OR INVOICING PURPOSES

30th May 2016

SJWAMNCH Research Ethics Committee Secretariat
Claire Haftin: Ph: 4142199
email: claire.haltin@amnch.ie

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
INCORPORATING THE NATIONAL CHILDREN'S HOSPITAL

TALLACHT, DUBLIN 24, IRELAND
TELEPHONE +353 1 41 42000

Dr. Daphne Halley .
SPR Oral Surgery
Trinity College Dublin
Dublin Dental University Hospital
Lincoln Place Dublin 2.

Re: The use of leucocyte-rick and platelet rich fibrin (la-PRF) for the treatment of oestochemoecrosis of the iaws: A randomised control trial

REC Reference: 2016/03/03 / 2016-05 List 19 (6) (Please quote reference on all correspondence)

Dear Dr. Halley,

Thank you for your recent correspondence in which you responded to the conditions as requested by the SJH/AMNCH Research Ethics Committee.

The Chairman of the Committee, Dr. Peter Lavin, has reviewed this correspondence, is satisfied with the responses and supporting documentation therein and advises that full ethical approval is now in place for this study.

Yours sincerely,

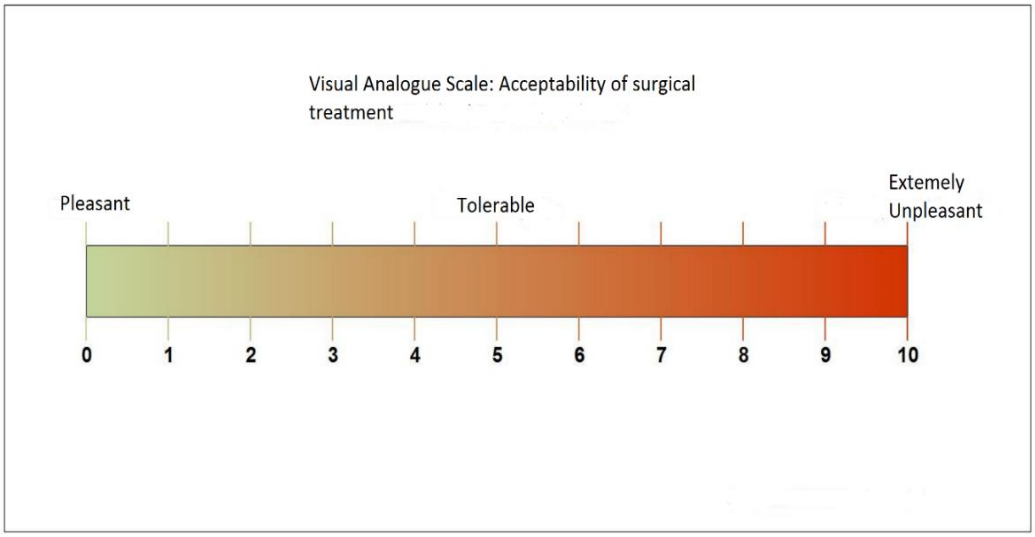
Clawe Hartin
Secretary
SJH/AMNCH Research Ethics Committee

The SJH/AMNCH Joint Research and Ethics Committee ope1 7tes in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.

NSV Code: WPA 00486

7.1.4 Appendix: 4 Visual analogue scale

At the appointment 10 days post-operatively, patients will be shown the scale below and asked to choose a point on the scale which best describes their intra-operative surgical experience. This result will be recorded within the patient's clinical notes.



7.1.5 Appendix: 5 Copy of the questionnaire

Title of Study:

Medication related osteonecrosis of the jaws: Prospective cohort study analysing the aetiology, microbiology, and response to surgical debridement with adjunctive leucocyte and platelet rich fibrin (L-PRF)

Introduction:

My name is Daphne Halley; I am a student in my third year of Oral Surgery postgraduate training in the Dublin Dental University Hospital. I am conducting research on the benefits of using leucocyte-platelet rich fibrin (L-PRF) as an adjunct to surgical debridement to promote healing in the management of medication related osteonecrosis of the jaws (MRONJ). As secondary objectives; I am looking at the microbes cultured from the specimens of necrotic bone, and also investigating current trends in treatment options adopted by Surgeons in Ireland for the management of MRONJ.

This part of the study involves the completion of a brief questionnaire on four clinical cases of patients with MRONJ, and how you would manage them.

Benefits of research:

This proposed research may highlight paucity in guidance for clinicians, and in turn help to form a consensus on how MRONJ should be managed in Ireland, with the aim of formulating a standardised evidence-based guidance pathway of care for these patients.

Confidentiality:

All information collected will be anonymous.

Permission:

This research has been approved by the St James's/Tallaght Research Ethics Committee and the Dublin Dental University Ethics Committee

Further Information:

You can get more information or answers to your questions about the study from the primary investigator, Daphne Halley, who can be contacted at the details below.

Name: Daphne Halley
Address: Dublin Dental University Hospital Lincoln Place, Dublin 2
Phone number: 083 1771255 **Email address:** daphne.halley@dental.tcd.ie

Questionnaire: Current management of patients diagnosed with medication related osteonecrosis of the jaws (MRONJ) in Ireland

The aim of this questionnaire is to look at the current management strategies employed for treating patients with established MRONJ in Ireland.

What is your title? (place a tick in the circle most applicable to your position)

- ○ Consultant Oral and Maxillofacial Surgeon
- ○ Specialist Oral Surgeon
- ○ Registrar in Maxillofacial Surgery
- ○ Registrar in Oral Surgery
- ○ NCHD
- ○ Other _____

What guidelines are you currently aware of for the management of established MRONJ of the jaws?

_____
_____
_____

Do you currently follow a guideline for the management of MRONJ of the jaws?

- ○ **Yes**
- ○ **No**

If yes, which guideline do you follow?

_____
_____

If no, what is your management protocol?

_____
_____

Do you feel the official guidelines that you mentioned above are relevant and comprehensive? (place a tick in the circle most applicable)

- Strongly agree
- Agree
- Neutral
- Disagree
- Strongly disagree

If not, why?

_____

_____

Do you feel confident managing patients with MRONJ? (place a tick in the circle most applicable)

- Strongly agree
- Agree
- Neutral
- Disagree
- Strongly disagree

If not, why?

_____

_____

CASE 1

A 68-year-old female presented with pain in the left anterior maxilla. She reports she had a tooth extracted 8 months previously at home in Latvia. She is currently on intravenous Zoledronic acid for metastatic breast cancer and has been on this medication for 14 months. She is a non-smoker. Clinically there is an erythematous sinus in the 2.1 region, which is painful and discharging pus on palpation. There is no visible exposed bone, but it is palpable on probing through the sinus. She reports no other symptoms. She reports that the area has previously been treated with antibiotics.

How would you manage this case? (place a tick in the circle with the most applicable answer)

- ○ Oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Surgical debridement, antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Other

If you answered other, please elaborate

_____

_____

If you were to prescribe antibiotics for non-surgical management, which one would you prescribe? Please specify duration and dosage.

Antibiotic _____

Dose _____

Duration _____

What is your rational for choosing this antibiotic?

_____

What is your rational for the duration of time prescribing this antibiotic?

_____

If you were to treat it surgically, would you prescribe antibiotics also? And if so what regime would you prescribe pre-operatively and post-operatively?

Preoperative antibiotic _____

Dose _____

Postoperative antibiotic _____

Duration _____

Dose _____

What is your rational for choosing this antibiotic?

_____

What is your rational for the duration of time prescribing this antibiotic?

_____

CASE 2

A 73-year-old female is reviewed on the clinic with a large area of exposed bone in the anterior maxilla. She had a tooth extracted two years previously, and subsequently developed pain, recurrent infections, halitosis and increasing exposure of necrotic bone. She has previously been managed with courses of antibiotics when suppuration was present, Chlorhexidine and saline mouth rinses. She was a non-smoker. She has a history of bi-annual intravenous Zoledronic acid for osteoporosis for 24 months. The infusions were stopped 14 months ago. She is also on Methotrexate for rheumatoid arthritis. At present, there is a 4.5cm x 1cm area of exposed firm bone, there is no suppuration, but the area is painful on examination with marked halitosis.

How would you manage this case? (place a tick in the circle with the most applicable answer)

- ○ Oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Surgical debridement, antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Other

If you answered other, please elaborate

_____

_____

If you were to prescribe antibiotics for non-surgical management, which one would you prescribe? Please specify duration and dosage.

Antibiotic _____

Dose _____

Duration _____

What is your rational for choosing this antibiotic?

_____

What is your rational for the duration of time prescribing this antibiotic?

_____

If you were to treat it surgically, would you prescribe antibiotics also? And if so what regime would you prescribe pre-operatively and post-operatively?

Preoperative antibiotic _____

Dose _____

Postoperative antibiotic _____

Duration _____

Dose _____

What is your rational for choosing this antibiotic?

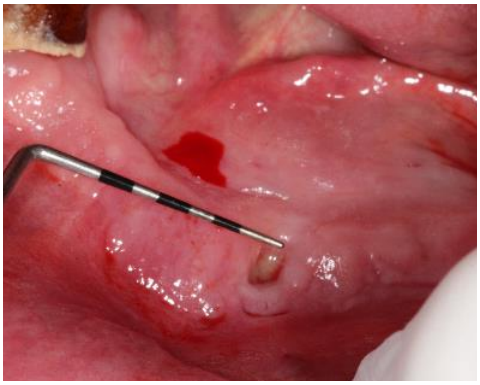
_____

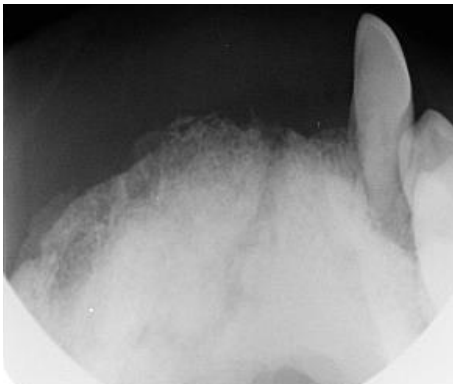
What is your rational for the duration of time prescribing this antibiotic?

_____

CASE 3

A 61-year-old male was reviewed on the clinic with a chronic sinus in the left anterior mandible. One year previously he reports a lower incisor spontaneously exfoliating, and since then he has had recurrent episodes of pain and ongoing suppuration. He has previously been managed with courses of antibiotics when indicated, Chlorhexidine and saline mouthwashes. He has a 9-month history of intravenous Zoledronic acid for metastatic prostate cancer, which was stopped 5 months ago. He currently smokes and has a 30-smoking pack year history. At present, he has poor oral hygiene and there is draining sinus in the 3.3 region which is discharging pus.





How would you manage this case? (place a tick in the circle with the most applicable answer)

- ○ Smoking cessation
- ○ Smoking cessation, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Smoking cessation, antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Smoking cessation, surgical debridement, antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Other

If you answered other, please elaborate

_____

_____

If you were to prescribe antibiotics for non-surgical management, which one would you prescribe? Please specify duration and dosage.

Antibiotic _____

Dose _____

Duration _____

What is your rational for choosing this antibiotic?

_____

What is your rational for the duration of time prescribing this antibiotic?

_____

If you were to treat it surgically, would you prescribe antibiotics also? And if so what regime would you prescribe pre-operatively and post-operatively?

Preoperative antibiotic _____

Dose _____

Postoperative antibiotic _____

Duration _____

Dose _____

What is your rational for choosing this antibiotic?

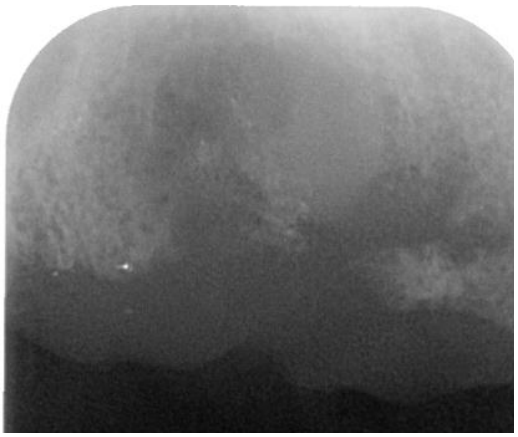
_____

What is your rational for the duration of time prescribing this antibiotic?

_____

CASE 4

A 78-year-old female presents as a new patient to the clinic with exposed bone, pain and suppuration in the right maxilla. She reports having the 1.3 extracted 8 months previously with her dentist, but the area has never healed. She has had 2 courses of antibiotics to date. She has a 4-year history of weekly oral Alendronic acid for osteoporosis, which was stopped 4 months ago. She is a non-smoker.



How would you manage this case? (place a tick in the circle with the most applicable answer)

- ○ Oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Surgical debridement, antibiotics, oral hygiene instruction, saline and Chlorhexidine mouthwashes
- ○ Other

If you answered other, please elaborate

_____

_____

If you were to prescribe antibiotics for non-surgical management, which one would you prescribe? Please specify duration and dosage.

Antibiotic _____

Dose _____

Duration _____

What is your rational for choosing this antibiotic?

_____

What is your rational for the duration of time prescribing this antibiotic?

_____

If you were to treat it surgically, would you prescribe antibiotics also? And if so what regime would you prescribe pre-operatively and post-operatively?

Preoperative antibiotic _____

Dose _____

Postoperative antibiotic _____

Duration _____

Dose _____

What is your rational for choosing this antibiotic?

_____

What is your rational for the duration of time prescribing this antibiotic?

_____

Thank you for taking the time to complete this questionnaire. If you would like feedback from the survey, I would be happy to disseminate the results. Please contact me via email: daphne.halley@dental.tcd.ie

7.1.6 Appendix: 6 Statistics for larger samples

Ideally, if a large number of patients was achievable the study design would be a controlled trial to investigate the benefit of L-PRF. A power calculation would be used to estimate the number of patients required to achieve significant results. The research question would be; “Is the proportion of healed versus non-healed different for conservative surgical debridement compared to conservative surgical debridement with adjunctive L-PRF”. Intra-operative randomization would not be feasible due to the nature of preparing L-PRF, but post-operative assessment of healing could be randomized which would reduce assessor bias. The data is discrete and can be categorized as healed, or not healed. If there were very large numbers, participants could be paired according to independent variables such as age, sex, antiresorptive history, medical co-factors etc., and a McNemars’s test could be performed. With smaller numbers, where pairing is not feasible a Chi-square test for counts ≥5, or a Fisher’s exact test for counts ≤5.

Similarly, if there was no control group the data is still looking at counts of patients with categorical dependent variables and hence Chi-square test is applicable for counts ≥5, or a Fisher’s exact test for counts ≤5.

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