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Abstract

Objectives: Our aim was to conduct a systematic review (SR) of the literature assessing the role of human biomarkers in the diagnosis or prognostication of medication-related osteonecrosis of the jaws (MRONJ).

Study design: An electronic search without date or language restriction was carried out in PubMed/MEDLINE, the *Cochrane Central Register of Controlled Trials*, Web of Science, and LILACS until March 2018. In addition, a manual search in the grey literature was conducted. The search process was performed by two independent reviewing authors. Eligibility criteria included randomised and non-random clinical trials, prospective or retrospective cohorts, case controls, and case series evaluating the association between biomarkers and MRONJ. The protocol of this SR was registered in PROSPERO under number CRD42018095886.

Results: The search and selection process yielded 19 studies (2 case series, 6 case-control studies, 9 prospective cohort studies and 2 retrospective studies) published between 2008 and 2018. Twenty-four biomarkers collected from serum, saliva, and urine were investigated by these studies. Eleven biomarkers were possibly related to MRONJ; however, no consensus is observed in the literature in regard to the sensitivity and clinical effectiveness of these biomarkers.

Conclusion: While many biomarkers have been associated with MRONJ, the present SR found scarce clinical evidence supporting the use of these biomarkers for the diagnosis and prognosis of MRONJ.

Keywords: Osteonecrosis, Medication-related osteonecrosis of the jaws, Biomarkers, Diagnosis.

Statement of clinical relevance

While many biomarkers have been associated with medication-related osteonecrosis of the jaws (MRONJ), scarce clinical evidence was identified in a systematic review to support the use of these biomarkers for the diagnosis and assessment of prognosis of MRONJ.

Introduction

Medication-related osteonecrosis of the jaw (MRONJ) is a severe complication that can occur in patients who have been treated with anti-resorptive medication¹. Bisphosphonate (BP) medications are the most frequently used class of anti-resorptive drugs for the treatment of bone disorders such as osteoporosis and cancer-associated skeletal complications². However, more recently, other drugs such as denosumab^{1,3} and anti-angiogenic drugs⁴ have been implicated in MRONJ.

MRONJ is characterised by the necrosis of jawbones in the oral cavity^{5,6} and affects the mandible approximately twice as often as the maxilla.⁷ According to the American Association of Oral and Maxillofacial Surgeons criteria⁸, most of the previous studies have diagnosed MRONJ as 'exposed bone in the maxillofacial area occurring in the absence of head and neck irradiation and showing no evidence of healing for at least eight weeks after identification in patients treated with BP therapy. The incidence of MRONJ after oral surgery involving bone is greater among patients receiving frequent intravenous BP infusions compared with patients taking oral BPs⁹. In relation to denosumab, the MRONJ risk in osteoporosis patients treated is estimated to be from 0.01% to 0.03% and in cancer patients to be from 1% to 2%¹⁰.

The pathogenesis of MRONJ is currently unclear but may result from extensive bone remodelling and the subsequent relative hypoxia of newly formed bone, typically involving oral surgery or trauma with subsequent delayed wound healing, altered jawbone remodelling, and a biofilm-mediated infection¹¹⁻¹³.

Intravenous BPs, which are used mostly for oncological indications, have been linked to MRONJ risk of 3-18%. On the other hand, oral BPs have been estimated to cause MRONJ at a significantly lower rate, 1.04 to 69 patients in 100,000 patients.¹⁴ The risk of MRONJ may increase in patients using corticosteroids^{15,16}.

Previous studies have attempted to identify useful biomarkers for prediction¹⁷⁻³², diagnosis²⁸ and severity¹.

Bone biomarkers identify products of bone resorption and formation⁹, indicate the bone-turnover process, and may be used to validate bone-mineral density testing when investigating patients for bone disease.¹⁴ Although one or more bone biomarker assays may help identify patients with increased bone resorption and/or formation rates, their efficacy in clinical practice with BP treatment is uncertain at this time¹⁷.

Currently, no clinical biomarkers exist to identify patients at risk for MRONJ. The bone biomarkers and other relevant endocrine assays discussed in the literature include C-telopeptide (CTX), N-telopeptide (NTX), bone-specific alkaline phosphatase (BAP), osteocalcin (OCN), intact parathyroid hormone (PTH), RANKL, osteoprotegerin (OPG), triiodothyronine, thyroxine, thyroid-stimulating hormone, and vitamin D 25-hydroxy. Recent studies suggest that serum-immune markers such as specific subsets of T-cells (Th-17 and $\gamma\delta$ -T) may serve as MRONJ risk predictors^{33,34}. On one hand, studies suggest that serum bone-turnover markers (CTX and NTX) can predict the risk of MRONJ or guide therapeutics^{35,36}. On the other hand, others have shown insufficient evidence for MRONJ-risk prediction using CTX and NTX markers^{9,37,21,26}. In a case-control study²⁴, the authors evaluated whether interleukin-17 (IL-17) and CTX correlate with MRONJ; they concluded that both markers were significantly higher in MRONJ, suggesting altered immune responses and bone remodelling may contribute to MRONJ development.

These contradictory results lead us to speculate whether any biomarker has the potential to mark MRONJ. Thus, the aim of the present study was to conduct a systematic review (SR) of the literature assessing potential biomarkers associated with MRONJ.

Materials and Methods

Protocol registration

The protocol of this SR was based primarily on the PRISMA-P³⁸ and registered in PROSPERO under number CRD42018095886. No deviation from the originally specified protocol was registered. This SR methodology followed the recommendations of the *Cochrane Handbook for Systematic Reviews of Interventions*³⁹. The PRISMA checklist⁴⁰ was followed to increase the quality and transparency of the study. Clinical questionnaires were separated and organised using the PICOS strategy⁴¹.

MRONJ case definition

To distinguish MRONJ from other delayed healing conditions, the present SR considered MRONJ if all the following three characteristics were present⁸:

1. Current or previous treatment with medication related to osteonecrosis of the jaws
2. Exposed bone in the maxillofacial region that has persisted for more than eight weeks

3. No history of radiation therapy to the jaws and no metastatic disease to the jaws

Focused question

Do human biomarkers contribute to the prediction, diagnosis, staging, or prognostication of MRONJ?

Search strategy

An electronic search without date or language restriction was performed in PubMed/MEDLINE, the *Cochrane Central Register of Controlled Trials*, Web of Science, and LILACS until March 2018. Furthermore, a specific electronic search was performed on the following journals' websites: *Journal of Periodontology*, *Journal of Clinical Periodontology*, *Clinical Oral Implant Research*, *Clinical Implant Dentistry and Related Research*, *International Journal of Oral & Maxillofacial Surgery*, *Journal of Oral and Maxillofacial Surgery*, *Oral Surgery*, *Oral Medicine*, *Oral Pathology and Oral Radiology*, *Head and Neck Pathology*, *Journal of Oral Pathology and Medicine*, and *Oral Oncology*. A search of the Grey Literature Report⁴² and OpenGrey⁴³ databases revealed unpublished studies. Furthermore, the reference lists/bibliographies of all candidate full-text articles (cross-referencing) and in the ClinicalTrials.gov database were searched.

A search strategy for all databases was developed as follows:

- 1) osteonecrosis[MeSH Terms] OR osteonecrosis of the jaw[Title/Abstract] OR bisphosphonate-associated osteonecrosis of the jaw[MeSH Terms] OR bone necrosis[Title/Abstract] OR bone necroses[Title/Abstract] OR biomarkers[MeSH Terms] OR biochemical markers[MeSH Terms] OR serum markers[MeSH Terms] OR salivary markers[Title/Abstract] OR immune markers[MeSH Terms] OR urine markers[Title/Abstract] OR C-terminal telopeptide[Title/Abstract] OR CTX[Title/Abstract] OR bone-specific alkaline phosphatase[Title/Abstract] OR BAP[Title/Abstract] OR osteocalcin[MeSH Terms]
- 2) bisphosphonates[MeSH Terms] OR etidronate[MeSH Terms] OR clodronate[MeSH Terms] OR risedronate[MeSH Terms] OR alendronate[MeSH Terms] OR ibandronate[MeSH Terms] OR pamidronate[MeSH Terms] OR zoledronic acid[MeSH Terms] OR denosumab[MeSH Terms] OR anti-RANKL antibody[Title/Abstract] OR anti-angiogenic[MeSH Terms] OR drug induced[Title/Abstract] OR drug related[Title/Abstract] OR drug associated[Title/Abstract] OR anti-resorptive treatment[Title/Abstract] OR anti-resorptive drug[MeSH Terms] OR anti-resorptive agents[MeSH Terms]
- 3) 1 and 2

The search strategy included appropriate changes in the keywords and followed the syntax rules of each database.

Eligibility criteria

The present SR included studies that analysed the association between biomarkers and MRONJ according to the PICOS strategy described

below:

Population: Adults or children with MRONJ

Interventions: Not applicable

Comparison: Not applicable

Outcome: To evaluate specific biomarkers associated with MRONJ

Study design: Observational studies (prospective or retrospective cohorts), case-control studies, and case series

The present SR excluded editorial letters, pre-clinical studies, in vitro studies, and case reports and reviews.

Screening process

The search-and-screening process was conducted by two independent reviewing authors (VM and DA), starting with the analysis of titles and abstracts. Next, full papers were selected for careful reading and analysed according to eligibility criteria (inclusion/exclusion) for future data extraction. Disagreements between the reviewing authors were resolved through careful discussion. When necessary, the authors of the included studies were contacted by email for clarification.

Data extraction

Two independent reviewing authors (VM and DA) extracted data using a form to record the following: study details (year of publication and authors), study design, clinical proposal (prediction, diagnosis, severity), participants, drug type and administration route, biomarkers, methods, results, and authors' conclusions.

Assessments of the risk of bias and quality

Risk-of-bias and study-quality analyses were performed independently by two reviewing authors (VM and MC). The Newcastle–Ottawa Scale⁴⁴ was used in the analysis of non-randomised studies. The assessment was conducted independently for cohort and case-control studies. Studies could obtain one star/point per item in the selection and ascertainment categories or two stars/points in the comparability category. According to the NOS, the maximum score for a given study is nine stars/points. Studies scoring six or more stars were considered to have low risk of bias.

Data synthesis

A meta-analysis of the findings was not possible given the high level of heterogeneity found across studies. Instead, a qualitative (narrative) synthesis was conducted.

Results

Study protocol

The search and data extraction were performed in adherence with the study protocol with no deviations. The search of titles, abstracts, and full-text articles as well as the risk of bias of selected studies were independently reviewed by two reviewers using a standard method to ensure internal validity.

Literature search

The initial search yielded 427 unique titles in MEDLINE/PubMed, 119 in the *Cochrane Central Register of Controlled Trials*, 222 in Web of Science, and 52 in LILACS. The first evaluation resulted in the selection of 25 full-text articles. Six studies⁴⁵⁻⁵⁰ were excluded after careful reading for failing to meet the eligibility criteria of the present SR. Thus, 19 studies^{1,17-32,51,52} published between 2008 and 2018 were included in the present SR. Searches of grey literature and the ClinicalTrials.gov database yielded no further studies. Only studies in the English language were found. Figure 1 presents the search and selection process of papers.

Study characteristics

Table 1 presents the characteristics of included studies. Two case series^{17,18}, six case-control studies^{1,19,23,27,30,52}, eight prospective cohort studies^{18, 21,24,25,29,31,32,51} and two retrospective studies^{20,32} were included in the current SR. The determination of biomarkers was conducted through the analysis of serum^{1,17-29,31,32,51,52}, saliva^{25,27,30,51} and urine^{26,28} samples. The total number of survey participants was 1,174. Marked methodological variability was observed among the studies. Five studies^{17,18,31,32,52} did not report the methodology used for the evaluation of biomarkers. Four studies^{27,29,30,32} did not report the type of BP used by the survey participants. Of the total of 19 included studies, 17 used biomarkers for MRONJ prediction^{17-32,51,52}, one for diagnosis²⁸, and one for assessing MRONJ severity¹.

Findings based on previous focused questions

Twenty-four possible biomarkers were investigated in the studies (Table 1). Only two studies^{31,52} investigated biomarkers in participants who underwent RANKL block therapy (denosumab). Eleven biomarkers were possibly related to MRONJ (CTX, BAP, IL-17, neutrophil function, oxidative stress [OS], NTX, metalloproteinase-9, desmoplakin, vascular endothelial growth factor, leukocyte count, and C-reactive protein [CRP]). In four case-control studies^{19,23,27,52}, five biomarkers (CTX, BAP, IL-17, OS, and NTX) were present in statistically significant levels in the MRONJ group compared with the control groups. Table 2 presents possible evidence of the relationship between biomarkers and MRONJ, while Figure 2 shows the levels of biomarkers detected in the studies and their respective associations with MRONJ (negative or positive).

Assessments of the risk of bias and quality

The results of the risk-of-bias and quality analyses of the included studies are presented in Table 3. After the analyses, no study scored the maximum NOS score. Seven studies^{17-19,21,23,27,32} scored less than six points, indicating the possibility of a high risk of bias.

Discussion

Summary of evidence

Bone tissue is a dynamic structure which undergoes continuous, life-long remodelling (formation and resorption) in response to physical load or metabolic environment²⁰. Biochemical turnover markers have long been used as an effective evaluation method during the bone-remodelling period²¹. However, pronounced variability in the markers is evident among individuals, thus complicating their use as an aid in MRONJ diagnosis and risk prediction³⁵.

Biomarkers indicating the degradation products of collagen I may indicate bone resorption. In 2000, the use of CTX to monitor patients undergoing anti-resorptive therapy was proposed because of its minimal spontaneous variation.⁵³ In most patients, on bone-turnover suppression, CTX levels tend to decrease²⁴.

CTX was the biomarker most investigated by the studies in the present SR, which showed evidence^{19,20,24} and no evidence^{17,18,22,21,26,28,29} for MRONJ prediction. Controversy and debate exist in the scientific literature regarding the recommendation of the CTX test for MRONJ risk⁵⁴. Only three studies^{19,20,24} (30%) reported a positive association between CTX and MRONJ. One of the difficulties in evaluating the association of CTX with MRONJ is the presence of confounding factors, such as fasting, that can alter CTX values among individuals^{55,56}. A previous SR revealed no significant differences in mean CTX values between MRONJ patients and control participants⁵⁴. Another recent SR also indicated that the CTX test has no predictive value in determining the risk of MRONJ in patients taking BPs⁵⁵.

Bone formation can be evaluated by the serum levels of BAP, an osteoblast-specific enzyme, the second most investigated biomarker in the included studies. Three studies^{20,28,49} showed significantly lower BAP levels in MRONJ patients taking oral BPs, while seven other studies^{17,18,23,26,29,31,30} lacked evidence associating BAP levels with MRONJ.

OCN is a biomarker of bone-remodelling activity and reflects bone formation in bone disease. Four of the included studies^{17-19,29} found that OCN values in MRONJ patients were significantly lower than normal reference values, but all studies lacked evidence to affirm an association between OCN and MRONJ and that OCN is relatively stable and less affected by BP therapy.

Bone resorption can be assessed by examining the level of PTH, which indirectly promotes bone resorption by osteoclasts. Four of the included studies^{17,18,20,25} report that PTH has no predictive value for MRONJ development.

Thyroid hormones and vitamin D 25-hydroxy are essential for skeletal development and in maintaining optimal bone strength⁵¹. Although a previous retrospective study⁵⁷ indicated hypothyroidism to be an important MRONJ risk factor, three of the included studies^{17,31,51} did not observe a significant association between triiodothyronine, thyroxine, thyroid-stimulating hormone, and vitamin D 25-hydroxy and MRONJ. Four included studies used NTX^{23,28,30,31} as a biomarker to measure the bone-turnover rate. NTX can be measured in the urine^{25,28}, serum^{17,18,23,25,28,31}, or saliva³⁰. While two included studies^{28,30} reported evidence between NTX and MRONJ patients, four other studies^{17,18,23,31} lacked evidence. The use of NTX for MRONJ diagnosis is complex because this biomarker can alter even without evidence of the improvement of bone-necrosis lesions. Systemic factors such as circadian rhythm, diet, bone disease, and bone metastases may be associated with changes in NTX levels³¹.

A high level of CRP in the blood is a marker of inflammation²⁹. Lack of specificity may hinder a direct association between CRP and MRONJ. In the present SR, one included study⁵¹ showed evidence of an association between CRP and MRONJ; on the other hand, other study³² found no evidence of such an association. CRP levels may be altered by various inflammatory disorders. Specifically, in MRONJ patients, the severity of bone necrosis and gingival inflammation and the progression of neoplastic lesions may increase CRP levels^{29,58}. In addition, psychological factors may alter CRP⁵⁹.

Although they are related to MRONJ, the mechanism of action of BPs and denosumab are different. Denosumab blocks RANKL, mimicking the physiological effects of osteoprotegerin, a tumour necrosis factor family member that prevents the differentiation of osteoclasts and promotes

their apoptosis, inhibiting bone resorption by depleting mature osteoclasts¹¹. However, two studies^{1,28} showed that the serum levels of RANKL and OPG and the RANKL/OPG ratio were not valuable biomarkers to determine the severity of oral lesions in MRONJ patients.

Studies included in the present SR showed evidence between IL-17²⁴, neutrophil function²⁷, OS⁴⁷, metalloproteinase-9⁵¹, desmoplakin⁵¹, vascular endothelial growth factors²⁹, and leukocyte count³² and MRONJ, while other authors found low evidence between deoxypyridinoline²⁵, amino-terminal propeptide of type I collagen²⁸, sclerostin²⁸, total alkaline phosphatase²⁹, RANKL¹, OPG¹, and RANKL/OPG ratio¹ and MRONJ. However, these hypotheses should be interpreted with caution given the deficiency in available studies related to these biomarkers.

Strengths and limitations

The present SR's several strengths include the achievement and prior registration of a protocol, an unrestricted search process (including grey literature), and duplicate review procedures for the search and assessment of the risk of bias. However, some limitations are also observed. After the risk-of-bias analysis (NOS) was conducted, seven studies^{17,18,21,23,27,32,52} were classified as having a high risk of bias; therefore, their results should be interpreted with caution. Another limitation is the wide variety of study designs and methodologies included in this review, which make it difficult to interpret and compare the data. For example, most studies^{27,29,30,32} did not individualise the analyses in relation to the drug type or administration route used and the therapy duration. Analyses regarding age and gender were also not conducted.

Currently, literature regarding MRONJ incidence in patients undergoing denosumab therapy is scarce. In this SR, only two studies^{31,52} included data on denosumab. However, neither study conducted a subgroup analyses, which makes it impossible to individually analyse data between BPs and denosumab.

Implications for clinical practice and recommendations for further research

To date, the literature fails to demonstrate reliable biomarkers for the diagnosis of predisposition and prognosis of MRONJ. Thus, the use of biomarkers for MRONJ diagnosis or clinical monitoring should be cautiously interpreted. Currently, no biomarker with MRONJ specificity and sensitivity exists. Biomarkers should be interpreted in relation to the patients' clinical, radiological, and systemic conditions.

The analysis of the protein-derivative biomarkers in the baseline is fundamental. In this way, the monitoring and comparison of these biomarkers could be conducted throughout the treatment. In addition, an individualised search for MRONJ biomarkers in relation to drug type, administration route, and treatment duration is encouraged. To reduce the risk of bias, the methodology for collecting and analysing the biomarkers should be carefully performed. For example, the current immunoassays available for bone BAP do not show perfect specificity and still display some degree of cross-reactivity toward liver BAP. Thus, increased bone BAP levels must be interpreted with caution in patients with liver disease. Also, among the controllable factors, bone resorption displays significant circadian variation, and serum CTX, NTX, and OC concentrations peak in the early morning between midnight and 8:00 a.m., with a nadir in the afternoon⁶⁰. This circadian effect can be generally

observed with most bone biomarkers, though CTX displays the highest amplitude in circadian variation. In this way, the collection of these biomarkers should preferably be done in the morning.

The potential of each body fluid should be better investigated. Most of the studies included in this SR searched for biomarkers present in the blood serum. However, the study by Thumbigere-Math et al.⁵¹ has identified through salivary proteomic analysis the significant change in expression of more than 200 proteins in MRONJ cases compared to controls. In this way, the understanding of the interrelationship between sensitive biomarkers for the MRONJ and the body fluid pathway is essential in the future.

Conclusion

In this SR, 22 biomarkers were listed, 11 showed evidence for MRONJ diagnosis, prediction, or severity (CTX, BAP, IL-17, neutrophil function, OS, NTX, metalloproteinase-9, desmoplakin, VEGF, CRP, leukocyte count), and 11 showed no evidence (OCN, PTH, T3, T4, TSH, vitamin D 25-hydroxy, DPD, P1NP, sclerostin, ALP, RANKL/OPG ratio). Little clinical evidence supports the use of these biomarkers for MRONJ diagnosis and prognosis and may allow confusion on MRONJ diagnosis. Thus, professionals should interpret the association between biomarkers and MRONJ with caution. A greater number of prospective studies is encouraged in the future.

ACCEPTED MANUSCRIPT

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Figure captions.

Figure 1. Flow diagram (PRISMA format) of the screening and selection process.

Figure 2. Number of occurrence of the biomarkers in the studies and their respective representations (negative or positive) with MRONJ.

Table 1. Main characteristics of included studies.

Author (year)	Study design Number of participants	Type and administration route of BP	Methods	Clinical proposal	Biomarkers	Samples	Origin	Disease
Lehrer et al., (2008) ¹⁷	Case-series 5	Zoledronic acid, pamidronate, alendronate Oral and IV	NR	Prediction	CTX, NTX, BAP, OCN, PTH, T3, T4, TSH, Vitamin D 25 hydroxy	Serum concentration	USA	Metastatic breast cancer, Osteoporosis, metastatic prostate cancer
Lehrer et al., (2009) ¹⁸	Case-series 7	Zoledronic acid, pamidronate; alendronate Oral and IV	NR	Prediction	CTX, NTX, BAP, OCN, PTH, T3, T4, TSH, vitamin D 25 hydroxy	Serum concentration	USA	Metastatic breast cancer, osteoporosis Gaucher's disease
Kwon et al., (2009) ¹⁹	Prospective 18	Alendronate and risendronate Oral	ELISA	Prediction	CTX	Serum concentration	Korea	Osteoporosis
Lazarovici et al., (2010) ²⁰	Prospective 78	Alendronate, risedronate, zoledronic acid, pamidronate Oral and	ELISA, MicroVue BAP EIA Kit, Immulite 2000 intact	Prediction	CTX, BAP, PTH	Serum concentration	Israel	Osteoporosis, breast carcinoma, multiple myeloma, Prostate carcinoma, neurogenic carcinoma

		IV	PTH in vitro diagnostic					
Lee et al., (2010) ²¹	Prospective 163	Alendronate, risendronate, ibandronate Oral	ELISA	Prediction	CTX	Serum concentration	USA	Osteoporosis, Osteopenia, breast cancer, prostate or colon cancer
Kwon et al., (2011) ²²	Retrospective 23 (< 6 months) 61 (> 6 months)	Alendronate Oral	ELISA, radioimmunoassay	Prediction	CTX and OCN	Serum concentration	Korea	Osteoporosis
Morris et al., (2012) ²³	Retrospective 56	Zoledronic acid, pamidronate, alendronate Oral and IV	ELISA	Prediction	NTX and BAP	Serum concentration	USA	Breast cancer, prostate cancer, myeloma, ovarian cancer, lung cancer
Lee et al., (2013) ²⁴	Case-control 9 (MRONJ) 9 (Control)	Alendronate, risedronate, ibandronate, alendronate Oral and IV	ELISA	Prediction	CTX and IL-17	Serum concentration	USA	Cancer, osteoporosis
Favot et al., (2013) ²⁵	Prospective 23	Alendronate, pamidronate, zoledronic Oral and IV	Spectrophotometer	Prediction	Neutrophil function	Serum concentration and saliva	Canada	NR

Kim et al., (2013) ²⁶	Case-control 37 (MRONJ) 37 (Control)	Alendronate, risendronate, pamidronate, zoledronic Oral and IV	ELISA	Prediction	OCN, DPD, CTX, NTX, BAP, PTH	Serum concentration and urine	Korea	Osteoporosis, bone metastasis
Bagan et al., (2014) ²⁷	Case-control 24 (IV BPs MRONJ) 20 (received IV BPs without MRONJ) 17 (Control)	NR IV	High- performanc e liquid chromatogr aphy, spectrophot ometer	Prediction	OS	Serum concentration and saliva	Spain	Breast câncer, multiple myeloma
Patel et al., (2014) ²⁸	Prospective 29	Pamidronate, zoledronic acid IV	ELISA	Diagnosis and prediction	NTX, CTX, P1NP, sclerostin, sRANKL, BAP	Serum concentration and urine	USA	Multiple myeloma
Thumbigere- Math et al., (2015) ⁵¹	Prospective 40	Zolendronate, pamidronate IV	Mass spectrometr y	Prediction	Metalloproteinase -9 and Desmoplakin	Serum concentration and saliva	USA	Breast cancer, multiple myeloma, prostate cancer, lung cancer, renal cell carcinoma
Kolokythas et al., (2015) ³⁰	Case-control 20 (MRONJ) 14 (Control)	NR IV	ELISA	Prediction	NTX and BAP	saliva	USA	Multiple myeloma, breast cancer, prostate cancer, Renal carcinoma

Thumbigere-Math et al., (2016) ²⁹	Prospective 25	NR IV	Immunodiagnostic Systems Inc., ELISA	Prediction	ALP, BAP, OCN, CTX, VEGF, T3, T4, TSH, Vitamin D 25 hydroxy, CRP	Serum concentration	USA	Breast cancer, multiple myeloma, prostate cancer, lung cancer, Renal cell carcinoma
Tohashi et al., (2016) ³¹	Prospective 56	Bisphosphonates and Denosumab Oral and IV	NR	Prediction	NTX and BAP	Serum concentration	Japan	Bone metastasis, Osteoporosis, multiple myeloma
Mücke et al., (2016) ³²	Prospective 212	NR Oral and IV	NR	Prediction	Leukocyte count CRP	Serum concentration	Germany	Metastatic breast cancer, multiple myeloma, metastatic prostate cancer, osteoporosis, metastatic renal cancer, Metastatic non-small cell lung cancer, Metastatic colonic cancer, lymphoma
Bagan et al., (2017) ¹	Case-control 41 (MRONJ) 44 (Control)	Zoledronic acid, alendronate, ibandronic acid, risedronate Oral and IV	ELISA	Severity	RANKL and OPG	Serum concentration	Spain	Multiple myeloma, breast cancer, multiple myeloma and breast cancer, prostate cancer, renal cancer, lung cancer, colon cancer, lymphoma, osteoporosis

Peisker et al., (2018) ⁵²	Case-control 20 (MRONJ) 20 (Control)	Zoledronic acid, ibandronate, risendronate, Denosumab Oral and IV	NR	Prediction	CTX, OCN, PTH, BAP	Serum concentration	Germany	Metastatic breast cancer, osteoporosis
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MRONJ, medication-related osteonecrosis of the jaw; IV, intravenous; BP, bisphosphonate; CTX, C-terminal telopeptide; BAP, bone-specific alkaline phosphatase; PTH, intact parathyroid hormone; NTX, N-telopeptide; OCN, Osteocalcin; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; IL-17, Interleucin-17; DPD, deoxypyridinoline; OS, oxidative stress; P1NP, amino-terminal propeptide of type I collagen; sRANKL, soluble receptor activator of NF- κ B ligand; VEGF, vascular-endothelial growth factor; CRP, C-reactive protein; BMD, bone mineral density; RANKL, receptor activator of NF- κ B ligand; OPG, osteoprotegerin; NR, not reported

Table 2. Relation of evidence (positive or negative) between biomarkers and MRONJ.

Biomarker	Studies that showed evidence	Studies that showed lack of evidence
CTX	Kwon et al. (2009) ¹⁹ ; Lazarovici et al. (2010) ²⁰ ; Lee et al. (2013) ²¹	Lehrer et al. (2008) ¹⁷ ; Lehrer et al. (2009) ¹⁸ ; Kwon et al. (2011) ²² ; Lee et al. (2010) ²¹ ; Kim et al. (2013) ²⁶ ; Patel et al. (2014) ²⁸ ; Thumbigere-Math et al. (2016) ²⁹ ; Peisker et al. (2018) ⁵²
BAP	Lazarovici et al. (2010) ²⁰ ; Patel et al. (2014) ²⁸ ; Peisker et al. (2018) ⁵²	Lehrer et al. (2008) ¹⁷ ; Lehrer et al. (2009) ¹⁸ ; Morris et al. (2012) ²³ ; Kim et al. (2013) ²⁶ ; Thumbigere-Math et al. (2016) ²⁹ ; Tohashi et al. (2016) ³¹ ; Kolokythas et al. (2015) ³⁰
IL-17	Lee et al. (2013) ²¹	
Neutrophil function	Favot et al. (2013) ²⁵	
OS	Bagan et al. (2014) ²⁷	
NTX	Patel et al. (2014) ²⁸ ; Kolokythas et al. (2015) ³⁰	Morris et al. (2012) ²³ ; Kim et al. (2013) ²⁶ ; Tohashi et al. (2016) ³¹ ; Lehrer et al. (2008) ¹⁷ ; Lehrer et al. (2009) ¹⁸
Metalloproteinase-9	Thumbigere-Math et al. (2015) ⁵¹	

Desmoplakin	Thumbigere-Math et al. (2015) ⁵¹	
VEGF	Thumbigere-Math et al. (2016) ²⁹	
CRP	Thumbigere-Math et al. (2016) ²⁹	Mücke et al. (2016) ³²
Leukocyte count	Mücke et al. (2016) ³²	
OCN	Lehrer et al. (2008) ¹⁷ ; Lehrer et al. (2009) ¹⁸ ; Kwon et al. (2011) ²² ; Kim et al. (2013) ²⁶ ; Thumbigere-Math et al. (2016) ²⁹ ; Peisker et al. (2018) ⁵²	
PTH	Lehrer et al. (2008) ¹⁷ ; Lehrer et al. (2009) ¹⁸ ; Lazarovici et al. (2010) ²⁰ ; Kim et al. (2013) ²⁶ ; Peisker et al. (2018) ⁵²	
T3, T4 and TSH	Lehrer et al. (2008) ¹⁷ ; Lehrer et al. (2009) ¹⁸ ; Thumbigere-Math et al. (2016) ²⁹	
Vitamin D 25 hydroxy	Lehrer et al. (2008) ¹⁷ ; Lehrer et al. (2009) ¹⁸ ; Thumbigere-Math et al. (2016) ²⁹	
DPD	Kim et al. (2013) ²⁶	
P1NP	Patel et al. (2014) ²⁸	
Sclerostin	Patel et al. (2014) ²⁸	

ALP	Thumbigere-Math et al. (2016) ²⁹
RANKL	Bagan et al. (2017) ¹ ; Patel et al. (2014) ²⁸
OPG	Bagan et al. (2017) ¹
RANKL / OPG ratio	Bagan et al. (2017) ¹

CTX, C-terminal telopeptide; BAP, bone-specific alkaline phosphatase; PTH, intact parathyroid hormone; NTX, N-telopeptide; OCN, Osteocalcin; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; IL-17, Interleucin-17; DPD, deoxypyridinoline; OS, oxidative stress; PINP, amino-terminal propeptide of type I collagen; VEGF, vascular-endothelial growth factor; CRP, C-reactive protein; BMD, bone mineral density; RANKL, receptor activator of NF- κ B ligand; OPG, osteoprotegerin

Table 3. Assessments of the risk of bias and quality (NOS Scale of case-control and cohort and studies).

NOS for case-control studies									
Authors (year)	Selection			Comparability			Outcome		Total 9/9
	Adequate definition	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non- Response rate	
Lee et al. (2013) ²⁴	0	★	★	★	★ ★	★	★	0	7/9
Kim et al. (2013) ²⁶	0	★	★	★	★ ★	★	★	0	7/9
Bagan et al. (2014) ²⁷	0	0	★	★	★ 0	★	★	0	5/9
Kolokythas et al. (2015) ³⁰	0	★	★	★	★ ★	★	★	0	7/9
Bagan et al. (2017) ¹	0	★	★	★	★ 0	★	★	0	6/9
Peisker et al. (2018) ⁵²	★	★	★	★	★ ★	★	★	0	8/9

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Table 3. Continuation**NOS for Cohort studies**

Author's (year)	Selection					Outcome			
	Representativeness of the exposed cohort	Selection of external control	Ascertainment of exposure	Outcome of interest not present at start	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes occur	Adequacy of follow-up of cohorts	Total score 9/9
Lehrer et al. (2008) ¹⁷	★	0	★	0	★ 0	★	0	0	4/9
Lehrer et al. (2009) ¹⁸	★	0	★	0	★ 0	★	0	0	4/9
Kwon et al. (2009) ¹⁹	★	0	★	0	★ 0	★	0	0	4/9
Kwon et al. (2011) ²²	★	★	★	0	★ ★	★	0	0	6/9
Lazarovici et al. (2010) ²⁰	★	0	★	★	★ 0	★	★	★	7/9
Lee et al. (2010) ²¹	★	0	0	★	★ 0	★	0	0	4/9

Morris et al. (2012) ²³	★	0	0	★	★ 0	★	★	0	5/9
Favot et al. (2013) ²⁵	★	★	★	0	★★	★	0	0	6/9
Patel et al. (2014) ²⁸	★	0	★	0	★ 0	★	★	★	6/9
Thumbigere-Math et al. (2015) ⁵¹	★	★	★	0	★★	★	★	★	8/9
Thumbigere-Math et al. (2016) ²⁹	★	★	★	0	★★	★	★	★	8/9
Tohashi et al. (2016) ³¹	★	★	★	0	★★	★	★	★	8/9
Mücke et al. (2016) ³²	★	0	★	0	★ 0	★	0	0	4/9

Studies could obtain one star/point per item in the selection and ascertainment categories or two stars/points in the comparability category. The maximum score for a given study is nine stars/points. Studies scoring six or more stars were considered to have low risk of bias.