A Systematic Review of Bone Anti-Resorptive Treatment Toxicity in Innate and Adaptive Immunity Cells: Osteonecrosis of the Jaws and Future Implications

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Abstract: *Introduction:* Bone anti-resorptive agents, namely bisphosphonates and denosumab are widely prescribed for the prevention of osteoporosis fractures and of cancer-related skeletal events. Osteonecrosis of the jaws (ONJ) has been reported for both drug categories. These agents have anti-proliferative effects in osteoclasts. We argue that because osteoclasts share their progenitor cells with macrophages, ONJ could be the result of reduced numbers and function of macrophages.

Methods: We systematically searched MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials (CENTRAL) from inception through September 2014, for studies reporting the effects of bone-antiresorptive agents in the innate and adapted immunity. No limitations pertaining to study type were set.

Results: Periosteal and endosteal tissues contain a discrete population of resident tissue macrophages. BPs cause apoptosis of macrophages in a dose and agent dependent manner. Increased cytotoxic activity by the γδ T cell population, could induce macrophage cell death causing local immune deficiency. Upon apoptotic stimuli, zoledronatepre-treated macrophages exhibit a significantly greater apoptotic rate. Long term BP exposed monocytes and macrophages may lead to functional impairment and reduced numbers of monocytic cells.

Conclusions: Bone-anti-resorptive agents have the capacity of both functionally impairing and reducing the macrophage population. The currently available limited evidence suggests a plausible role of these agents in the pathogenesis of ONJ. Future studies both clinical and experimental should focus on the effects of these drugs in immune homeostasis.

Keywords: Bisphosphonates, denosumab, osteonecrosis of the jaws, osteoclasts, macrophages, infection risk.

1. INTRODUCTION

Today, bone antiresorptive drugs, namely bisphosphonates (BPs) and denosumab are the most widely used drugs in the management of osteoporosis [1-4]. Apart from BPs, the blockade of receptor activator of nuclear factor - B ligand (RANKL) has been an advancement in the treatment of diseases that affect bone remodeling. Denosumab (DSB), which is the only drug in this class until nowadays, is a highly specific fully human IgG2 monoclonal antibody to RANKL. RANK, which is a member of the tumor necrosis factor super family of mediators, plays a prominent role in the regulation of osteoclastogenesis and osteoclast's survival [5-8]. DSB targets the RANKL and inhibits its interaction with the receptor activator of nuclear factor- B (RANK). Oncologists also prescribe intravenous bisphosphonates and DSB in higher doses compared to those prescribed for non-malignant indications, for the management of cancer related skeletal events [8].

Osteonecrosis of the jaw (ONJ) is a complication associated with the use of bone antiresorptive agents [9], which has been described over the last years [1, 9, 10]. It has been suggested that the development of ONJ accompanies much higher doses of IV bisphosphonates that oncologists use [11]. Nonetheless, recent good quality evidence suggests that ONJ is also common among patients receiving BPs (per os or intravenous) for non-malignant indications [12-16]. The hallmark of ONJ development is the finding of necrotic exposed bone in the oral cavity [9, 17]. In many cases, the precipitating event appears to be a dental extraction or other dental invasive procedures [9, 17]. Evidence supports the notion that ONJ is associated with dental extraction and use of dentures [9, 18, 19]. Another 40% of ONJ appears to be unrelated to dental

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treatment and develops spontaneously [13, 20]. The majority of lesions do not exhibit progress under antibacterial therapy and some even heal [16, 21-23].

Infection may be the common factor involved in the aetiopathogenesis of bone antiresoprtive drugs related ONJ. Both BPs and DSB exhibit anti-proliferative effects in osteoclasts and it is through those effects that they exert their therapeutic indication as bone anti-resorptive agents. However, osteoclasts and macrophages stems from a common progenitor cell lineage [24]. Thus, compromised local defense due to insufficient numbers or reduced functional capacity of macrophages along with the impaired oral mucosa that has been reported in patients receiving BPs [25], could allow oral pathogens to reach the bone surface of the jaws [26]. In a status of low bone turnover caused by potent anti-resorptives such as bisphosphonates and denosumab, the colonization of oral pathogens would be encouraged and fulminantinfection could develop [24, 26].

The scope of this review is to systematically collect and contextualizecurrently available evidence to support or reject this hypothesis.

2. MATERIALS AND METHODS

2.1. Selection of Relevant Studies

Eligible studies for the systematic review were all forms of publications, including clinical trials, observational cohort studies, case control studies, case series, case reports, reviews, abstracts, letters to the editor and cross sectional studies. Experimental studies were considered as well, in an attempt to collectively review currently existing evidence.

2.2. Search Strategy

To identify eligible studies, the main search was conducted in the electronic databases MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials (CENTRAL) from inception through March 2015, using any one of the terms:

"*Denosumab* OR AMG 162" (MeSH), " RANKLinhibition " (MeSH), " *bisphosphonates* " (MeSH), " (*bisphosphonates* OR pamidronate OR zoledronate OR etidronate OR ibandronate OR clodronate OR "Clodronic Acid" [Mesh] OR "pamidronate " [Substance Name] OR "Etidronic Acid" [Mesh] OR "zoledronic acid " [Substance Name] OR "ibandronic acid " [Substance Name]) OR ("pamidronate " [Substance Name] OR "Etidronic Acid" [Mesh]) OR "ibandronic acid" [Substance Name]) OR "Clodronic Acid" [Mesh]) OR "zoledronic acid" [Substance Name])) OR "Alendronate" [Mesh]) OR "risedronic acid "[Substance Name]) OR "tiludronic acid" [Substance Name)", "osteonecrosis " (MesH), "Jaw Diseases" (Mesh), "bone necrosis", "osteochemonecrosis", "jaw" (Mesh), "mandible" (MeSH) "maxilla" (MeSH), "antiresorptive", "macrophages (Mesh)", "Monocyte-Macrophage Precursor Cells (Mesh)", "Granulocyte-Macrophage Progenitor Cells (Mesh)", "Macrophage-Activating Factors (Mesh)", "Macrophage Activation (Mesh)", "Myeloid Progenitor Cells (Mesh)" without language restriction. The manual search was concluded by the perusal of the reference sections of all relevant studies or reviews and a contact with experts on the subject in an effort to identify relevant unpublished data. The main search as well as screening of titles and abstracts was completed independently by two authors.

2.3. Eligibility of Relevant Studies / Selection of Studies

In order for a study to be eligible the one of the following criteria need to apply: (**a**) the study should report on infectious complications in patients treated with bone anti-resorptive agents OR (**b**) the study should examine immune system components in a cell culture or animal design; (**c**) the study should discuss the side effects of bone – antiresorptive therapy in the immune system.

2.4. Data Extraction

Information from each study was extracted independently by two authors. Study general characteristics and outcomes were recorded.

2.5. Outcomes

Any reported infectious complication in patients under bone anti-resorptive agents was the outcome of this study.

Recorded changes to patients' immune system were also primary endpoint.

Secondary endpoints were information about infection risk or changes in immune homeostasis in animal experimental models.

Effects of bone-anti-resorptive agents in cell cultures of immune-mediating cells were also a secondary endpoint.

2.6. Role of the Funding Source

The study was supported in part by the IKY Fellowships of Excellence for Postgraduate studies in Greece – Siemens Program. The funding source had no role in the study design, collection, analysis and interpretation of the data, nor in the writing of the manuscript. A.K., and M.Y. had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

3. RESULTS – SYNTHESIS OF THE EVIDENCE

The search led to the identification of 58 relevant publications. Of these, there were 7 duplicates while 15 were reviews or synopses. 36 studies were eligible for the purpose of this review and have been reviewed in full text. Of these, 26 were included in the qualitative synthesis (PRISMA Flow Chart, Figure **1**). Those more important to support the theory in study (10) were tabularized for reader convenience (Table **1**). Due to the scarcity of evidence, the studies have been narratively summarized to synthesize the possible aetiopathogenetic theory in study. In this resume, we elected to present the data under discrete subheading, in which we summarize the evidence supporting each subheading. The complete theory which is based on the evidence under the subheadings, is presented in Table **1**.

3.1. ANTI-RESORPTIVE TREATMENT CONTRIBUTES INCREASED INFECTION RISK

Anastasilakis, Toulis *et al*. [27, 28], in their meta-analysis of RCTs reported that denosumab was associated with increased serious infections risk in women assigned to denosumab as compared to controls [HR (95% CI) 4.45, (1.15 to 17.14), p=0.03] [28]. On a follow up of the FREEDOM study publication, the authors updated their meta-analysis and reported the risk of serious infections to have remained significantly higher for the denosumab group [Mantel – Haenzel risk ratio (M – H RR)= 1.26, confidence

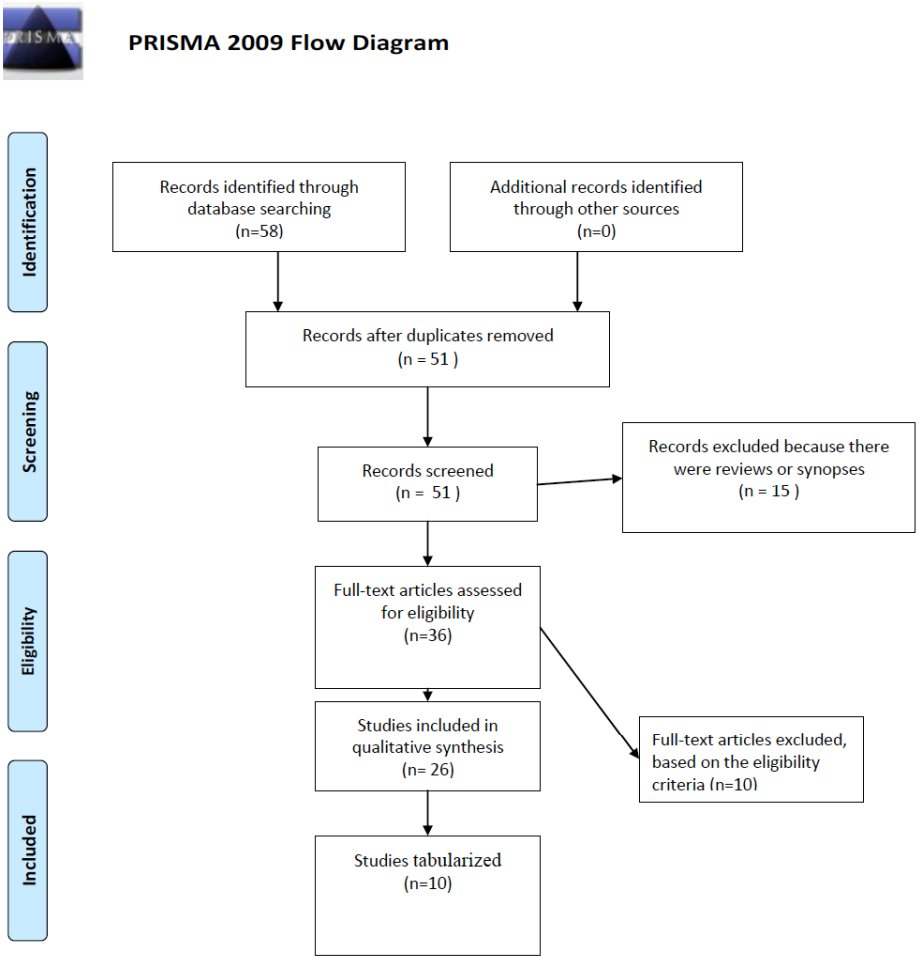

Figure 1: Results of the search. PRISMA flow diagram. (From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. Doi:10.1371/journal.pmed1000097).

Table 1: The Macrophage ONJ Hypothesis: Contemporary Evidence

Study	Year	Type	Conclusion	How conclusion fits hypothesis
Anastasilakiset al [28]	2009	Meta-analysis of RCTs	Denosumab confers increased infection risk	There is some kind of impairment in host immunity from DSB
Zhou *et al.* [29]	2014	Meta-analysis of RCTs	Denosumab confers increased infection risk	
Curtis *et al*. [30]	2015	Cohort study of RA patients	DSB and ZA cause similar infection risk	This impairment also exists for ZA
Chang *et al*. [32]	2008	Experimental animal model (mice)	Periosteal and endosteal tissues contain a discrete population of resident tissue macrophages.	Macrophages do exist in bone, where they exercise their immune role; in addition they may also play a role in tissue homeostasis
Moreau *et al*. [34]	2007	Experimental cell culture	BPs cause apoptosis of macrophages in a dose and agent dependent manner.	Macrophages population is reduced by BPs.
Vermeer *et al.* [46]	2013	Experimental cell culture	Pamidronate alters the cellular balance in the cultures from the osteoclast lineage towards macrophages and osteoblasts.	Inhibition of osteoclastogenesis changes the balance in favor of macrophages. We suggest that these cells could have been destined to be osteoclasts but they were not able to properly mature.
Ji *et al.* [47]	2012	Case series clinical study	No significant differences in bacterial diversity of ONJ tissues from patients treated with and without antibiotics	Lack of efficacy of antibiotic treatment in ONJ patients may be suggestive of compromised macrophage function.
Roelofs *et al*. [49]	2009	Experimental cell culture	Zoledronic acid-treated monocytes were able to trigger the activation of γδ T cells which act as antigen presenting cells (APCs) and induce cytotoxic activity.	Increased CD14/CD68 ratio is a marker of immune homeostasis alteration by BPs. The increased cytotoxic activity by γδ T cells induces macrophage cell death thereby causing local immune deficiency.
Hoefert *et al*. [56]	2015	Case series clinical study	Increased population of CD14+ cells in tissues from patients with ONJ.	
Muratsu *et al.* [55]	2013	Experimental cell culture	Macrophages pretreated with zoledronate have an increased cytokine and nitric oxide content.	Upon apoptotic stimuli, zoledronate treated macrophages may exhibit a significantly greater apoptotic rate.

interval (CI)=0.01-1.57; p = 0.04) [27]. A more recent meta-analysis, presented similar ratios and verified the increased infection risk in this group of patients [29]. Despite the limitations of the latter study pertaining to the small sample sizes and the heterogeneity [29]; this finding from meta-analyses of clinical trials suggests the possibility of an impaired –even minimally- immune system in patients receiving DSB. A recent study in patients with Rheumatoid Arthritis (RA), suggests that both DSB and zolendronate–the most potent bisphosphonate exhibit a similar increased infection risk [30].

3.2. MACROPHAGES ARE PRESENT IN THE JAW BONE

The presence of F4/80 + macrophage-like cells on osteal surfaces has been reported since 1984 [31]. Pettit and colleagues reported that periosteal and endosteal tissues contain a discrete population of resident tissue macrophages [32]. The authors reported that in an experimental system of enriched osteoblasts and macrophages, macrophages were required for efficient osteoblast mineralization in response to elevated extracellular calcium, which is the physiological remodeling stimulus [32]. Further they confirmed that macrophage depletion impaired osteoblast differentiation and mineralization [32]. Macrophages have been reported to be present in the oral mucosa as well [25].

Blood monocytes migrate into every tissue of the body, where they differentiate into resident tissue macrophages. Infection, inflammation or tissue injury trigger a rapid matriculation of monocytes from peripheral blood to the affected area. These monocytes then differentiate to become immune macrophages that play their role in innate and adaptive immune responses [33].

3.3. BISPHOSPHONATES INDUCE APOPTOSIS OF MACROPHAGES

In 2007, Moreau *et al*. showed that in the murine macrophage cell line J774A. BPs inhibited proliferation and induced apoptosis (programmed cell death). The efficiency of inducing apoptosis by the different BPs was zoledronate>risedronate> alendronate >pamidronate>etidronate, but they considered the serum concentration of BPs not sufficient to induce and sustain macrophage apoptosis over a long period [34]. Importantly, it has been reported that nitrogen containing BPs, and especially zoledronic acid (ZOL), can exert antimyeloma activity both *in vitro* and *in vivo* [35, 36]. Tumor macrophages have been reported to be targets of BP treatment in cancer patients [36].

3.4. MACROPHAGES ARE AFFECTED BY ANTI-RESORPTIVE AGENTS

Macrophages have been reported to exhibit marked similarities with osteoclasts. Activated macrophages display increased membrane ruffling, spreading, adhesion and lysosomal enzyme activity. Both macrophages and osteoclasts contain phagolysosomes, acidic compartments full of hydrolases. Furthermore, both macrophages and osteoclasts contain tartrate-resistant acid phosphatase (TRAP), a lysosomal protein that participates both in bone resorption and in the inflammatory responses exerted by the macrophages [37].

It has been proposed that, except for osteoclasts, monocytes and macrophages may be the cells that are most likely to be affected by the administration of bisphosphonates [38]. Indeed, the team by Roellofs *et al.* reported that when J774 macrophages were co-cultured with rabbit osteoclasts, J774 cells that were adjacent to resorbing osteoclasts frequently internalized more fluorescently-labelled alendronate analogue (FL-ALN) than J774 cells more distant to the osteoclasts. Moreover, J774 macrophages that occupied resorption pits internalized more FL-ALN than those on unresorbed surfaces [39, 40]. Thus macrophages can internalize BPs in a similar manner and in a same extent with osteoclasts.

Roelofs *et al*. demonstrated high levels of risedronate uptake into bone marrow monocytes. Analysis of intracellular uptake of fluorescent risedronate analogues demonstrated that CD14 labelled monocytes internalized relatively large amounts of BPs both *in vitro* and vivo compared to other bone marrow cell populations. Highly endocytic cell types such as macrophages and monocytes demonstrated much higher levels of intracellular uptake and were more sensitive to BPs than less endocytic cell types such as lymphocytes and osteoblasts [38].

Of note, J774 macrophage-like cells were actually used as an experimental cell culture model to study the effects of bisphosphonates on osteoclasts [24]. We discussed above that bisphosphonates are internalized into J774 macrophage-like cells in the same way that they are internalized by the osteoclasts [41]. Within the cell, BPs inhibit cholesterol synthesis [42]. What is more, protein prenylationhas been reported to be inhibited in J774 cells by risedronate at a concentration of 10^5 M, which is similar to the concentration of BPs that affects osteoclast viability *in vitro*; such a concentration can be achieved within the osteoclast resorption area [43].

Cytotoxic and migration inhibitory effects of BPs on macrophages have been described as early as 1996. Migration inhibition appeared to be a more sensitive indicator of BP activity than cytotoxicity [44]. Tsurushima *et al*. injected Aggregatibacteractinomycetemcomitans and Freund ' s adjuvant into drill holes of the jaws and femurs of Wistar rats. The rats treated with zoledronate showed significant wider osteonecrosis areas in mandible and femurs. They concluded that bacterial infection in BP affected bones leads to a rapid advance of osteonecrosis. Because of the same result by Freund's adjuvant, a highly antigenic agent, they considered the inflammatory stimulus as a trigger of ONJ [45].

3.5. COULD COMMON MACROPHAGE-OSTEOCLAST PROGENITORS FORM MACROPHAGES INSTEAD OF OSTEOCLASTS?

Vermeer *et al.* isolated bone marrow cells from jaw and long bone from mice and primed the cells with the cytokines M-CSF and RANKL in order to cause them differentiate into osteoclasts. In those cells, they investigated the expressionof the transcription factors V-mafmusculoaponeuroticfibrosarcoma oncogene homolog B (MafB) and interferon regulatory factor 8 (Irf8), which are highly expressed in macrophages and inhibit osteoclastogenesis. They analyzed the expression of the macrophage marker F4/80 and the osteoblast marker tissue non-specific alkaline phosphatase (ALP). They showed that, expression of ALP and F4/80was higher after treatment with 100μMpamidronate (PAM) from days 3–8 in jaw cells [46]. They suggested that the potent inhibition of osteoclastogenesis by PAM alters the cellular balance in the cultures from the osteoclast lineage towards

macrophages and osteoblasts [46] Their findings may suggest that (1) a common progenitor lineage exists for bone macrophages and osteoclasts. Anti-resorptive drugs may skew this relation in favor of macrophages. (2) Those resulting macrophages may not be adequately mature to cope with their role.

We suggest that the impairment of macrophage progenitor lineage after administration of anti-resorptive agents might result in mature cells, which were destined to become osteoclasts but now they have matured to cells having similarities to macrophages but are less functional.

3.6. ANTIBIOTICS DO NOT RESOLVE ONJ AND THIS MAY BE DUE TO COMPROMISED MACROPHAGE FUNCTION

Ji *et al*. indicated that oral antibiotic therapies are of limited efficacy on bacterial population associated with ONJ lesions, showing no significant differences in bacterial diversity of ONJ tissue from patients treated with and without antibiotics [47]. This indeed could also be triggered by compromised macrophage functions. Species affiliated to genera Pavimonas and Peptostreptococcus were more prevalent in antibiotic-administered groups, whereas Fusobacterium, Atrobium, and Streptococcus predominantly existed in groups without antibiotics [47]. The lack of efficacy of antibiotic treatment in ONJ patients to –at least partially- eliminate bacterial populations may be suggestive of the compromised macrophage function in this group of patients.

3.7. BISPHOSPHONATES MAY ALTER THE IMMUNE RESPONSE TOWARDS A *Th2* TYPE

In addition to their anti-resorptive effects, it has become apparent that nitrogen-containing BPs also have immunomodulatory properties. These drugs activate immune cells called gamma, delta T lymphocytes (γδT cells) [48]. Treatment of human peripheral blood mononuclear cells with zoledronic acid induced accumulation of isopentenyl diphosphate (IPP) and its stereo- isomer dimethylallyl diphosphate (DMAPP). These zoledronic acid-pretreated monocytes were able to trigger the activation of γδT cells [49]. This phenomenon was described as an acute-phase reaction after BP intravenous administration of nitrogen-containing BP involving the activation of γδ T cells [48]. Roelofs *et al*. reported that low concentrations of zoledronate(0.5μmol/l) were sufficient to cause detectable accumulation of IPP/DMAPP. They hypothesized that a concentration of 1μmol/l for 2 hoursis equivalent to circulating concentration of one standard dose of zoledronate and they detected increased IPP/DMPP concentrations [49]. They suggested that the same γδ T cells, when stimulated by IPP might also behave like mature antigen presenting cells (APCs) and present antigens to the other γδ T cells. This would mean that naive CD4 + and CD8 + γδ T cells turn into effector cells [48]. ATh1 or Th2 response of T cells is the result. Th1 responses are pro- inflammatory with subsequent production of IFN- γ and TNF- α, against bacterial infections. A Th2 response is cytotoxic with induction of CD8 + cytotoxic T cells needed for killing infected cells or tumour cells [50]. Activated macrophages (activated by intracellular bacterium like Listeria monocytogenes) have been reported to interact with γδ T cells to acquire cytotoxic activity, [51] which could subsequently induce macrophage cell death. This immunological result for intracellular pathogens could be stimulated by BPs and their metabolites, thus shifting the immune response towards a Th2type and causing local immune deficiency.

Kalayan *et al*. suggested that a chronic immune stimulation by IPP of γδ T cells may be resulting in a decrease of these cells in circulation. This might lead to a depletion of these innate T cells and loss of function [52]. Hoefert *et al*. reported an increased population of CD14 + cells in tissues from patients with ONJ. This changed ratio of CD68/CD14 expression in ONJ patients is in contrast to patients with osteoradio-necrosis or osteomyelitis and could be further supportive of the above theory. Concordantly, Zhang *et al*. [53] described lower CD68 macrophage infiltration in mucosal tissues of ONJ-lesions and in BP-exposed patients a finding which is opposite to marked CD 68 infiltrations noted in tissue adjacent to extraction sockets of periodontitis affected teeth in controls. They also noted that CD68 macrophages were lower in healthy controls when compared to either periodontitis or ONJ patients [53]. In this regard Pietschmann *et al*. observed a decreased CD14 expression on peripheral blood monocytes/macrophages exposed to alendronate in an *in vitro* model [54]. Alendronate treatment has also been reported to resultin an increased production of interleukin-1 α, tumornecrosis factor (TNF) and interferon-γ [54]. Pazianas concluded that long term BP exposed monocytes and macrophages may lead to functional impairment and reduced numbers of monocytic cells [24].

Muratsu *et al*. observed that in macrophages pretreated with zoledronate an increased cytokine and nitric oxide level was found [55]. Lipopolysaccharide (LPS)-induced apoptosis also increased after zoledronate treatment. More specifically, LPS-stimulated production of IL-1 β, IL-6, and TNF- α from the osteoclastic-like cells RAW254.7 increased significantly after zoledronate pretreatment. The nitric oxide production was significantly higher in zoledronate pretreated cells than in LPS only treated controls, suggesting that zoledronate accelerates nitric oxide release and finally a significant apoptosis was seen after LPS and zoledronate pretreatment and additional LPS exposition. After LPS binding to Toll –like receptor 4 (TLR4) and activating NF- κ B, an over- production of nitric oxide and pro-inflammatory cytokines like IL-1 β, IL-6, and TNF- α and other detrimental mediators may occur that could activate apoptosis. As a result, immune cells produce large amounts of inflammatory cytokines to give raise to a hyper-inflammatory state [55]. Changes of macrophages could appear in that pre-apoptotic state and therefore have an effect in CD68/CD14 ratio as reported by Hoefert *et al.* [56].

4. DISCUSSION

Through this review, we were able to summarize available evidence that bone anti-resorptive treatment is associated with increased infection risk. This effect is clearer for denosumab, for which two meta-analyses of RCTs [28, 29] concluded in agreement. A recent cohort study published this year, reported a similar infection risk in hospitalized RA patients under either DSB or ZA treatment [30]. Thus there is sufficient evidence to suggest an increased infection risk for patients in bone anti-resorptive treatment, thereby justifying the scope of this review. Which is to explore the etiology of this increased infection risk and provide further insight in the etiology of ONJ.

Chang *et al.* [32] reported that periosteal and endosteal tissues contain a discrete population of resident tissue macrophages. Since macrophages do exist in bone, where they exercise their immune role they may be the target of bone anti-resorptive induced ONJ and infection in general. Moreau *et al.* [34] reported that BPs cause apoptosis of macrophages in a dose and agent dependent manner. Thus the macrophage population is a target for BPs. Vermeer *et al.* [46] concluded that inhibition of osteoclastogenesis changes the balance in favor of macrophages. We further suggest that these cells could have been destined to be osteoclasts but they were not able to properly mature. This argument needs to be tested in future experimental models. A similar experiment with the one conducted by Vermeer *et al.* [46] needs to be designed, in which the resulting population of cells will be compared to a control macrophage cell population, with regard to their ability to produce enzymes, cytokines and a variety of paracrine mediator molecules. Roelofs *et al*. [49] and Hoefert *et al*. [56] conducted studies that suggest an increased cytotoxic activity by the γδ T cell population, which could in turn induce macrophage cell death thus leading to local immune deficiency. The immune response in ONJ patients is probably shifted towards a Th2 type due to the pharmacodynamics of bone-antiresorptive agents [50]. Muratsu *et al*. [55] concluded that upon apoptotic stimuli, zoledronate treated macrophages may exhibit a significantly greater apoptotic rate. Pazianas previously suggested that long term BP exposed monocytes and macrophages may lead to functional impairment and reduced numbers of monocytic cells [24]. It appears that bone-anti-resorptive agents have the capacity of both functionally impairing and reducing the macrophage population. Future studies should also focus on the numbers of monocytic cells in the tissues and blood of patients under such treatment, to detect differences in population subsets that may further elucidate the etiology of osteonecrosis of the jaws.

To conclude, the currently available limited evidence suggests that the apparent role of bone-anti-resorptive agents in the pathogenesis of ONJ may be mediated through their effects in the macrophage population. To further prove or disprove this argument, future studies either clinical or experimental should focus on the effects of these drugs in the macrophage population.

CORETIP

Periosteal and endosteal tissues contain a discrete population of resident tissue macrophages. Bisphosphonates (BPs) cause apoptosis of macrophages in a dose and agent dependent manner, causing local immune deficiency. Long term BP exposed monocytes and macrophages may lead to functional impairment and reduced numbers of monocytic cells. Bone-anti-resorptive agents have the capacity of both functionally impairing and reducing the macrophage population. Currently available evidence suggests a plausible role of these agents in the pathogenesis of osteonecrosis of the jaws (ONJ), with impairment of local immunity consisting the key component of its pathogenesis.

AUTHOR CONTRIBUTIONS

Kyrgidis A and Yavropoulou M selected the theme to be reviewed, performed the literature search, wrote the text and draw Table 1; Tilaveridis I, Andreadis Ch, Antoniades K, Kouvelas D designed the text structure and made critical corrections and revisions until the submitted version was achieved.

CONFLICT-OF-INTEREST STATEMENT

The above-mentioned authors of this manuscript hereby declare that they do not have any conflict-of-interest (including but not limited to commercial, personal, political, intellectual, or religious interests) related to the work submitted herein.

REFERENCES

[1] Kyrgidis A, Triaridis S and Antoniades K. Effects of bisphosphonates on keratinocytes and fibroblasts may have a role in the development of osteonecrosis of the jaw. Bioscience Hypotheses 2009; 2: 153-159. http://dx.doi.org/10.1016/j.bihy.2009.02.005

[2] Torring O. Effects of denosumab on bone density, mass and strength in women with postmenopausal osteoporosis. Therapeutic Advances in Musculoskeletal Disease 2015; 7: 88-102. http://dx.doi.org/10.1177/1759720X15579189

[3] Min YK. Update on Denosumab Treatment in Postmenopausal Women with Osteoporosis. Endocrinol Metab 2015; 30: 19. http://dx.doi.org/10.3803/EnM.2015.30.1.19

[4] Hou Y, Gu K, Xu C, Ding H, Liu C and Tuoheti Y. Dose-Effectiveness Relationships Determining the Efficacy of Ibandronate for Management of Osteoporosis. Medicine 2015; 94: e1007. http://dx.doi.org/10.1097/MD.0000000000001007

[5] Kyrgidis A. Denosumab, osteoporosis, and prevention of fractures. N Engl J Med 2009; 361: 2189.

[6] Kyrgidis A and Andreadis C. Epidemiologic Studies Are Needed To Clarify Whether Dental Modalities Could Be Predictors Of Bisphosphonates Osteonecrosis Of The Jaw In Breast Cancer Patient. Oncologist 2009; 14: 101-102. http://dx.doi.org/10.1634/theoncologist.2008-0155

[7] Kyrgidis A, Triaridis S and Vahtsevanos K. New evidence on bisphosphonate related osteonecrosis of the jaws suggests dental referral prior to commencement of treatment. Hippokratia 2009; 13: 64.

[8] Kyrgidis A, Tzellos TG, Toulis K and Antoniades K. The facial skeleton in patients with osteoporosis: a field for disease signs and treatment complications. J Osteoporos 2011; 2011: 147689. http://dx.doi.org/10.4061/2011/147689

[9] Khan AA, Morrison A, Hanley DA, Felsenberg D, McCauley LK, O'Ryan F, et al. Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. J Bone Miner Res 2015; 30: 3-23. http://dx.doi.org/10.1002/jbmr.2405

[10] Kyrgidis A, Triaridis S, Vahtsevanos K and Antoniades K. Osteonecrosis of the jaw and bisphosphonate use in breast cancer patients. Expert Review of Anticancer Therapy 2009; 9: 1125-1134. http://dx.doi.org/10.1586/era.09.74

[11] Kyrgidis A, Tzellos T-G, Toulis K and Antoniades K. The Facial Skeleton in Patients with Osteoporosis: A Field for Disease Signs and Treatment Complications. Journal of Osteoporosis 2011; Article ID 147689, 147610 pages, doi:147610.144061/142011/147689.

[12] Kyrgidis A and Barbas A. Osteonecrosis of the jaw among bisphosphonate users. Am J Med 2008; 121: e21. http://dx.doi.org/10.1016/j.amjmed.2008.08.013

[13] Hess LM, Jeter JM, Benham-Hutchins M and Alberts DS. Factors associated with osteonecrosis of the jaw among bisphosphonate users. Am J Med 2008;121: 475-483 e473.

[14] Conwell LS and Chang AB. Bisphosphonates for osteoporosis in people with cystic fibrosis. The Cochrane database of systematic reviews 2014; 3: CD002010. http://dx.doi.org/10.1002/14651858.cd002010.pub4

[15] Sharma A, Einstein AJ, Vallakati A, Arbab-Zadeh A, Walker MD, Mukherjee D, et al. Risk of Atrial Fibrillation With Use of Oral and Intravenous Bisphosphonates. The American Journal of Cardiology 2014; 113: 1815-1821. http://dx.doi.org/10.1016/j.amjcard.2014.03.008

[16] Lee SH, Chang SS, Lee M, Chan RC and Lee CC. Risk of osteonecrosis in patients taking bisphosphonates for prevention of osteoporosis: a systematic review and meta-analysis. Osteoporosis International 2013; 25: 1131-1139. http://dx.doi.org/10.1007/s00198-013-2575-3

[17] Ruggiero SL. Bisphosphonate-related osteonecrosis of the jaw: an overview. Annals of the New York Academy of Sciences 2010; no-no.

[18] Kyrgidis A, Vahtsevanos K, Koloutsos G, Andreadis C, Boukovinas I, Teleioudis Z, et al. Biphosphonate related osteonecrosis of the jaws: risk factors in breast cancer patients. A case control study. J Clin Oncol 2008; 26: 4634-4638. http://dx.doi.org/10.1200/JCO.2008.16.2768

[19] Vahtsevanos K, Kyrgidis A, Verrou E, Katodritou E, Triaridis S, Andreadis C, et al. Longitudinal Cohort Study of Risk Factors in Cancer Patients of Bisphosphonate-Related Osteonecrosis of the Jaw. J Clin Oncol 2009; 27: 5356-5362. http://dx.doi.org/10.1200/JCO.2009.21.9584

[20] Badros A, Terpos E, Katodritou E, Goloubeva O, Kastritis E, Verrou E, et al. Natural History of Osteonecrosis of the Jaw in Patients With Multiple Myeloma. Journal of Clinical Oncology 2008; 26: 5904-5909. http://dx.doi.org/10.1200/JCO.2008.16.9300

[21] Otto S, Marx RE, Tröltzsch M, Ristow O, Ziebart T, Al-Nawas B, et al. Comments on "Diagnosis and Management of Osteonecrosis of the Jaw: A Systematic Review and International Consensus". J Bone Miner Res 2015; 30: 1113-1115. http://dx.doi.org/10.1002/jbmr.2525

[22] Cardona F, Bagán JV, Sáinz E, Figuerido J, Giner F and Vidán FJ. Osteonecrosis de los maxilares por bisfosfonatos: Actualización y puesta al día. Anales del Sistema Sanitario de Navarra 2009; 32. http://dx.doi.org/10.4321/s1137-66272009000500012

[23] Khan AA, Morrison A, Hanley DA, Felsenberg D, McCauley LK, O'Ryan F, et al. Diagnosis and Management of Osteonecrosis of the Jaw: A Systematic Review and International Consensus. J Bone Miner Res 2014; 30: 3-23. http://dx.doi.org/10.1002/jbmr.2405

[24] Pazianas M. Osteonecrosis of the Jaw and the Role of Macrophages J Natl Cancer Inst 2011; 103: 232-240. http://dx.doi.org/10.1093/jnci/djq516

[25] Kyrgidis A, Triaridis S and Antoniades K. Effects of bisphosphonates on keratinocytes and fibroblasts may have a role in the development of osteonecrosis of the jaw. Bioscience Hypotheses 2009; 2: 153-159. http://dx.doi.org/10.1016/j.bihy.2009.02.005

[26] Katsarelis H, Shah NP, Dhariwal DK and Pazianas M. Infection and Medication-related Osteonecrosis of the Jaw. J Dent Res 2015; 94: 534-539. http://dx.doi.org/10.1177/0022034515572021

[27] Toulis K and Anastasilakis A. Increased risk of serious infections in women with osteopenia or osteoporosis treated with denosumab. Osteoporosis International 2010; 21: 1963-1964. http://dx.doi.org/10.1007/s00198-009-1145-1

[28] Anastasilakis AD, Toulis KA, Goulis DG, Polyzos SA, Delaroudis S, Giomisi A, et al. Efficacy and safety of denosumab in postmenopausal women with osteopenia or osteoporosis: a systematic review and a meta-analysis. Horm Metab Res 2009; 41: 721-729. http://dx.doi.org/10.1055/s-0029-1224109

[29] Zhou Z, Chen C, Zhang J, Ji X, Liu L, Zhang G, et al. Safety of denosumab in postmenopausal women with osteoporosis or low bone mineral density: a meta-analysis. International journal of clinical and experimental pathology 2014; 7: 2113-2122.

[30] Curtis JR, Xie F, Yun H, Saag KG, Chen L and Delzell E. Risk of Hospitalized Infection Among Rheumatoid Arthritis Patients Concurrently Treated With a Biologic Agent and Denosumab. Arthritis and Rheumatology 2015; 67: 1456-1464. http://dx.doi.org/10.1002/art.39075

[31] Hume DA, Loutit JF and Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: macrophages of bone and associated connective tissue. Journal of Cell Science 1984; 66: 189-194.

[32] Chang MK, Raggatt L-J, Alexander KA, Kuliwaba JS, Fazzalari NL, Schroder K, et al. Osteal Tissue Macrophages Are Intercalated throughout Human and Mouse Bone Lining Tissues and Regulate Osteoblast Function In Vitro and In Vivo. The Journal of Immunology 2008; 181: 1232-1244. http://dx.doi.org/10.4049/jimmunol.181.2.1232

[33] Gordon S and Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005; 5: 953-964. http://dx.doi.org/10.1038/nri1733

[34] Moreau MF, Guillet C, Massin P, Chevalier S, Gascan H, Baslé MF, et al. Comparative effects of five bisphosphonates on apoptosis of macrophage cells in vitro. Biochemical Pharmacology 2007; 73: 718-723. http://dx.doi.org/10.1016/j.bcp.2006.09.031

[35] Green JR and Guenther A. The backbone of progress-preclinical studies and innovations with zoledronic acid. Crit Rev Oncol Hematol 2011; 77(1): S3-S12. http://dx.doi.org/10.1016/S1040-8428(11)70003-8

[36] Rogers TL and Holen I. Tumour macrophages as potential targets of bisphosphonates. Journal of Translational Medicine 2011; 9: 177-177. http://dx.doi.org/10.1186/1479-5876-9-177

[37] Bune AJ, Hayman AR, Evans MJ and Cox TM. Mice lacking tartrate-resistant acid phosphatase (Acp 5) have disordered macrophage inflammatory responses and reduced clearance of the pathogen, Staphylococcus aureus. Immunology 2001; 102: 103-113. http://dx.doi.org/10.1046/j.1365-2567.2001.01145.x

[38] Roelofs AJ, Coxon FP, Ebetino FH, Lundy MW, Henneman ZJ, Nancollas GH, et al. Fluorescent risedronate analogues reveal bisphosphonate uptake by bone marrow monocytes and localization around osteocytes in vivo. Journal of Bone and Mineral Research 2010; 25: 606-616. http://dx.doi.org/10.1359/jbmr.091009

[39] Roelofs AJ, Thompson K, Ebetino FH, Rogers MJ and Coxon FP. Bisphosphonates: molecular mechanisms of action and effects on bone cells, monocytes and macrophages. Current pharmaceutical design 2010; 16: 2950-2960. http://dx.doi.org/10.2174/138161210793563635

[40] Coxon FP, Thompson K, Roelofs AJ, Ebetino FH and Rogers MJ. Visualizing mineral binding and uptake of bisphosphonate by osteoclasts and non-resorbing cells. Bone 2008; 42: 848-860. http://dx.doi.org/10.1016/j.bone.2007.12.225

[41] Thompson K, Rogers MJ, Coxon FP and Crockett JC. Cytosolic Entry of Bisphosphonate Drugs Requires Acidification of Vesicles after Fluid-Phase Endocytosis. Molecular Pharmacology 2006; 69: 1624-1632. http://dx.doi.org/10.1124/mol.105.020776

[42] Amin D, Cornell SA, Gustafson SK, Needle SJ, Ullrich JW, Bilder GE, et al. Bisphosphonates used for the treatment of bone disorders inhibit squalene synthase and cholesterol biosynthesis. Journal of Lipid Research 1992; 33: 1657-1663.

[43] Rogers MJ. From Molds and Macrophages to Mevalonate: A Decade of Progress in Understanding the Molecular Mode of Action of Bisphosphonates. Calcified Tissue International 2004; 75: 451-461. http://dx.doi.org/10.1007/s00223-004-0024-1

[44] Rogers MJ, Chilton KM, Coxon FP, Lawry J, Smith MO, Suri S, et al. Bisphosphonates induce apoptosis in mouse macrophage-like cells in vitro by a nitric oxide-independent mechanism. Journal of Bone and Mineral Research 1996; 11: 1482-1491. http://dx.doi.org/10.1002/jbmr.5650111015

[45] Tsurushima H, Kokuryo S, Sakaguchi O, Tanaka J and Tominaga K. Bacterial promotion of bisphosphonate-induced osteonecrosis in Wistar rats. International Journal of Oral and Maxillofacial Surgery 42; 1481-1487. http://dx.doi.org/10.1016/j.ijom.2013.06.011

[46] Vermeer JA, Jansen ID, Marthi M, Coxon FP, McKenna CE, Sun S, et al. Jaw bone marrow-derived osteoclast precursors internalize more bisphosphonate than long-bone marrow precursors. Bone 2013; 57: 242-251. http://dx.doi.org/10.1016/j.bone.2013.08.007

[47] Ji X, Pushalkar S, Li Y, Glickman R, Fleisher K and Saxena D. Antibiotic effects on bacterial profile in osteonecrosis of the jaw. Oral Dis 2012; 18: 85-95. http://dx.doi.org/10.1111/j.1601-0825.2011.01848.x

[48] Thompson K, Roelofs AJ, Jauhiainen M, Monkkonen H, Monkkonen J and Rogers MJ. Activation of gammadelta T cells by bisphosphonates. Adv Exp Med Biol 2010; 658: 11-20. http://dx.doi.org/10.1007/978-1-4419-1050-9_2

[49] Roelofs AJ, Jauhiainen M, Monkkonen H, Rogers MJ, Monkkonen J and Thompson K. Peripheral blood monocytes are responsible for gammadelta T cell activation induced by zoledronic acid through accumulation of IPP/DMAPP. Br J Haematol 2009; 144: 245-250. http://dx.doi.org/10.1111/j.1365-2141.2008.07435.x

[50] Moser B and Eberl M. gammadelta T-APCs: a novel tool for immunotherapy? Cellular and molecular life sciences : CMLS 2011; 68: 2443-2452. http://dx.doi.org/10.1007/s00018-011-0706-6

[51] Egan PJ and Carding SR. Downmodulation of the inflammatory response to bacterial infection by gammadelta T cells cytotoxic for activated macrophages. The Journal of experimental medicine 2000; 191: 2145-2158. http://dx.doi.org/10.1084/jem.191.12.2145

[52] Kalyan S, Quabius ES, Wiltfang J, Monig H and Kabelitz D. Can peripheral blood gammadelta T cells predict osteonecrosis of the jaw? An immunological perspective on the adverse drug effects of aminobisphosphonate therapy. J Bone Miner Res 2013; 28: 728-735. http://dx.doi.org/10.1002/jbmr.1769

[53] Zhang Q, Atsuta I, Liu S, Chen C, Shi S and Le AD. IL-17-mediated M1/M2 macrophage alteration contributes to pathogenesis of bisphosphonate-related osteonecrosis of the jaws. Clin Cancer Res 2013; 19: 3176-3188. http://dx.doi.org/10.1158/1078-0432.CCR-13-0042

[54] Pietschmann P, Stohlawetz P, Brosch S, Steiner G, Smolen JS and Peterlik M. The effect of alendronate on cytokine production, adhesion molecule expression, and transendothelial migration of human peripheral blood

mononuclear cells. Calcif Tissue Int 1998; 63: 325-330. http://dx.doi.org/10.1007/s002239900535

[55] Muratsu D, Yoshiga D, Taketomi T, Onimura T, Seki Y, Matsumoto A, et al. Zoledronic acid enhances lipopolysaccharide-stimulated proinflammatory reactions through controlled expression of SOCS1 in macrophages. PloS one 2013; 8: e67906. http://dx.doi.org/10.1371/journal.pone.0067906

[56] Hoefert S, Schmitz I, Weichert F, Gaspar M and Eufinger H. Macrophages and bisphosphonate-related osteonecrosis of the jaw (BRONJ): evidence of local immunosuppression of macrophages in contrast to other infectious jaw diseases. Clin Oral Investig 2015; 19: 497-508. http://dx.doi.org/10.1007/s00784-014-1273-7

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