Ghizlane Chafai

The predicting factors of bisphosphonate-related osteonecrosis of the jaw

Universidade Fernando Pessoa

Faculdade de Ciências da Saúde

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Candidate signature
Abstract

Osteonecrosis of the jaw (ONJ) is a main adverse effect related to Bisphosphonates (BPs). Bisphosphonates are potent inhibitors of bone resorption and are most widely prescribed drugs for the treatment of osteoporosis, and are also used in malignant bone metastases, multiple myeloma, and Paget’s disease. With increasing use, evidence is emerging that patients taking BPs are at risk of developing bisphosphonate related-osteonecrosis of the jaw (BRONJ), sometimes occurring spontaneously, but more commonly following dental extractions or oral bone surgery. However, the pathogenesis of BRONJ remains unknown. Attempts have been made to predict the development of BRONJ. The aim of this study is to review and to discuss if the biomarkers and the systematic markers of bone turnover can be used to predict the risk of BRONJ.

The methodology used in the accomplishment of this work was based on a bibliographic review for the subject under study.

Key-words: bisphosphonates, osteonecrosis of the jaw, CTX, systematic markers of bone turnover.
Resumo

Osteonecrose da mandíbula (ONJ) é um dos principais efeitos adversos relacionados com a utilização dos bifosfonatos (BPs). Os bifosfonatos são inibidores potentes da reabsorção óssea e são drogas amplamente prescritas para o tratamento da osteoporose, sendo também usadas nas metástases ósseas, mieloma múltiplo, e doença de Paget. Com o seu uso crescente, há uma crescente evidência de que os doentes que tomam BPs estão associados a um maior risco de desenvolver osteonecrose da mandíbula relacionada com bifosfonatos (BRONJ). Esta lesão surge, por vezes, de forma espontânea, ou, mais vulgarmente após extracção dentária ou cirurgia óssea oral. Apesar destas evidências, a patogénese da BRONJ permanece desconhecida. Diversos estudos têm tentado prever o desenvolvimento desta patologia.

O objetivo deste estudo é analisar e discutir se os biomarcadores e os marcadores sistémicos de renovação óssea podem ser usados para prever o risco de BRONJ.

A metodologia utilizada na realização deste trabalho foi baseada numa revisão bibliográfica sobre o tema em estudo.

Palavras-chave: bifosfonatos, osteonecrose da mandíbula, CTX, marcadores sistémicos de renovação óssea.
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<tr>
<td>AAOMS</td>
<td>The American Association of Oral and Maxillofacial Surgeons</td>
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<tr>
<td>ALP</td>
<td>alkaline phosphatase</td>
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<tr>
<td>BAP</td>
<td>bone specific alkaline phosphatase</td>
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<td>BPs</td>
<td>bisphosphonates</td>
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<td>BRONJ</td>
<td>bisphosphonate-related osteonecrosis of the jaw</td>
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<td>CI</td>
<td>confidence intervals</td>
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<tr>
<td>CTX</td>
<td>C-terminal telopeptide</td>
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<tr>
<td>DPD</td>
<td>deoxypyridinoline</td>
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<tr>
<td>ivBPs</td>
<td>intravenous bisphosphonates</td>
</tr>
<tr>
<td>LD</td>
<td>linkage disequilibrium</td>
</tr>
<tr>
<td>MMP2</td>
<td>matrix metalloproteinase 2</td>
</tr>
<tr>
<td>NTX</td>
<td>N-terminal telopeptide</td>
</tr>
<tr>
<td>ONJ</td>
<td>osteonecrosis of the jaw</td>
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<tr>
<td>OC</td>
<td>osteocalcin</td>
</tr>
<tr>
<td>OPG</td>
<td>osteoprotegerin</td>
</tr>
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<td>OPN</td>
<td>osteopontin</td>
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<tr>
<td>SNPs</td>
<td>single nucleotide polymorphisms</td>
</tr>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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I. Introduction

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) has become one of the most prominent enigmas not only in the dental community but in the field of skeletal as a whole. Confusion surrounding BRONJ exists for several reasons, including a lack of understanding about how and why this condition manifests. (Allen and Burr, 2009).

The incidence of BRONJ in the USA report was 0.8 - 12% with intravenous BPs and 0.7/100,000 person-years of exposure with oral BPs. In Europe, the incidence was 95/100,000 person-years of exposure with intravenous administration and 1/100,000 person-years of exposure with oral administration. BRONJ occurred in 1.15% of intravenously administered patients and in 0.04% of the orally administered patients in Australia. Another report from the USA indicated that the incidence of BRONJ was 0.1% with oral BPs administration (Ikebe, 2013).

Bisphosphonates (BPs) are antiresorptive agents that have been used for more than a decade, mainly for the treatment of metabolic bone diseases, such as osteoporosis and osteopenia, and to control the skeletal complications associated with multiple myeloma and metastases of solid tumors to the bone (Glover et al., 1994; Boissier et al., 2000; Lazarovici et al., 2010).

Intravenous BPs are primarily used and effective in the treatment and management of cancer-related conditions, including hypercalcemia of malignancy, skeletal-related events associated with bone metastases in the context of solid tumors such as breast cancer, prostate cancer, and lung cancer, and management of lytic lesions in the setting of multiple myeloma (Berenson et al., 2002). Although BPs have not been shown to improve cancer-specific survival, they have had a significant positive effect on the quality of life for patients with advanced cancer involving the skeleton. Before 2001, pamidronate was the only drug approved in the United States for the treatment of metastatic bone disease. In 2002, zoledronic acid was approved for this indication by the US Food and Drug Administration (FDA). More recently, a once-yearly infusion of zoledronate and a parenteral formulation of ibandronate administered every 3 months have been approved by FDA for management of osteoporosis (Ruggiero et al., 2009). Oral BPs are approved to treat osteoporosis and frequently used to treat osteopenia as well. They are also used for a variety of less common conditions such as Paget’s disease of bone.
and osteogenesis imperfecta of childhood. By far the most prevalent and common indication, however, is osteoporosis (Ruggeiro et al., 2009).

BPs, which are potent inhibitors of osteoclast-mediated bone resorption, are associated with bisphosphonate-related osteonecrosis of the jaw (BRONJ). To date, the etiology and pathogenesis of BRONJ development remain unknown. It is hypothesized that long-term therapy may impair bone turnover and angiogenesis processes, predisposing to BRONJ development (Thumbigere-Math et al., 2016).

The American Association of Oral and Maxillofacial Surgeons (AAOMS) published a position paper in 2007, with revision in 2009 (Ruggiero et al., 2009) and 2014 (Ruggiero et al., 2014), to address the need for clarity to diagnosis the BRONJ. The AAOMS suggest the following criteria to establish the diagnosis of BRONJ: 1) current or previous treatment with BPs; 2) exposed necrotic bone in the maxillofacial region that has persisted for more than 8 weeks; 3) and no history of radiation to the jaws. The 2014 AAOMS position paper update recommended a change of the term BRONJ to medication-related osteonecrosis of the jaw (MRONJ) to reflect the growing number of osteonecrosis cases associated with non-BP antiresorptive drugs and antiangiogenic therapies (Enciso et al., 2016; Ruggiero et al., 2014; Frietas et al., 2016).

However, in 2015 the International Task Force on Osteonecrosis of the Jaw (Gavalda and Bagan, 2016) defined ONJ as follows:

- Exposed bone in the maxillofacial region that fails to heal in 8 weeks after identification by a health professional.
- Exposure to an antiresorptive agent.
- No history of craniofacial radiotherapy.

Patients taking BPs have risk factors associated with dental surgery involving the jaw, including tooth extraction, dental implant, and other alveolar surgeries, all of which can initiate osteonecrosis (Dal Pra et al., 2017; Yamashita et al., 2010).

Several cross-sectional studies have attempted to investigate that predictive value of bone turnover markers (eg, C-terminal telopeptide (CTX), N-terminal telopeptide (NTX),
osteocalcin (OC), and alkaline phosphatase (ALP) and angiogenesis markers (eg, VEGF) in BRONJ development (Thumbigere-Math et al., 2016).

This study aims to review the available literature about the possible predictive factors and markers such as markers of bone turnover, angiogenesis, endocrine function, and inflammation for the development of bisphosphonate-related osteonecrosis of the jaw.

1. Materials and methods

Search Strategies: The Pub Med, Medline and B-on databases was electronically researched for articles published to February 2017 without date restrictions. The following keywords was using jointly or individually: Bisphosphonates, osteonecrosis, bisphosphonate-related osteonecrosis of the jaw, CTX, biomarkers of bone turnover. In additional, manual search was made by each one of the researchers.

Exclusion criteria: articles published in another language other than English, studies that the main topic was not the relation between osteonecrosis of the jaw and the bisphosphonates, full text articles were not available on the database, single case report, duplicated articles, and commentaries.

The initial search resulted in a list of 293 articles. In turn, titles were analyzed and based on exclusion criteria only 87 abstracts were included. After reading of the available abstracts, 45 articles were included in the study.
II. Development

1. Chemistry and Mechanism of Action of the Bisphosphonates

BPs are synthetic compounds with a chemical structure similar in that of inorganic pyrophosphate, and endogenous regulator of bone mineralization. While pyrophosphate is comprised of two phosphate groups linked by phosphoanhydride bonds (P-O-P structure), BPs are compromise of two phosphonate groups linked by phosphoether bonds (P-C-P structure). There are two main types of BPs: Nitrogen containing (alendronate, risedronate, pamidronate) and non-nitrogen containing (etidronate, tiludronate). The BPs act almost exclusively on bone when administered at physiological doses because of specific affinity to bone, where they deposit both in newly formed bone and in proximity to the osteoclasts. BPs act on bone through several mechanisms simultaneously. The first is exemplified by internalization by osteoclasts, causing disruption of osteoclast-mediated bone resorption, the second by inhibiting osteoclasts recruitment and accelerating programmed cell death (apoptosis) of osteoclasts, thus reducing osteoclast numbers. Both of mechanisms lead to reduction of bone and to a decrease in bone turnover (Gupta et al., 2013). BPs are hydrophilic molecules thereby not passing directly through biological membranes. Evidence suggests that BPs are held in bone several years even after the drug therapy is discontinued. BPs bind tightly to hydroxapatite crystals situated in bone surfaces and there remain for long time, since they are not susceptible to enzymatic degradation by osseous pyrophosphatase. BPs are constantly and gradually released from bone tissue into haematic circulation, entering in osteoclasts by endocytosis (Izzotti et al., 2013).

2. Pathogenesis

There have been many pre-clinical and clinical studies on the pathophysiology of BRONJ, but the exact mechanism of why osteonecrosis occurs is under investigation. In particular, there are many theories being presented on why this type of osteonecrosis only occurs on the jaw and not in other areas. Several review articles propose a relationship to excessive of the jaw bone turnover, infection/inflammation, angiogenesis inhibition, soft tissue toxicity, the immune system, and accumulation of micro-fractures (Kim et al., 2015; Allen and Burr, 2009).
2.1 Suppression of bone turnover

BPs inhibits the differentiation and promotes apoptosis of osteoclasts, so that the resorption and formation of bone is decreased (Russell et al., 2008). Based on the action mechanisms of these medications, it had been reported that bone turnover plays an important role in osteonecrosis. The reason why osteonecrosis occurs in the jaw rather than in other long bones is explained by the strong suppression of the bone turnover in jaw after experimental BPs administration in preclinical study (Allen, 2011), and more rapid cortical bone turnover in the human alveolar bone than in the long bones (Allen et al., 2010). However, there is contradictory opinion based on the facts that bone turnover is not decreased in the ONJ lesions (Lesclous et al., 2009), osteoclasts exist in the osteonecrotic areas, and that active bone resorption is occurring in these areas (Kim et al., 2015).

2.2 Soft tissues toxicity

BPs act primarily on osteoclasts, they also have direct toxicity towards soft tissue such as oral epithelial cells (Kim et al., 2015). In vitro, BPs were indeed shown to inhibit proliferation of endothelial cells and angiogenesis (Wood et al., 2002), and furthermore, reduce the viability of periodontal ligament fibroblasts and macrophages (Vemeer et al., 2016). BPs suppresses the proliferation and transportation of oral keratinocytes, which can increase the chances of latent bone exposure and subsequent infection (Yamashita and McCauley, 2012).

2.3 Infection/Inflammation

Apparently, soft tissue toxicity and infection are two closely related phenomena possibly explaining the pathophysiology of ONJ. Unlike long bones, the jaw is constantly exposed to pathogens present in dental plaque, particularly the actinomyces species commonly found in ONJ (Hansen et al., 2007). According to the study of (Silveira et al., 2016) showed that zoledronic acid was associated with non vital bone and that there was no statistically significant association between the presence of microbial colonies and the presence of non-vital bone, they suggested that the presence of microbial colonies is not involved with osteonecrosis (Silveira et al., 2016). Barros and colleagues, (2016) conclude that BRONJ is characterized by increases in empty osteocyte lacunae, osteoclast numbers, polymorphonuclear neutrophils, CD68 mononuclear-positive cells, immunostaining for TNF-
α, IL-1β, iNOS, and NF-Kb but not mast cells. BRONJ is also inversely associated with cells exhibiting IL-18 bp.

2.4 Angiogenesis inhibition

BPs have an angiogenic effect (Santini et al., 2003). There are several reports about osteonecrosis of the jaw which happened after administration of anti-angiogenic agents; moreover, it is difficult to explain why the osteonecrosis develops in circulation-rich upper jaw rather other long bones (Kim et al., 2015).

2.5 Immune-related or fracture-related theories

BPs controls the activity of various cells which involve in the immune response (Oizumi et al., 2010). The risk of osteonecrosis after tooth extraction becomes significantly higher if steroids or chemotherapeutic agents, which may influence the innate/acquired immune system, are given during bisphosphonates administration (Thumbierger-Math et al., 2012). Bone tissue is constantly undergoing the repetitive micro-fractures and healing process throughout the life, and such micro-trauma is slowly accumulated by age, and due to the suppressive effect of bisphosphonates on osteoclasts or osteoblasts, resulting in latent osteonecrosis lesions (Kim et al., 2015). Where high concentrations of BPs are present, the remodelling is inhibited; with a consequent increase is bone density (Rosini et al., 2015). Therefore, BRONJ is probably caused by multiple, combined factors that cannot be explained by single path physiologic mechanism.

3. Biomarkers for predicting BRONJ

3.1 Bone turnover markers (CTX, NTX, bone-specific alkaline phosphatase (BAP))

Marx and colleagues, (2007) took 30 consecutive cases with presence of no healing expose bone in maxilla or mandible that has persisted for more than 8 weeks in a patient who has received a systemic bisphosphonates but has not received local radiation therapy. Each included patient was asked for detailed history concerning the BPs: dose, the frequency, the duration of the BPs therapy, co morbidities and indication for the BPs. Results in part noted a higher incidence related to alendronate, 94.7% predilection for the posterior mandible and a 50% occurrence spontaneously, with the remaining 50% resulting from an oral surgical
procedure, mostly tooth removals. Just over 53% of patients were taking their oral BPs for osteopenia, 33.3% for documented osteoporosis, and 13.4% for steroid-induced osteoporosis related to 4 or more years of prednisone therapy for an autoimmune condition. There was a direct exponential relationship between the size of the exposed bone and the duration of oral BPs use. There was also a direct correlation between reports of pain and clinical evidence of infection. The morning fasting serum C-terminal telopeptide (CTX) test results were observed to correlate to the duration of oral BPs use and could indicate recovery of bone remodelling with increased values if the oral BPs was discontinued. A stratification of relative risk was seen as CTX values less than 100 pg/ml representing high risk, CTX values between 100 pg/ml and 150 pg/ml representing moderate risk, and CTX values above 150 pg/ml representing minimal risk. The CTX values were noted to increase between 25.9 pg/ml and 26.4 pg/ml for each month of a drug holiday indicating a recovery of bone remodelling and a guideline as to when oral surgical procedures can be accomplished with the least risk. In addition, drug holidays associated with CTX values rising above the 150 pg/ml threshold were observed to correlate to either spontaneous bone healing or a complete healing response after an office-based debridement procedure.

Kunchur and colleagues, (2009) reported that of 348 patients, 215 were taking oral BPs alendronate (63.5%) and taking long-term oral BPs were older (71 ± 11.6 years), were predominantly women with osteoporosis, and were medically compromised. The average CTX value was 238 ± 144 pg/ml, with 98 having a value less than 200 pg/ml. One patient with a CTX value of 126 pg/ml developed ONJ after an extraction. Seven intravenous BPs patients underwent extractions with no cases of ONJ developing. The CTX value was 329 ±354, with 4 less than 200 pg/ml. Fifteen patients developed ONJ, 12 after extractions and 3 spontaneously. Of these, 7, who were still taking a BPs at presentation, had a CTX value of 116 pg/ml. A CTX value of less than 150 pg/ml did not correlate with the clinical risk factors of age, gender, comorbidities, bone disease, or BPs duration. A statistically significant difference in the CTX value was found for those taking alendronate compared with those taking risedronate (p < 0.0001). If the BPs was ceased, the CTX value increased at approximately 25 pg/ml per month. The CTX test is not predictive of the development of ONJ for an individual patient but does identify those in the “risk zone,” which is a value of less than 150 pg/ml to 200 pg/ml. If medically appropriate, the BPs can be ceased so that the CTX value increases to bring the patient out of the “risk zone.”
Lazarovici and colleagues, (2010) investigated the predictive value of serum levels of CTX, bone specific alkaline phosphatase (BAP), and parathyroid hormone (PTH) for the development of BRONJ. The Data on the demographics, comorbidities, and BPs treatment were collected from 78 patients scheduled for dentoalveolar surgery. Of the 78 patients, 51 had been treated with oral BPs and 27 had been treated with frequent intravenous infusions of BPs. Blood samples for CTX, BAP, and PTH measurements were taken preoperatively. Surgery was performed conservatively, and antibiotic medications were prescribed for 7 days. Of the 78 patients, 4 patients taking oral BPs (7.8%) and 14 receiving intravenous BPs (51.8%) developed BRONJ. A CTX levels less than 150 pg/ml was significantly associated with BRONJ development, with an increased odds ratio of 5.268 (p = 0.004). The BAP levels were significantly lower in patients taking oral BPs who developed BRONJ. The PTH levels were similar in patients who did and did not develop BRONJ. They found that the CTX serum level is not a definitive predictor factor for the development of BRONJ; it might have an important role in the risk assessment before oral surgery. Serum BAP levels might beneficial, but their true value awaits additional investigation. PTH does not appear to have any predictive value for the development of BRONJ.

O’connell and colleagues, (2012) evaluated 23 patients underwent a morning fasting CTX test. 19 patients were taking alendronic acid orally, 2 risedronate sodium orally and 2 zoledronic acid intravenously. The indications for BPs were as follow: osteoporosis, multiple myeloma, osteopenia, breast cancer. The mean duration of BPs treatment was 30 months. The mean CTX value at or less than 180 pg/ml, with 11 patients having a value at or less than 150 pg/ml. The mean follow-up period was 5 months. None of the patients, who underwent removal of one or more teeth, subsequently developed BRONJ. They concluded that the CTX test was not predictive for the development of BRONJ following oral surgery.

Hutcheson and colleagues, (2014) reported the value of CTX test at the moment of the extraction and after extraction in cases of BRONJ in a large prospective cohort. All patients took an oral BPs for osteoporosis and had extractions from January 2007 to June 2013. 950 patients on oral BPs for osteoporosis had 2,461 extractions performed under local anesthesia. 181 patients had a CTX level lower than 150pg/ml. Four patients developed BRONJ, all had a CTX level lower than 150pg/ml. All were older than 70 years, and were on alendronate, and had medical comorbidities, but were not immunocompromised. The case – control comparison approached significance (< 150 pg/ml; p = 0.073). Alendronate was
associated with a low CTX level (p< 0.05). A CTX levels lower than 150pg/ml had a sensitivity of 100% and specificity of 81%. They conclude that the risk of BRONJ for patients with osteoporosis on BPs having extractions is approximately 0.2%. A CTX level lower than 150 pg/ml, is sensitive and is associated with an approximately 3-fold greater risk of BRONJ.

As a biomarker of bone resorption, N-telopeptide of type I collagen (NTX) is responsible for cross-linking of the bone matrix and bone-specific alkaline phosphatase (BAP) is a highly specific isoenzyme in bone and reflects osteoblastic cellular activity.

Morris and colleagues, (2012) investigated N-telopeptide of type I collagen (NTX) and bone-specific alkaline phosphatase (BAP) as potential predictors of ONJ onset. The Patients with ONJ were identified from a prospectively maintained database from the Dental Service at Memorial Sloan-Kettering Cancer Centre. Therefore, the medical records were used to identify possible stored serum from up to 4 approximate points: 12 months before ONJ, 6 months before ONJ, 1 month before ONJ, and at the ONJ diagnosis. Using commercially available enzyme-linked immunosorbent assays, the available serum was used to measure both NTX (Osteomark; normal range 5.5 to 19.5 nmol/L BCE) and BAP (Quidel; normal range 14.2 to 42.7U/L). The absolute values and trends in NTX and BAP over time were examined as possible predictors for ONJ. From March 1998 to September 2009, they identified 122 patients with ONJ. Of these, 56 (46%) had one or more serum samples available. Overall, 55 patients (98%) received BPs. Using the exact dates; no obvious patterns in either NTX or BAP were noted. Similarly, using the ordinal points, no evidence of suppression of NTX or BAP over time was seen. The consecutive median values were as follows: The median NTX values were 8.0 nmol/L (range, 3.8 - 32.9) at 12 months before ONJ; 9.5 nmol/L (range, 4.7 - 42.7) at 6 months; 9.5 nmol/L (range, 4.5 - 24.6) at 1 month, and 10.4 nmol/L (range, 4.4 - 32.5) at the ONJ diagnosis. The median BAP values were 18.0 U/L (range, 7.0 - 74) at 12 months before ONJ; 18.0 U/L (range, 4.0 - 134) at 6 months; 14.0 U/L (range, 4.0 - 132) at 1 month, and 18.0 U/L (range, 0.7 - 375) at the ONJ diagnosis. Only 2 patients (4%) had NTX and 17 (30%) had BAP below the normal range at the ONJ diagnosis. They had seen no trend in the NTX and the BAP levels before the ONJ diagnosis.

Kim and colleagues, (2013) investigate in the possible associations of the bone biomarkers osteocalcin (OC), deoxypyridinoline (DPD), CTX, NTX, BAP, PTH and BRONJ development. 37 patients had at least 1 sample available at the time of BRONJ diagnosis and
were included in the present study (age, 73.6 ± 11.2 years, 3 men and 34 women). Then, 37 age- and gender-matched patients composed the control group. The patient’s baseline characteristics (patient’s personal information and type of BPs taken, dose, dosage instructions, duration of medication use, and indication) were recorded. Through an examination, the location and size of the exposed necrotic bone, the presence of infection and pain, and the extension of lesions were recorded. Possible comorbidities, including patient-related factors (Diabetes, Obesity, and renal failure) and iatrogenic factors (steroid use, chemotherapy), were recorded. Of the 37 patients in the BRONJ group, 35 were taking BPs for osteoporosis and 2 patients for bone metastasis. Two patients had a history of chemotherapy use, 8 patients had been using steroid, and 6 patients had a diagnosis of diabetes. The median duration of BP exposure was 73 months (range, 21 – 87 months) for the BRONJ group and 67 months (range, 27 – 94 months) for the control group (p > 0.05). The levels of the bone formation markers serum OC and serum BAP, and those of the bone degradation markers urine DPD, urine NTX, and serum CTX were not significantly different between the 2 groups; only levels of serum PTH showed a significant difference (p < 0.05). The OC, DPD, CTX, NTX and BAP levels were not significant between the 2 groups (p > 0.05). The serum CTX level in reference to a 150 pg/ml cut-off was also not significant for the development of BRONJ (p > 0.05). They did not find evidence for the risk prediction of BRONJ of those current bone biomarkers.

3.2 Angiogenesis Markers

Vincenzi and colleagues, (2012) evaluated the role of vascular endothelial growth factor (VEGF) as predictive marker of BRONJ. Of 81 patients, 6 (7.4%) developed BRONJ following intravenous BPs administration (1 under treatment with pamidronate, 3 under treatment with zoledronic acid on standard schedule and 2 under treatment with zoledronic acid on metronomic administration). These 6 patients were affected by breast cancer (n = 3), prostate cancer (n = 2) and renal cancer (n = 1). The median number of BPs administrations was 9 in BRONJ group vs. 11 in non-BRONJ group (p = 0.196) and the median time of BRONJ development was 9 months (range 6 - 22). Compared to the patients who didn’t develop BRONJ, these 6 patients showed the strongest decrease in VEGF circulating levels at day 7 (median = 540.81, confidence intervals (CI) 95% 129.35-634.64 vs. median = 788.55, CI 95% 728.96-870.44, p < 0.0001) and at day 21 (median = 458.00, CI 95% 369.05 - 601.29 vs. median = 710.81, CI 95% 638.66 - 955.53, P < 0.0001) after the first administration of
either zoledronic acid or pamidronate. The detection limit of the VEGF was 62.5 pg/ml. If confirmed, serum VEGF levels at day 7 and 21 after the first administration of intravenous BPs could represent an effective early predictive marker of BRONJ.

### 3.3 Other markers

Mücke and colleagues, (2016) in their study examined the value of preoperative measurements of inflammatory mediators in blood in 212 patients with BRONJ who were studied prospectively. Multiple logistic regression analysis was used to assess the importance of the amounts of substance in the blood that are independently associated with the dependent variable recurrence of BRONJ. The only factor that significantly influenced the development of recurrence BRONJ was reduction in the white cell count ($p < 0.0001$, hazard ratio 5.324; 95% CI 2.373 to 11.945). Neither white cell counts nor C-reactive protein concentration within or above the reference ranges were significant associated with recurrence BRONJ. Patients whose white cell counts were lower than the reference range were at increased risk of recurrence BRONJ.

Thumbigere-Math and colleagues, (2016) analyzed serum markers of bone turnover, angiogenesis, endocrine function, and inflammation in patients with BRONJ who discontinued long-term intravenous BPs therapy. Serum samples were obtained from 25 BRONJ patients who had discontinued long-term ivBPs for an average of 11.4 ± 8.7 months and 48 non-BRONJ controls who continued receiving ivBPs therapy. Samples were analyzed for total alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin, C-telopeptide, vascular endothelial growth factor, triiodothyronine, thyroxine, thyroid-stimulating hormone, 25-hydroxuvitamin D, and C-reactive protein. They compared serum markers between a subgroup of BRONJ patients ($n = 10$) and controls ($n = 48$) whom they closely matched for mean age (64 years) and number of BPs infusions (mean, 19 infusions). Within this well-matched subgroup, and consistent with the unmatched results, they found no significant differences in levels of bone turnover or endocrine markers between BRONJ patients and controls (table I). However, log$_{10}$ VEGF (2.92 ± 0.38 pg/ml vs. 2.41 ± 0.35 pg/ml, $p < 0.001$) and CRP (34 ± 26 mg/l vs. 13 ± 8 mg/l, $p < 0.01$) levels were significantly higher in BRONJ patients compared with controls. Of the 10 BRONJ patients in the subgroup analysis, 5 had discontinued BPs for 6 months or less (range, 2 to 6 months) and 5 had discontinued BPs for more than 6 months (range, 7 to 22 months). Both in this subset of BRONJ patients and the
entire BRONJ group, none of the serum markers were correlated with the duration of intravenous BP discontinuation.

Table I. Serum biochemical markers matched according to mean age and number of bisphosphonate. (Adapted from Thumbigere-Math et al., 2016).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>BRONJ (n=10)</th>
<th>Non BRONJ(n=48)</th>
<th>Reference Range</th>
<th>P Value*</th>
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</thead>
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<tr>
<td>Age, yr</td>
<td>64.4</td>
<td>64.4</td>
<td>—</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>No. of BP infusions</td>
<td>19 ± 8</td>
<td>19 ± 7</td>
<td>—</td>
<td>0.92</td>
</tr>
<tr>
<td>BP discontinuation, mo</td>
<td>9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Biochemical markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total alkaline phosphatase, U/L</td>
<td>101 ± 52</td>
<td>85 ± 76</td>
<td>40-150</td>
<td>0.54</td>
</tr>
<tr>
<td>Bone-specific alkaline phosphatase, ng/L</td>
<td>12 ± 6</td>
<td>14 ± 8</td>
<td>6.5-22</td>
<td>0.47</td>
</tr>
<tr>
<td>Osteocalcin, ng/mL</td>
<td>13 ± 4</td>
<td>14 ± 10</td>
<td>11-50</td>
<td>0.56</td>
</tr>
<tr>
<td>Bone resorption: C-telopeptide, pg/mL</td>
<td>271 ± 167</td>
<td>222 ± 119</td>
<td>50-580</td>
<td>0.27</td>
</tr>
<tr>
<td>Angiogenesis: log10 vascular endothelial growth factor, pg/mL</td>
<td>2.92 ± 0.38</td>
<td>2.41 ± 0.35</td>
<td>1.78-2.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triiodothyronine, ng/dL</td>
<td>120 ± 26</td>
<td>110 ± 30</td>
<td>60 ± 180</td>
<td>0.33</td>
</tr>
<tr>
<td>Thyroxine, mg/dL</td>
<td>10 ± 2</td>
<td>9 ± 2</td>
<td>5 ± 11</td>
<td>0.40</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone, mIU/mL</td>
<td>2 ± 1</td>
<td>2 ± 2</td>
<td>0.4-3.0</td>
<td>0.29</td>
</tr>
<tr>
<td>25-hydroxyvitamin D, mg/L</td>
<td>33 ± 14</td>
<td>31 ± 10</td>
<td>30-75</td>
<td>0.81</td>
</tr>
<tr>
<td>Inflammation: C-reactive protein, mg/L</td>
<td>34 ± 26</td>
<td>13 ± 8</td>
<td>0-10</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* P values were calculated from 2-sample t tests.

Choi and colleagues, (2015) recruited 45 individuals (2 men, 43 women; age: 68.7± 12.3). All visited the Yonsei University Dental Hospital for surgical intervention from January 2012 to January 2013 and had a history of BP prescription (oral and/or intravenous). The following inclusion criteria were employed: Case group: received BPs; none healing, exposed bone in the maxilla or mandible that had persisted for more than 8 weeks; no history of local radiation therapy. Control group: received BPs; history of surgical dental intervention including extraction, implant placement, and periodontal surgery involving osseous injury; no none-healing exposed bone in the maxilla or mandible that had persisted for more than 8 weeks. Exclusion criteria for their study were as follows: uncontrolled systemic disease; uncontrolled hemorrhagic disease; psychiatric disorder; pregnancy; and any factor that compromised the
appropriateness of a patient for the study, according to the medical judgment of the investigators. As a result, 26 individuals diagnosed with BRONJ were designated cases, and 19 without BRONJ were designated controls. They analyzed three VEGF single nucleotide polymorphisms (SNPs) rs699947 (in promoter region), rs2010963 (in the 5' -UTR), and rs3025039 (in the 3' UTR) to investigate the association between VEGF polymorphisms and BRONJ. The genotype permutation test showed all genotypes were within the Hardy–Weinberg equilibrium (P>0.05). Of all three SNPs, rs2010963 and rs3025039 differed significantly between cases and controls. The frequency of genotype CC in rs2010963 and rs3025039 was significantly different between cases and controls (P= 0.04, P= 0.03, respectively) when multiple logistic regression analysis was applied. The rs699947 and rs2010963 polymorphisms were in tight linkage disequilibrium (LD), while the rs3025039 polymorphism was only weakly associated with the other polymorphisms. Haplotypes were reconstructed using LD coefficient values, and their frequencies in cases and controls were compared. The frequency of haplotype C-C (-2578/-634) and C-G (-2578/-634) was found to show no statistical difference between case and control. They also selected extended haplotypes, predicted using genotype data, for analysis they excluded haplotype C-C-T (-2578/-634/+936) due to a frequency <3%. Extended VEGF haplotype C-G-T (-2578/-634/+936) was inversely associated with BRONJ, more common among controls than cases (P=0.039). By contrast, haplotype C-C-C (-2578/-634/+936) was more common among cases (P=0.177), though the difference was not statistically significant. The CC homozygotes of rs2010963 and rs3025039 polymorphisms in the VEGF gene were associated with an increased risk of BRONJ in the Korean population.

III. Discussion

The morbidity of BRONJ, its high incidence among patients receiving frequent intravenous infusions of BPs, and the large number of patients using oral BPs has spurred a vigorous search for predictive and prognostic factors for the development of BRONJ (Marx et al., 2007; Kunchur et al., 2009). This bibliographic review evaluated the efficacy of the CTX test, biomarkers of bone suppression and formation, angiogenesis markers, inflammation markers, endocrine hormones, as predictors of the development of BRONJ. Despite the initial work of Marx and colleagues, (2007) the cut-off values for the CTX is not established till today, no consensus in the literature was been shown. Some works confirmed that the serum fasting value below 150 pg/ml was associated to high risk of BRONJ (Hutcheson et al., 2014;
Lazarovici et al., 2010; Kunchur et al., 2009), other studies was controversial and reported that CTX test did not predict the development of BRONJ (Bagan. 2008; Baim and Miller. 2009; O’Connell et al., 2012; Thumbigere-Math et al., 2016; Dal Pra et al., 2017). For those reasons the American Society for Bone and Mineral Research Task Force on BRONJ and American Dental Association, have not endorsed CTX test findings as a predictor for BRONJ development (Schwartz. 2008; Thumbigere-Math et al., 2016). Finally, the validity of CTX test as a predictor of BRONJ must be explored in future studies. Due of the relatively low incidence of BRONJ among patients taking oral BPs, more cohort study should be conduct.

For the other biomarkers NTX, BAP, OC, DPD, PTH, no consensus in the literature was found regarding on the use of biomarkers levels in clinical practice, with conflicting and controversial findings reported. The small number of cases studied in the published series and the dispersion of the results do not make possible to establish a relation between articles, to date. No trends were seen in the NTX and BAP levels before the ONJ diagnosis (Morris et al., 2012). Kim and colleagues, (2013) investigated in the possible associations of the bone biomarkers OC, DPD, NTX, BAP, PTH and BRONJ development, they did not find evidence for the risk prediction of BRONJ of those current bone biomarkers. Thumbigere-Math and colleagues, 2016 do not find significant difference in mean BAP, OC levels between BRONJ patients and controls.

The role of VEGF as predictive angiogenic marker in BRONJ development was not concluding, Vincenzi and colleagues, (2012) in their study showed a significant decrease in serum VEGF levels at day 7 and day 21 after the first BPs administration, but they didn’t have any control group. Thumbigere-Math and colleagues, (2016) didn’t find significant difference in the serum VEGF levels between BRONJ patients who discontinued long-term ivBPs therapy and controls. More prospective and extensive studies should be done.

The endocrine markers like PTH, triiodothyronine, thyroxine, and thyroid - stimulating hormone didn’t have a significant association with the BRONJ development. Thumbigere-Math and colleagues, (2016) related in their study that the levels of bone turnover and endocrine markers in BRONJ patients who discontinue long-term intravenous BP therapy are similar to those in non-BRONJ controls receiving intravenous BP therapy. Further studies containing a greater number of patients are needed to prove the utility of those markers in the prediction of the BRONJ.
Recently there have been several cases reports indicating that genetic variation is strongly associated with BRONJ risk. Genetic variation between individuals may increase or decrease the BRONJ susceptibility. Sarasquete and colleagues, (2008) were the first to report an association between cytochrome P450-2C (CYP2C8) polymorphisms and ONJ. Katz and colleagues, (2011) identified a potential association between a combined COL1A1, RANK, matrix metalloproteinase 2 (MMP2), osteoprotegerin (OPG), and osteopontin (OPN) genotype score and BRONJ risk in multiple myeloma patients undergoing ivBPs therapy. Choi and colleagues, (2015) found that the CC homozygote of rs2010963 and rs3025039 polymorphism in the VEGF gene were associated with an increased risk of BRONJ in the Korean population. However, as genetic variation among different ethnic groups may have different effects, it is worthwhile conducting genetic association studies on the different ethnic groups to which future genetic diagnostic methods will be applied. Therefore, additional epidemiological cohort studies with larger sample size and functional biological research would be required. The genetic test might be helpful to predict the BRONJ but more investigations should be done.

IV. Conclusions

This bibliographic review finds the follow conclusions:

- The CTX test has no predictive ability to detect the risk of BRONJ.
- The bone turnover biomarkers (BAP, OC, NTX, DPD), endocrine markers (thyroid hormones and PTH), inflammation marker (C-reactive protein) were unable to establish an evident association with BRONJ.
- The angiogenesis marker (VEGF) could represent an effective early predictive marker of BRONJ but more studies should be done.
- The genetic tests may be useful as a diagnostic tool to predict the BRONJ; additional investigations are needed to explore the real potential of those markers and their impact for the clinical practice.
V. References


