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THE ROLE OF HIGH-DOSE VITAMIN D IN RISK REDUCTION OF OSTEONECROSIS OF THE JAW IN CANCER PATIENTS RECEIVING ZOLEDRONIC ACID

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Abstract

ABSTRACT

Osteonecrosis of the jaw (ONJ) is a serious complication of oncological patients after or during drug therapy, whose manifestations range from asymptomatic to aspects requiring extensive operative treatment and adversely affecting patient's qualify of life. Taking in account that the lack of supplementation vitamin D causes hypovitaminosis, increasing bone renewal, losing bone mass, and in severe cases determing osteomalacia (one of the risk factors for ONJ) and that the probability of developing ONJ increases significantly during the first 3 years of treatment, the main objective of the present study is to assess whether the implementation of high-dose vitamin D in oncologic patients treated with zoledronic acid plus the well-known primary prevention protocols for elimination of potential risk factors could effectively reduce the risk of ONJ.

Given the simplicity, safety and low costs of vitamin D supplementation, the finding of a real protective effect on the development of ONJ may have important implications in clinical practice, making safer the administration of zoledronic acid.

Summary

SUMMARY

Background. Osteonecrosis of the jaws (ONJ) is a potentially severe adverse effect of oral disease of some medications such as bisphosphonates, antiresorptive and antigenic drugs. ONJ pathogenesis is yet unclear and some risk factors have been recognized. Among these, vitamin D deficiency has been hypothesized as a potential risk factors for ONJ development.

Objectives. The primary objective of the present study is to estimate the incidence of ONJ in a group of oncologic patients treated with zoledronic acid receiving high-dose vitamin D supplementation after application of well-known prevention strategies for elimination of potential risk factors and risk reduction of ONJ.

Methods. Between January 2014 and December 2015, a total of 82 oncologic patients were collected. Patients have been allocated into two groups above mentioned. Group T patients received vitamin D implementation during the treatment with zoledronic acid. Group C patients did not received vitamin D implementation during the treatment with zoledronic acid.

Results. In the group T the median duration of antiresorptive therapy was of 14,03 (SD=±2,77). In the group C the median period of BPs assumption was 17,78 months (SD=±3,00). To date, no ONJ cases have been observed in group T; on the contrary 6 (11,1%) patients developed ONJ in group C.

Conclusions. The findings of this study support the value that primary preventive measures plus the implementation of high-dose vitamin D could reduce the risk of occurrence of ONJ in a high-risk oncological population. However, further investigation to assess the real benefit of high dose vitamin D supplementation are needed.

CHAPTER 1

Background, Rationale and Objectives

BACKGROUND

Osteonecrosis of the jaw (ONJ) is a severe adverse drug reaction, that has been defined as a progressive destruction and death of bone that affects the mandible and/or maxilla of patients exposed to the treatment particularly with nitrogen-containing bisphosphonates (BPs), in the absence of a previous radiation treatment [1].

Since 2003, such lesion have been initially termed bisphosphonate-related osteonecrosis of the jaw (BRONJ) as it usually followed the administration of different types of bisphosphonates (BPs) [2, 3].

The first definition of ONJ was introduced by the American Association of Oral and Maxillofacial Surgery (AAOMS) [10] and comprised the following criteria:

- Current or previous treatment with BPs;
- Exposed bone in the maxillofacial region that has persisted for more than 8 weeks;
- No history of radiation therapy to the jaw.

This definition, which relies heavily upon the presence of clinically evident necrotic bone exposed through the oral mucosa or facial skin, has been adopted by the vast majority of clinical and epidemiological studies, and commonly used in clinical trials for case adjudication. However, several independent reports have recently highlighted that ONJ does not always present with oral mucosa fenestration and necrotic bone exposure; indeed, the non-exposed variant of jaw osteonecrosis, initially reported in 2008, has been characterized by other clinical

features of the jaw, in the absence of frank bone exposure [4-6]. Of note, diagnosis of non-exposed ONJ is based on exclusion of common jawbone diseases, such as odontogenic infections and other bone disorders known to cause similar manifestations.

In 2012 the SICMF (Italian Society of Maxillofacial Surgery) and the SIPMO (Italian Society of Oral Pathology and Medicine) proposed a new definition [1]: "Bisphosphonate related osteonecrosis of the jaw (BRONJ) is an adverse drug reaction described as the progressive destruction and death of bone that affects the mandible or maxilla of patients exposed to the treatment with nitrogen-containing bisphosphonates, in the absence of a previous radiation treatment.

In last years, the term Medication Related Osteonecrosis of Jaw (MRONJ) has been recently introduced in medical literature, in consideration of the fact that ONJ also occurs for other class of drugs such as denosumab, a bone resorption inhibitor of the receptor activator of nuclear factor-kappa B ligand (RANKL) antibody family, anti-angiogenesis inhibitor (e.g. bevacizumab, sunitinib, everolimus), [7].

Bisphosphonates (BPs) are antiresorptive agents widely prescribed in the treatment of skeletal diseases. BPs inhibit bone resorption mainly conditioning osteoclast activity and they are effective in the prevention of bone complications in patients with multiple myeloma and bone metastases from solid tumors. While the potential for bisphosphonates to improve cancer-specific survival remains controversial, these medications had a significant positive effect on the quality of life for patients with advanced cancer involving the skeleton.

Denosumab, a RANK ligand inhibitor is an antiresorptive medication that inhibits osteoclast function, decreases bone resorption, and increases bone density. Denosumab therapy is not indicated for the treatment of multiple myeloma. Interestingly, in contrast to bisphosphonates, denosumab do not bind to bone and its effects on bone remodeling are mostly diminished within 6 months of treatment cessation.

However, evidence shows that bisphosphonates reduce skeletal morbidity in multiple myeloma and solid tumors affecting bone by 30–50% [8, 9]. Otherwise, recent studies have shown that denosumab can be even more effective than BPs in reducing the incidence and delaying the time to SREs [10].

1.1.1 Epidemiology

The real epidemiology of ONJ remains unclear due to inconsistency and limitations of available studies, including a lack of a specific ICD code, under-reporting in surveillance drug systems, a recent introduction of preventive measures, case adjudication restricted to exposed ONJ, short-term observation and a lack of cumulative long-term incidence rates.

In patients with cancer exposed to zoledronate, the cumulative incidence of ONJ ranges from 0.7 to 6.7%. The risk of ONJ in patients with cancer exposed to zoledronate ranges from 50 to 100 times higher than in patients with cancer treated with placebo [7].

1.1.2 Pathophysiology

Even if ONJ pathogenesis is not defined, there are several hypothesis that could explain its main localization to the jaws: inflammation or infection, chronic microtrauma, altered bone remodeling or over suppression of bone resorption, angiogenesis inhibition, soft tissue medication toxicity, terminal vascularization of the mandible, suppression of immunity [11, 12].

A. Inhibition of osteoclastic bone resorption and remodeling

Bisphosphonates and denosumab inhibit osteoclast differentiation and function, and increase apoptosis, all leading to decreased bone resorption and remodeling [13, 14].

Nitrogen-containing bisphosphonates (pamidronate, alendronate, risedronate, incadronate, zoledronate) target the intracellular enzyme farnesyl diphosphonate synthase, inhibiting the mevalonate pathway, resulting in disruption of post-translational intracellular signaling proteins such as Ras). This would alter cytoskeletal organization and cell motility, resulting in osteoclast apoptosis. Furthermore, bisphosphonates disrupt normal bone homeostasis, resulting in impaired healing, especially in bone exposed to constant trauma, which may determine necrosis. In contrast, the effect of denosumab as an antibody is temporary. It has been shown that remodelling of the jaw bone resumes when denosumab is discontinued.

Based on the action mechanisms of these medications, it has been reported that bone turnover plays an important role in osteonecrosis. Indeed, 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 bone after experimental bisphosphonate administration in preclinical study and more

rapid cortical bone turnover in the human alveolar bone [15]. The central role of bone remodeling inhibition is further corroborated by a similar incidence of ONJ observed with other antiresorptive medications such as denosumab [7].

B. Inflammation/Infection

Both systemic and local oral risk factors have been implicated in ONJ pathogenesis, where several human studies have implicated dental disease or bacterial infection. Although tooth extraction has been performed in most of the initial reported cases of ONJ, these teeth commonly had existing periodontal or periapical disease [9]. However, it is not clearly defined whether osteonecrosis occurs first and then the necrotic lesion becomes to be infected, or infected lesion becomes to undergo osteonecrosis. Since the active resorption does not occur in bisphosphonate-containing bone, the infected tissue is not readily removed completely and can easily progress to chronic osteomyelitis.

Inflammation or infection has long been considered an important component of ONJ. Early studies identified bacteria, especially Actinomyces species, in biopsied specimens of necrotic bone removed in patients with ONJ [16].

C. Inhibition of Angiogenesis

Angiogenesis is a process that involves growth, migration and differentiation of endothelial cells to form new blood vessels. Angiogenesis favorably influences tumor growth and also influences tumor invasion of vessels, resulting in tumor metastasis. Angiogenesis requires binding of signaling molecules such as vascular endothelial growth factor (VEGF) to receptors on the endothelial cells. This signaling promotes new blood vessel growth.

Bisphosphonates have an anti-angiogenic effect [17]. Osteonecrosis is classically considered an interruption in vascular supply or avascular necrosis, and therefore, it is not surprising that inhibition of angiogenesis is a leading hypothesis in ONJ pathophysiology [18]. In vitro experiments consistently demonstrate a reduction in angiogenesis in response to zoledronic acid. Studies in cancer patients treated with zoledronic acid support these data with decreased circulating VEGF levels [19]. Moreover, there is a growing body of literature linking osteonecrosis of the jaw and other bones in patients receiving novel antiangiogenic drugs (tyrosine kinase inhibitors and monoclonal antibody targeting VEGF) [17].

D. Other Hypotheses

Soft tissue toxicity

Although bisphosphonates primarily act on osteoclasts, they also have direct toxicity towards soft tissues such as oral epithelial cells. BPs suppress the proliferation and transportation of oral keratinocytes [18, 20], which can increase the chances of latent bone exposure and subsequent infection. However, after reaching the bloodstream, bisphosphonates are mostly excreted through the kidneys after a few hours, and the concentration of bisphosphonates in tissues other than the bone are reported to be quite low.

Immune-related, or hair-line fracture-related theories

Bisphosphonates control the activity of various cells that are involved in the immune response [21]. 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 bisphosphonate administration [22-24]. Bone tissue is constantly undergoing the repetitive micro-fractures and healing process throughout the life, and such micro-trauma is slowly accumulated by age [25]. Micro-fractures caused by normal mastication are slowly accumulated due to the suppressive effect of bisphosphonates on osteoclasts or osteoblasts, resulting in latent osteonecrosis lesions [26]. Bacterial invasion of these lesions may cause progression to a deeper infection [27]. The results of various animal studies would support above-mentioned hypotheses. However, there are also many contradictory evidences that do not support such theories. Therefore, ONJ is probably caused by multiple, combined factors that cannot be explained by a single pathophysiologic mechanism.

1.1.3 Clinical manifestation

Exposed ONJ is characterized by the presence of clinically evident necrotic bone, which is exposed through the oral mucosa or facial skin, tending to affect the mandible more frequently than the maxilla [28]. Common associated manifestations include soft tissue swelling and

erythema, pus discharge, fistula/sinus tracts, tooth loss, jaw deformity, pain and sensory disturbances. The dimension of exposed bone in ONJ can vary from a few millimeters to several centimeters. Of note, little is known regarding the true extension of necrotic bone surrounding the superficial exposed areas, as very few studies have reported data from radiographs, computed tomography (CT) or MRI scans [29]. It seems that the vast majority of patients present with localized bone disease, which often is painful and infected.

Non-exposed clinical variant of ONJ include otherwise unexplained jawbone pain, fistula/sinus tract, swelling, loose teeth and pathological fractures [4, 6, 30, 31]. Notably, these clinical signs and symptoms are not correlated to dental, periodontal or other possible jawbone disorders causes and indeed the diagnosis of non-exposed ONJ is one of exclusion.

1.1.4 Risk factors

Many risk factors are well recognized and are usually categorized in three groups:

- Drug-related: type, duration and dosage, administration route.
- Systemic: age, diabetes, vitamin D deficiency, suppressed bone turnover markers, genetic factors, concomitant steroids.
- Local: intraoral surgery (e.g. tooth extraction), local anatomical factors, concomitant oral disease (e.g. periodontitis).

A. Drug-related risk factor

Type of drugs

Administered drugs: in hematological and oncological patients, zoledronic acid (administered to the majority of ONJ patients but also the drug most commonly used, at least after 2002) seems to result in a statistically higher risk of ONJ. Among cancer patients exposed to zoledronate, the cumulative incidence of ONJ is in the low single digits (range = 0.7% - 6.7%) [32]. When limited to studies with Level 1 evidence (i.e. systematic reviews or RCTs), the risk of ONJ in subjects exposed to zoledronate approximates 1% (100 cases per 10,000 patients) [33]. The risk of ONJ among cancer patients exposed to zoledronate ranges between 50-100 times higher than cancer patients treated with placebo [7].

Duration of bisphosphonate use

On average, ONJ patients were treated for longer periods than those without ONJ. The duration of intravenous treatment with BPs is generally correlated with the total dose of drug administered, given the type of monthly administration, continuous and indefinite in time, recommended by major guidelines, at least until 2007 [34, 35]. Regardless of indications for therapy, the duration of BP or antiresorptive therapy continues to be a risk factor for developing ONJ. Among cancer patients exposed to zoledronate or denosumab, the incidence of developing ONJ was, respectively, 0.6 and 0.5% at 1 year, 0.9 and 1.1% at 2 years, and 1.3 and 1.1% at 3 years with the risk for ONJ among denosumab-exposed subjects plateauing between years 2 and 3 [36]. In a study by Saad, et al, the investigators combined three-blinded phase three trials and found similar results, including a plateau after 2-years for patients exposed to denosumab [23]. Among cancer patients exposed to zoledronate or denosumab (n=5723), the incidence of developing ONJ was, respectively, 0.5 and 0.8% at 1 year, 1.0 and 1.8% at 2 years, and 1.3 and 1.8% at 3 years [7].

Regarding total dose of administered (cumulative doses), available data indicate a higher risk of developing ONJ with an increase in total BPs dose, which is intravenously administered monthly to cancer and hematological patients, both for zoledronate and pamidronato [9].

Administration route

There is a higher risk for intravenous injection of BPs but this factor may be closely related to the greater drug bioavailability and their prevalent use in cancer patients (at significantly higher total doses and durations) [9].

B. Systemic risk factor

Old age

ONJ shows an increasing trend in patients of old age. It has been reported that the prevalence increases in patients older than 65 years of age, and a similar trend has been reported in local studies, with the highest prevalence seen in patients 75 to 79 years of age [23].

Diabetes

The risk of ONJ is increased in diabetes patients [23]. This is thought to be due to decreased bone quality following ischemia of capillaries, decreased function of vascular endothelial cells, and increased apoptosis of osteoblasts and osteocytes caused by diabetes, in addition to decreased function of immune cells and increased inflammation seen in diabetes.

Vitamin D deficiency

When a vitamin D deficiency is present, calcium absorption decreases by 15% (phosphorous absorption by 60%), reducing serum ionized calcium. This decrease is detected by parathyroid gland calcium sensors, which respond with an increase in parathyroid hormone (PTH) whose function is to maintain serum calcium levels, acting on the liver and on bone where it stimulates bone resorption.

When the calcium supply to the organism is inadequate, the vitamin D hormonally active metabolite (1,25 (OH)2D) helps maintain calcium homeostasis, acting on vitamin D receptors (VDR) of the osteoblasts in which receptor activator of nuclear factor kappa-B ligand (RANKL) formation is induced in a similar way as with PTH, so producing bone resorption for calcium homeostasis. When the major circulating vitamin D metabolite (25(OH)D) levels are very reduced a failure to produce correct calcium absorption in the intestine occurs, with insufficient substrate for converting 1,25(OH)2D, even when PTH levels are high.

The optimal level of vitamin D has recently been described as follows: sufficient vitamin D concentration so that serum PTH levels are neither elevated nor decreased when vitamin D supplements are taken [37].

It has been indicated that hypovitaminosis D changes bone metabolism, increases bone renewal, losing bone mass, and in severe cases can lead to osteomalacia. Severe hypovitaminosis D (i.e. osteomalacia) is characterized by an impairment of bone mineralization and is commonly caused by a decrease of the serum calcium-phosphate product, which has multiple causes.

Bedogni et al. evaluated histomorphometric parameters in bone samples taken from patients treated with bisphosphonates. 77% of patients with ONJ suffered of osteomalacia compared with 5% of those who did not have ONJ. Given that osteomalacia was found almost exclusively among patients with osteonecrosis, it can be said that osteomalacia could represents a risk factor for osteonecrosis pathogenesis [38].

Further studies indicated that vitamin D insufficiency was related to ONJ [39, 40]. Hokugo et al. developed a vitamin D-deficient rat model and tested combinations of risk factors for ONJ. They suggested that the group with intravenous zoledronic acid and vitamin D deficiency showed a higher rate of ONJ. Mastaglia et al. indicated that a vitamin D insufficiency plus BPs therapy group showed significantly lower values of bone mass and bone volume.

In addition, the results of recent study indicated that vitamin D affects also histopathological parameters, such as the numbers of osteoblasts and osteoclasts, as well as histological and

macroscopic osteonecrosis, especially in the group administered vitamin D after tooth extraction, and it can be concluded that vitamin D diminished the suppressive effects of BPs [41].

Genetic factors

Individual genetic susceptibility to ONJ development has been investigated in a small number of genome-wide association and candidate gene studies. The largest study performed so far (n = 94 ONJ cases) suggests that MHC class II polymorphisms may represent genetic risk factors related to the development of ONJ [9].

Steroid therapy

The risk of ONJ increases in patients on steroids [23, 24]. The reason for this is thought to be due to decreased immune cells and delayed wound healing related to steroid use, which in turn exacerbates oral inflammation and increases the risk of ONJ. However, the difference in incidence of ONJ caused by the use of medications is mostly based on the results of retrospective studies, therefore, further prospective studies are needed.

C. Local risk factors

Dentoalveolar surgery

In the international literature, dental extraction is considered one of the most significant risk factors associated with ONJ in cancer patients taking antiresorptive drugs. Several studies report that among patients with ONJ, tooth extraction is a common predisposing event ranging from 52 to 61% of patients reporting tooth extraction as the precipitating event [7, 23]. Interestingly, some patients develop ONJ without surgical dental procedures or other risk factors during treatment with bisphosphonates or denosumab. Therefore, the true incidence, etiology, and risk factors that contribute to ONJ pathogenesis are unknown in cancer patients [42].

The risk of developing ONJ among patients who have been exposed to antiresorptive medications for other dentoalveolar procedures such as dental implant placement and endodontic or periodontal procedures is unknown. Absent data, the committee considers the risk for ONJ after dental implant placement and endodontic or periodontal procedures that require exposure and manipulation of bone to comparable to the risk associated with tooth extraction.

Local anatomical factors

Protruded bone surfaces are covered by relatively thin mucous membranes, so that continuous irritation by dentures, etc. can lead to exposure of the surface and contribute to the pathogenesis of ONJ. Other anatomical landmarks such as a mandibular torus, the mylohyoid ridge, or a palatine torus can be vulnerable anatomic structures and act as local risk factors.

Concomitant oral disease

Dental and periodontal infection significantly increases the risk of ONJ in cancer patients exposed to antiresorptive therapy. Indeed, periodontal disease was diagnosed in 84% of cases in a large sample of patients with ONJ. However, periodontal disease is commonly observed in the general population in individuals >40 years of age, which may represent a confounding factor in assessing epidemiological association. Also, early clinical stages of ONJ are known to include non-exposed alveolar bone necrosis that can mimic clinical and radiological manifestations [9].

1.2 RATIONALE

Medication-related osteonecrosis of the jaw is a well-documented adverse event of therapy with specific drugs (e.g. BPs, denosumab, bevacizumab, sunitinib).

The pathogenesis is multifactorial and several risk factors are recognized. As described in 1.1.4 paragraph, the deficit of vitamin D and especially severe hypovitaminosis D modify bone metabolism with bone impairment and have been detected in ONJ lesions [38-41].

Up to date, in literature there are not prospective studies evaluating the effect of vitamin D implementation in oncologic patients before ONJ development. About this, arguing in term of prevention, we supposed that the well-known strategy for primary prevention of zoledronic acid -related ONJ and the high-dose vitamin D implementation together could have a greater effect in ONJ development.

1.3 OBJECTIVES

The primary objective of the present study is to estimate the incidence of ONJ in a group of oncologic patients treated with zoledronic acid receiving high-dose vitamin D supplementation after application of well-known prevention strategies for elimination of potential local risk factors and risk reduction of ONJ.

Moreover, secondary aims include the evaluation of changes in bone metabolism during treatment with vitamin D and of periodontal oral health by *plaque index, bleeding on probing (BoP), clinical attachment level* (CAL) and *tooth loss* (TL).

CHAPTER 2

Materials/Patients and Methods

2.1 Study design

Prospective cohort study:

- Group T: naïve patients in primary prevention for ONJ receiving zoledronic acid and high dose vitamin D supplementation.
- Group C: naïve patients in primary prevention for ONJ receiving zoledronic acid without high dose vitamin D supplementation.

2.2 Patients

Between January 2014 and December 2015, a total of 82 oncologic naïve patients were collected with the following inclusion criteria:

- age ≥ 18;
- diagnosis of cancer (in which antiresorptive therapy is required);
- signed informed consent.

Exclusion criteria were:

- previously or currently treatment with bisphosphonates, denosumab and/or other antiangiogenic drugs (e.g. bevacizumab);
- previous diagnosis of ONJ;
- history of head-neck radiotherapy.

The ethics committee approved for the study (Internal Ethical Committee of the University Hospital A.U.O.P "P. Giaccone" of Palermo -Internal registry: 9/2015) and the patient's consent to participate was obtained where specifically required. The study was performed in accordance with the ethical standards of the Declaration of Helsinki.

2.3 Data collection and variables

Medical notes of patients were collected. Following data (demographical, pathological anamnesis, local clinical and radiological indexes) were registered by clinical experts and entered into a pre-defined electronic database.

Demographical and Medical measurements: 1) age, 2) sex, 3) bone disorders and co-morbidities. During follow up 4) zoledronic acid assumption, 5) zoledronic acid duration and 6) local risk factors for ONJ were also registered; in case of ONJ development 7) ONJ site, 8) clinical manifestation of ONJ (bone exposure or non exposure), 9) abscess, 10) pain, 11) ONJ stadiation (according to SICMF – SIPMO).

Hematologic measurements: D25 (OH) D, Ca, P, PTH, nitrogen and creatinine.

Periodontal measurement: plaque index, bleeding on probing (BoP), clinical attachment level (CAL) and tooth loss (TL).

Radiologic measurements: baseline dental panoramic x-ray to identify any relevant pathology and to provide a baseline radiograph of the jaws and teeth.

All data of two groups were shown respectively in Table 1-2.

2.4 Description of clinical protocol

After having signed an informed consent, patients were included into primary prevention strategies for elimination of potential local risk factors and risk reduction of ONJ.

Then patients have been submitted to a preliminary oral evaluation, clinically and radiologically in order to evaluate the state of hard and soft tissues.

In case of gingivitis or periodontal disease, patients were underwent to causal therapy for eliminating status of inflammation and infection.

In case of invasive dental treatments (i.e. tooth extractions), the administration of antiresorptive therapy for both groups have been postponed till a complete recovery of biological tissues.

All patients underwent to a blood sampling for the assessment of serum levels of D 25 (OH) D, Ca, P, PTH, nitrogen and creatinine.

Patients have been allocated into two groups above mentioned. Group T patients received vitamin D implementation during the treatment with zoledronic acid. Group C patients did not receive vitamin D implementation during the treatment with zoledronic acid.

Oral vitamin D administration was scheduled as follows:

- *loading dose* of 100,000 IU/day for the first 3 days;
- *maintenance dose* of 100,000 IU of vitamin D once monthly for the duration of the study.

Every 4 months after the enrolment and for the entire duration of the observation period the patients underwent a dental examination and re-evaluation of the dental-periodontal status to exclude the presence of ONJ and to intercept any inflammatory processes / infectious in order to establish early prevention therapies.

2.5 Statistical analysis

Incidence rates per 1,000 person-months were calculated for both experimental and control groups.

Survival rates were calculated by the Kaplan-Meier method at 3, 6, 12, 18 and 24 months from the start of bisphosphonates therapy. The log-rank test was used to compare survival rates between experimental and control groups. A 2-sided P value of 0.05 or less was considered to assess statistical significance. All statistical analyses were carried out using Stata/SE 14.1 (StataCorp LP, Texas US).

CHAPTER 3

Results

82 naïve oncological patients were collected from January 2014 and December 2015. The patients ranged in age from 32 to 81 years. The 29 patients of group T had a mean age of 64.78 years (range 48-81) and group C consisted of 53 patients who were 59,62 years old (range 32-81). Data of each patients were registered and patients have followed up for a mean time of 14,03 months group T and 17,78 group C. All data of two groups were summarized respectively in Table 1-2.

All patients received zoledronic acid for malignant diseases. Breast and prostate cancer were the two most frequent cancer of the two group (T and C), 50,0% and 28,0% respectively. Other indications were multiple myeloma and solid tumors (lung, colon, liver).

In particular, in the group T the median duration of zoledronic acid therapy was of 14,03 months (SD=±2,77). Vitamin D supplementation started with BP administration according to our protocol as reported in paragraph 2.4. In the group C the median period of zoledronic acid assumption was 17,78 months (SD=±3,00).

During treatment with zoledronic acid, 5 patients received other drug-related ONJ (Tab. 1 and 2). In particular, among group T patients, only 1 (3.1%) patient received bevacizumab from August 2015 to January 2016; on the other hand in group C 4 (7,4%) patients have been taking bevacizumab since zoledronic therapy started.

Eight patients were in treatment with systemic steroids, 1 (3.4%) and 5 (9.4%) in group T and C respectively. On one hand, 3 patients have been taking steroid for months: group T patient for 19 months, group C patients (i.e. ID 14 and 33) for 16 and 12 months respectively; on the other

hand, 3 group C patients (i.e. ID 5, 6 and 41) received steroid for 15, 17 and 19 months respectively.

Every 4 months, a dental-periodontal evaluation has carried out to intercept any inflammatory processes / infectious and to apply secondary prevention protocol of ONJ. Local data of each patient was collected and registered in a periodontal chart, evaluating *plaque index, bleeding on probing (BoP), clinical attachment level* (CAL) and *tooth loss* (TL). No statistically significant differences were observed until now. Patients underwent to oral hygiene session every and not more than 6 months after clinical evaluation. Chlorexidine 0,2% mouthwashes 30 ml swished up to 60 seconds, 3 times/ daily for 7 days were prescribed.

In both groups, no dental extractions were performed during our study.

After a 14,03 month mean period of observation, no ONJ cases have been observed in group T; on the contrary, 6 (11,3%) patients developed ONJ in group C during 17,78 months. The mandible was affected in the totally of cases. The most frequent sign at presentation was bone exposure (83.3%), generally asymptomatic.

ONJ was defined and classified according to SICMF – SIPMO classification [1], as follows: 4 patients with stage 1 (66.7%) and 2 patients with stage 2 (33.3%). All data were shown in Tab. 3.

Overall in the time-span Jan.2014-Dec.2016, 408 person-months were included in the experimental group (N=32 people) and no cases of ONJ were diagnosed. Conversely, 960 person-months were included in the control group (N=54 people) and 6 new cases of ONJ were diagnosed, giving a crude incidence rate of 6 per 1,000 person-months. For all cases in the experimental group, the 20-months survival rate (no ONJ) was 100% compared to 87% in the control group ($p>0.05$) (See figure).

CHAPTER 4

Discussion

Antiresorptive agents that target osteoclasts, thereby inhibiting bone resorption and subsequent bone loss, currently are considered the cornerstone for the treatment and prevention of bone metastases of solid tumors [16, 17]. In the past, intravenous BPs (i.e. zoledronic acid) were considered the gold standard for the treatment of such conditions. Since 2003, there has been an increase in number of case reports regarding ONJ induced by BPs, especially with intravenous administration. ONJ is described as adverse drug reaction described as the progressive destruction and death of bone that affects the mandible or maxilla of patients exposed to the treatment with nitrogen-containing bisphosphonates, in the absence of a previous radiation treatment. This an uncommon drug-related complication ranges from asymptomatic to requiring extensive operative treatment and adversely affecting patients' qualify of life.

Generally, the incidence of ONJ with intravenous bisphosphonates has been reported 0.8–1.2 % on average, increasing up to 21 % after injection of bisphosphonate for 3 years or more [43].

In particular, in literature, among cancer patients exposed to zoledronate, the incidence of developing ONJ was, 0.5 at 1 year, 1,0 at 2 years, and 1.3 at 3 years [7].

In our prospective study, the median period of observation with zoledronic acid and high-dose of vitamin D supplementation was of 14,03 (SD=±2,77).

About risk factors, it is well known that in solid cancer patients, such as breast cancer and prostate cancer, or in patients with multiple myeloma, the risk of ONJ is higher than in patients with other diseases [43-45]. In our sample, breast and prostate cancer were the two major

cancer involved the two group (T and C) of patients with 50,0% and 28,0% respectively. Other indications were multiple myeloma, lung, colon, liver.

Regarding local risk factors, dentoalveolar surgery is considered one of the major risk factor for developing of ONJ; according to the literature, it is estimated that the incidence of the side effect after tooth extraction ranges from 1.6% to 14.8% of cases [46]. It is well known that the application of dental preventive measures before the initiation of the bisphosphonate therapy reduced the incidence of jaw osteonecrosis [47]. In fact, compared to retrospective studies on patients who did not receive dental examination before bisphosphonate administration, there are numerous prospective studies that show a decrease in ONJ when a dental examination is performed before treatment [23, 48, 49]. According to this, the population enrolled in our sample received an appropriate dental examination before antiresorptive assumption and thanks to our accurate preventive screening no dental extractions have been needed in any two groups until now.

Nowadays, pre-existing inflammatory dental disease, such as periodontal disease or periapical pathology, is a well recognized risk factor [11]. In patients with cancer and ONJ, pre-existing inflammatory dental disease was a risk factor in 50% of cases [11, 23]. Given that a common treatment of inflammatory dental disease is tooth extraction, pre-existing dental disease may confound the relation between tooth extraction and the risk for ONJ noted earlier. To exclude periodontal disease, patients underwent an oral hygiene session every and not more than 6 months after clinical evaluation and chlorexidine (0,2% mouthwashes 30 ml swished up to 60 seconds, 3 times/ daily for 7 days) were prescribed. Data of each patient was collected and registered in a periodontal chart, evaluating *plaque index, bleeding on probing (BoP), clinical attachment level* (CAL) and *tooth loss* (TL).

Regarding vitamin D levels and dental health, Ferrari HA Bischoff, showed that circulating levels of 25 (OH) D are strongly correlated with gingival health, dental care and the risk of developing periodontitis and gingivitis. Moreover, vitamin D may also reduce both gingivitis and periodontal disease through its antiinflammatory effect [50]. Thanks to close dental-periodontal re-evaluation, patients showed good oral hygiene during follow up. In any case, no statistically significant differences were observed until now.

CONCLUSION

The present study is the first prospective study investigating the role of high-dose vitamin D and evaluating the incidence of ONJ in a group of oncologic patients treated with zoledronic acid and high-dose vitamin D supplementation.

Although many variables exist in ONJ pathogenesis, given the simplicity, safety and low costs of vitamin D supplementation, the potential protective effect on the development of ONJ may have important implications in clinical practice, making safer the administration of antiresorptive drugs.

However, further investigation to assess the real benefit of high dose vitamin D supplementation in incidence of ONJ development in cancer patients treated with antiresorptive drugs are needed.

CHAPTER 5

Tables and Figures

ID Patient	Ptazient	Age (yrs)	Gender	Cancer	Antiresorptive therapy	Antiresorptive duration	Systemic steroid therapy	Other drug-related ONJ	Local risk factors
1	TC	48	F	Breast cancer	Zoledronic acid	19	Yes	No	No
2	GB	68	M	Lung cancer	Zoledronic acid	13	No	No	No
3	AC	64	F	Breast cancer	Zoledronic acid	18	No	No	No
4	CS	54	M	Prostate cancer	Zoledronic acid	18	No	No	No
5	LS	52	F	Breast cancer	Zoledronic acid	17	No	No	No
6	FR	54	M	Microcitoma	Zoledronic acid	16	No	No	No
7	MP	79	M	Multiple Myeloma	Zoledronic acid	16	No	No	No
8	FL	69	F	Colon cancer	Zoledronic acid	12	No	No	No
9	FP	55	F	Breast cancer	Zoledronic acid	13	No	Bevacizumab	No
10	MC	65	M	Prostate cancer	Zoledronic acid	13	No	No	No
11	RN	81	M	Prostate cancer	Zoledronic acid	13	No	No	No
12	PDM	79	M	Prostate cancer	Zoledronic acid	16	No	No	No
13	DM	51	F	Breast cancer	Zoledronic acid	12	No	No	No
14	RF	72	M	Prostate cancer	Zoledronic acid	11	No	No	No
15	SC	73	M	Prostate cancer	Zoledronic acid	11	No	No	No
16	CP	69	M	Prostate cancer	Zoledronic acid	17	No	No	No
17	SM	64	F	Breast cancer	Zoledronic acid	16	No	No	No
18	VS	70	M	Prostate cancer	Zoledronic acid	10	No	No	No
19	GM	53	F	Breast cancer	Zoledronic acid	16	No	No	No
20	GP	70	F	Breast cancer	Zoledronic acid	16	No	No	No
21	FC	79	F	Breast cancer	Zoledronic acid	16	No	No	No
22	DDS	81	M	Prostate cancer	Zoledronic acid	16	No	No	No
23	BS	59	M	Prostate cancer	Zoledronic acid	16	No	No	No
24	SP	45	F	Breast cancer	Zoledronic acid	11	No	No	No
25	CB	55	F	Breast cancer	Zoledronic acid	12	No	No	No
26	RM	58	F	Breast cancer	Zoledronic acid	12	No	No	No
27	GS	67	F	Multiple Myeloma	Zoledronic acid	12	No	No	No
28	TV	75	M	Prostate cancer	Zoledronic acid	9	No	No	No
29	BG	80	M	Prostate cancer	Zoledronic acid	10	No	No	No

Tab 1. Group T: patients in primary prevention for antiresorptive-related ONJ receiving high dose vitamin D supplementation.

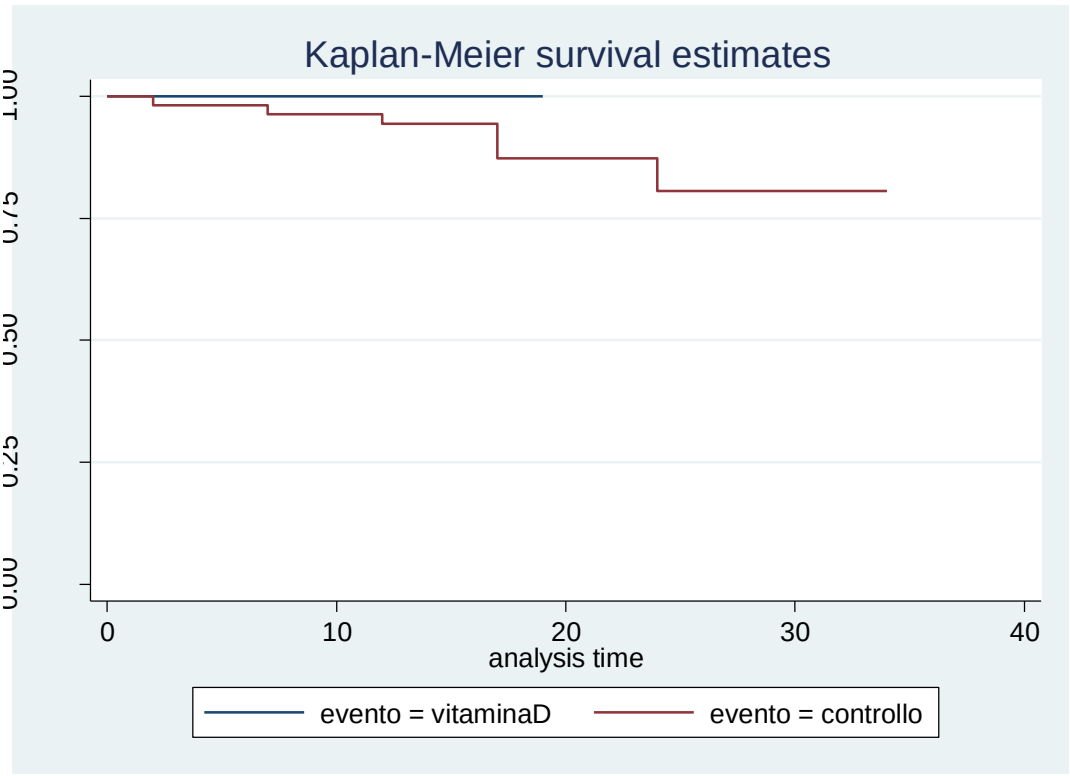
ID Patient	Ptazient	Age (yrs)	Gender	Cancer	Antiresorptive therapy	Antiresorptive duration	Systemic steroid therapy	Other drug-related ONJ	Local risk factors
1	MA	36	F	Multiple Myeloma	Zoledronic acid	18	No	No	No
2	CA	55	F	Breast cancer	Zoledronic acid	24	No	No	No
3	PA	41	F	Breast cancer	Zoledronic acid	18	No	No	No
4	LB	63	F	Breast cancer	Zoledronic acid	11	No	Bevacizumab	No
5	TAB	60	M	Multiple Myeloma	Zoledronic acid	20	Yes	No	No
6	CB	46	M	Colon cancer	Zoledronic acid	25	Yes	No	No
7	PC	71	M	Prostate cancer	Zoledronic acid	14	No	No	No
8	BC	64	F	Breast cancer	Zoledronic acid	12	No	No	No
9	RC	47	F	Breast cancer	Zoledronic acid	17	No	No	No
10	GC	67	M	Lung cancer	Zoledronic acid	13	No	No	No
11	AC	32	F	Breast cancer	Zoledronic acid	12	No	Bevacizumab	No
12	CDG	78	M	Prostate cancer	Zoledronic acid	25	No	No	No
13	EF	62	F	Breast cancer	Zoledronic acid	13	No	No	No
14	GF	63	F	Breast cancer	Zoledronic acid	16	Yes	No	No
15	GF	68	M	Lung cancer	Zoledronic acid	15	No	No	No
16	GF	70	M	Prostate cancer	Zoledronic acid	14	No	No	No
17	PF	78	M	Colon cancer	Zoledronic acid	20	No	No	No
18	RG	67	F	Breast cancer	Zoledronic acid	15	No	No	No
19	ALB	76	F	Multiple Myeloma	Zoledronic acid	23	No	No	No
20	GLS	65	M	Prostate cancer	Zoledronic acid	25	No	No	No
21	SLN	81	M	Lung cancer	Zoledronic acid	15	No	No	No
22	CM	55	F	Breast cancer	Zoledronic acid	21	No	No	No
23	VM	58	F	Breast cancer	Zoledronic acid	16	No	No	No
24	SM	68	M	Prostate cancer	Zoledronic acid	14	No	No	No
25	MRM	56	F	Breast cancer	Zoledronic acid	26	No	No	No
26	MGM	60	F	Breast cancer	Zoledronic acid	8	No	No	No
27	AM	65	F	Colon cancer	Zoledronic acid	14	No	Bevacizumab	No
28	SM	67	F	Breast cancer	Zoledronic acid	24	No	No	No
29	MM	65	F	Breast cancer	Zoledronic acid	27	No	No	No
30	AP	71	F	Breast cancer	Zoledronic acid	12	No	No	No
31	PP	52	F	Breast cancer	Zoledronic acid	12	No	Bevacizumab	No
32	GP	43	F	Breast cancer	Zoledronic acid	10	No	No	No
33	FR	50	M	Lung cancer	Zoledronic acid	12	Yes	No	No
34	GS	75	M	Prostate cancer	Zoledronic acid	16	No	No	No
35	GS	49	F	Breast cancer	Zoledronic acid	15	No	No	No
36	MS	45	F	Breast cancer	Zoledronic acid	25	No	No	No
37	CS	51	M	Prostate cancer	Zoledronic acid	18	No	No	No
38	VS	65	F	Multiple Myeloma	Zoledronic acid	12	No	No	No
39	AMT	50	F	Breast cancer	Zoledronic acid	17	No	No	No
40	AT	44	F	Breast cancer	Zoledronic acid	21	No	No	No
41	GV	50	F	Breast cancer	Zoledronic acid	27	Yes	No	No
42	EZ	45	F	Breast cancer	Zoledronic acid	12	No	No	No
43	MA	69	F	Breast cancer	Zoledronic acid	29	No	No	No
44	FPB	63	M	Prostate cancer	Zoledronic acid	16	No	No	No
45	MC	66	M	Multiple Myeloma	Zoledronic acid	34	No	No	No
46	MDB	55	F	Breast cancer	Zoledronic acid	34	No	No	No
47	MGN	62	F	Breast cancer	Zoledronic acid	23	No	No	No
48	GP	72	M	Prostate cancer	Zoledronic acid	24	No	No	No
49	SE	68	M	Liver cancer	Zoledronic acid	2	No	No	No
50	AG	60	F	Breast cancer	Zoledronic acid	17	No	No	No
51	FF	43	M	Prostate cancer	Zoledronic acid	7	No	No	No
52	RC	72	M	Prostate cancer	Zoledronic acid	12	No	No	No
53	RDS	58	F	Breast cancer	Zoledronic acid	17	No	No	No

Tab 2. Group C: patients in primary prevention for antiresorptive-related ONJ without high dose vitamin D supplementation.

ID Patient	Ptazient	Cancer	ONJ localization	ZLD duration (months)	Clinical sign (E/NE)	Abscess	Pain	SICMF-SIPMO Stadiation
48	GP	Prostate cancer	Mandibular	24	E	No	No	2
49	SE	Liver cancer	Mandibular	2	E	No	No	1
50	AG	Breast cancer	Mandibular	17	E	No	Yes	1
51	FF	Prostate cancer	Mandibular	7	E	Yes	No	2
52	RC	Prostate cancer	Mandibular	12	NE	Yes	No	1
53	RDS	Breast cancer	Mandibular	17	E	No	No	1

Tab 3. ONJ clinical characteristics

Legend
ZDL: Zoledronic acid
E/NE: bon exposure / non exposure



Tab 3. Survival analysis

Survival rates		
Months	Control group	Experimental group
3	98.0	100.0
6	98.0	100.0
12	94.0	100.0
18	87.0	100.0
24	81.0	100.0

Tab 4. Incidence rates

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