Nationwide Multicentric Retrospective and Prospective Epidemiologic Survey of Bisphosphonate-related Osteonecrosis of the Jaws

Ph.D Thesis

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Szeged, 2020

LIST OF PAPERS RELATED TO THE SUBJECT OF THE THESIS

I. **Vereb T**, Boda K, Czakó L, Vaszilkó M, Fülöp G, Klenk G, Janovszky Á, Oberna F, Piffkó J, Seres L.: Cloud-based multicenter data collection and epidemiologic analysis of bisphosphonate-related osteonecrosis of the jaws in a Central European population. J Clin Med 2020; 9: 426.
IF (2019): 3,303 Q1

II. **Vereb T**, Janovszky Á, Mucsi M, Piffkó J, Seres L.: Aktualitások a gyógyszer okozta állcsontelhalás primer és szekunder prevenciójának stratégiájában az evidenciák és a nemzetközi ajánlások tükrében [Current evidence-based approaches and international guidelines in primary and secondary prevention strategies of medication-related osteonecrosis of the jaws]. Orv Hetil 2020; 161: 214-223. [Hungarian]
IF(2019): 0,497 Q3

III. **Vereb T**, Vetró É, Piffkó J, Seres L.: A biszfoszfonát okozta állcsontnekrózisok. [Bisphosphonate-related osteonecrosis of the jaws] Magyar Urológia, 2012, 24: 153-158. [Hungarian]

1. Introduction

Bisphosphonates (BPs) are considered to be the "gold standard" of the treatment of some osteologic and metastatic oncologic diseases. BPs have an antiresorptive effect on bones and they effectively reduce pathological bone pain and the frequency of skeletal related events. They also decrease intraosseous tumor growth and tumor-induced hypercalcaemia; and finally, they improve the patient's quality of life. The first known cases of medication-related osteonecrosis of the jaw (MRONJ) were published in the literature in 2003. These cases have been related to BP treatments, therefore, this adverse drug reaction was named bisphosphonate-related osteonecrosis of the jaw (BRONJ).

Osteonecrosis of the jaw has been observed in connection with several new groups of drugs since 2009, so it became necessary to amend the terminology, definition and description of the disease. This condition has been referred to as medication-related osteonecrosis of the jaws (MRONJ) by the AAOMS since 2014. The changes in definition are illustrated in Table 1.

Definition of BRONJ (AAOMS 2009)	**Definition of MRONJ (AAOMS 2014)**
1. Current or previous treatment with a bisphosphonate	1. Current or previous treatment with ***antiresorptive or antiangiogenic agents***
2. Exposed bone in the maxillofacial region that has persisted for more than 8 weeks	2. Exposed bone or ***bone that can be probed through an intraoral or extraoral fistula(e) in the maxillofacial region*** that has persisted for more than eight weeks
3. No history of radiation therapy to the jaws	3. No history of radiation therapy to the jaws or ***obvious metastatic disease to the jaws***.

Table 1. Changes in the nomenclature and definition of bisphosphonate/medication related osteonecrosis of the jaws.

The pathomechanism of MRONJ has not been completely clarified so far. Several hypotheses were imposed to elucidate the full process of osteonecrosis that develops in the maxilla or in

the mandible. It is most likely that the multifactorial disease is a consequence of the interaction of drugs acting at different biochemical points of attack and signaling pathways with trigger factors from the external environment. The possible pathophysiological theories of MRONJ development are discussed in more detail in the following subsections.

1.) Bone remodeling is a complex mechanism regulated by paracrine, autocrine and endocrine hormones. Antiresorptive drugs inhibit not only osteoclast activity directly and indirectly, but osteoblast functional activity is also injured, as demonstrated by numerous studies.

2.) Bone regeneration depends not only on the bone remodeling, but also on the blood supply and angiogenesis. Tooth extraction initiates an inflammatory cascade, which promotes the expression of different hypoxia-induced angiogenic factors. The role of VEGF in the pathogenesis of MRONJ can be presumed, since suppression of this protein or its signaling pathway alone can provoke the development of MRONJ. However, antiresorptive treatment in combination with antiangiogenic drugs can significantly increase the incidence of this severe disorder.

3.) Local contamination and infection after invasive dental procedures in bisphosphonate-treated patients have also been emphasized in the pathogenesis of MRONJ. BP administration can upregulate the expression of pro-inflammatory cytokines (e.g. Il-1 or TNF-alpha), consequently an enhancement of leukocyte–endothelial cell interactions in the mandibular and maxillary periosteum has been observed.

4.) Depending on the administration way of BPs, the biological utilization can significantly differ (GI absorption about 1%, iv. absorption about 60%), followed by binding to the bone surface. After prolonged use, BPs accumulate in the skeleton, and reach the highest concentration in the mandible. Potentially a toxic level may develop, which may affect cellular mechanism and bony regeneration, leading to the development of MRONJ.

5.) Immune system is responsible not only for defensive mechanisms against infective agents, but also for regenerative mechanisms. These signaling pathways can be affected by BPs in different manners. Clinical studies have revealed that some BPs can elevate the level

of pro-inflammatory cytokines, such as TNF-alpha. Polymorphonuclear leukocytes activated by inflammatory cascade showed alteration in number and functional activity, which were investigated as a biomarker for MRONJ susceptibility. After chronic BPs treatment, altered chemotaxis, enhanced leukocyte-endothelial interactions can be observed in the mandibular periosteum, presumably mediated by different degrees of endothelium-derived adhesion molecule expression at the different anatomical locations.

Based on the Position Paper of AAOMS 5 stages of MRONJ can be distinguished according to their severity:

At risk: no apparent necrotic bone in patients who have been treated with oral or intravenous bisphosphonates

Stage 0*:* no clinical evidence of necrotic bone but nonspecific clinical findings, radiographic changes, and symptoms

Stage 1: exposed and necrotic bone or fistulas that probes to bone in patients who are asymptomatic and have no evidence of infection

Stage 2: exposed and necrotic bone or fistulas that probes to bone associated with infection as evidenced by pain and erythema in the region of exposed bone with or without purulent drainage

Stage 3: exposed and necrotic bone or a fistula that probes to bone in patients with pain, infection, and one of the following: exposed and necrotic bone extending beyond the region of alveolar bone (ie, inferior border and ramus in mandible, maxillary sinus, and zygoma in maxilla) resulting in pathologic fracture, extraoral fistula, oral antral or oral nasal communication, or osteolysis extending to inferior border of the mandible or sinus floor.

As a result of modern oncologic treatments, increased life expectancies and significantly improved quality of life have become available to patients. However, studies have reported a decrease in bone density and bone loss as a result of long-term hormone ablation treatments (e.g.: breast and prostate cancer).

In addition to bisphosphonates, other antiresorptive agents with better pharmacokinetics have been developed to treat these side effects, but these drugs also lead to developing MRONJ (RANKL inhibitors - denosumab). Explosive development of biological target therapies have

led the detection of additional MRONJ cases in association with non-antiresorptive medications, such as VEGF inhibitors (e.g. bevacizumab), mTOR inhibitors (e.g. everolimus), and CTLA-4 inhibitors (e.g. ipilimumab).

The incidence and prevalence of MRONJ show an increasing trend due to the broadened indications, the increased number of patients, the unexpected interactions and synergistic effects between different groups of drugs (bisphosphonate - denosumab, bisphosphonate - VEGF inhibitors, denosumab - VEGF inhibitors). The incidence of the disease may vary widely depending on the type of BPs used, route of administration (iv/orally), cumulative dose, underlying disease and comorbidities, concomitant medication therapies and surgical interventions.

Many authors agree that the reported data in the literature tend to underestimate the real incidence and prevalence. The incidence of BRONJ among BP users in Hungary is 0.9% in malignant indications, 0.1% in the non-malignant indication, and the odds ratio (OR) to develop BRONJ was 9.7 (95% CI) between them. Factors contributing to the development of MRONJ include underlying malignancy, intravenous drug administration, high cumulative dose, long-term drug use, concomitant use of drugs with different attack points, poor oral hygiene, poorly fitting dentures, and inadequate compliance.

Diagnosis and staging are established based on clinical and radiological findings. The main purposes of radiological examinations are to assess the extent of osteonecrosis, to facilitate individual surgical treatment planning, to follow up cases, and to evaluate prognosis. Effective and reliable therapy is not currently available. Treatment options include prevention of the disease, delaying or blocking progression, conservative, surgical and complementary therapies of soft and hard tissues.

2. Main objectives

To the author's best knowledge, no comprehensive nationwide epidemiological study has previously been performed in Hungary among BRONJ patients. The main objectives of the study are detailed below:

1) nationwide retrospective and prospective data collection of symptomatic BRONJ patients in Hungary
2) preparing an epidemiological description of the gender and age distribution of involved patients based on the database
3) investigation of underlying and concomitant diseases potentially related to the development of BRONJ
4) examination of the type, concentration, route and frequency of administration of BPs causing BRONJ
5) analysis of triggering factors taking part in the formation of osteonecrosis of the jaws
6) investigation the relationships between disease severity and the localization and extent of necrosis
7) identification of associations between disease severity and environmental effects

3. Material and methods

In 2012, following the approval of the local Scientific and Research Ethics Committee of the Medical Research Council (license number: 17773/2012/EKU 320/PI/12.) an online spreadsheet-based questionnaire and database have been set up and made available for non-profit use to voluntary researchers and clinicians in Hungary and Slovakia. The questionnaire has been made available to cooperating maxillofacial units. To date, seven maxillofacial units from Hungary and one from Slovakia have joined the study. Altogether 15 colleagues participated in the data collection. Data have been collected on gender, age on onset of the BRONJ, underlying and concomitant diseases, medical and dental history, smoking and alcohol consumption, type of bisphosphonate taken, treatment duration,

frequency and route of administration, presumed trigger factors, clinical stage of disease, location and extent of lesions.

The ***inclusion criteria*** of the study were determined as follows:

1) Previous or current BP treatment regardless of drug type, dose or route of administration

2) Consecutive or concomitant BP treatment with different bisphosphonate agents

3) Intra- or extraoral lesion following or after BP therapy

The ***exclusion criteria*** of the study were the following:

1) Asymptomatic patients with previous or current bisphosphonate treatment
2) Symptomatic patients consecutive and/or concomitant combined antiresorptive and antiangiogenic therapies
3) Patients with previous or undergoing radiation therapy of head and neck region

To achieve the purposes of the study a free-accessible Google Sheets (Google LLC, CA, USA) was applied. Google Sheets is a spreadsheet program which is available as a web and mobile application for each platform. The web based data collection system enables not only detailed data collection but also the analysis of the data gained. This system is suitable for controlling access permissions and user restrictions in different levels. These properties allowed easy real-time collaboration between multiple users working on the same document at the same time regardless of distance.

Statistical analysis was performed with the Statistical Program for Social Sciences version 23.0 for Windows (SPSS, Chicago, IL, USA). A p-value of less than 0.05 was interpreted to imply statistical significance. Means and standard deviations (SD) were calculated. Unpaired student's t-test was used for evaluation of statistical significance. Pearson Chi-square test was performed to examine the relationships between different biological variables.

4. Results

The data of 180 symptomatic BRONJ cases have been recorded during the data collection period (from 2012-2016). The number of reported cases decreased from year to year; 64 patients, 41 patients, 34 patients, 23 patients, 18 patients respectively. Full data were obtained for 148 (82.2%) patients. In 32 cases (17.8%) data collection was incomplete. The data on age, sex, underlying disease, method of drug administration, staging were complete in all cases. All data were included in the statistical analysis.

4.1. Age and gender distribution

There was a female predominance in the distribution of BRONJ. 122 women (67.8%) and 58 men (32.2%) were affected. Male-to-female ratio was 1:2.1, this correlates well with the results of other authors.

The mean age at the time of the diagnosis was 66.80 years; 66.22 years in women (range 37-85 years; SD 10.29 years) and 68.02 years in men (range 42-89 years; SD 9.33 years). There was no significant difference between the ages of males and females (p=0.246). Patients suffering from non-malignant diseases (osteoporosis, rheumatoid arthritis) were generally older (n=36; mean 68.57 years; range 38-84 years, SD 9.79 years) than patients with malignant disease (n=140; mean 66.32 years; range 37-89 years, SD 9.43 years).

Within the malignant group renal cancer patients were generally younger (n=13; mean 62.92 years; range 51-77 years, SD 8.45 years) than the rest of the group (n=127; mean 66.67 years; range 37-89 years, SD 9.49 years) but the difference was not significant (p=0.153). Breast cancer patients were only slightly younger (n=66; mean 64.68 years; range 37-85 years, SD 10.16 years) but the age difference was statistically significant (p=0.045) when compared with the rest of the group (n=74; mean 67.86 years; range 48-89 years, SD 8.49 years). The mean age of multiple myeloma and lung cancer patients were 67.62 (n=16; range 57-80 years, SD 6.9 years) and 65.00 (n=7; range 48-81 years, SD 10.77 years), respectively. Prostate cancer patients (n=30; mean 71.57 years; range 61-89 years, SD 7.00 years) were significantly

older (p=0.000075) than the other malignant cases (n=110; mean 64.93 years; range 37-85 years, SD 9.53 years).

4.2. Underlying diseases

The vast majority of BRONJ cases occurred in patients with malignant diseases (n=140; 77.8%). 34 patients (18.9%) received bisphosphonate for osteoporosis; 2 patients (1.1%) were diagnosed with rheumatoid arthritis. In 4 cases (2.2 %) the reasons for treatment remained unknown because of the nature of retrospective studies.

4.3. Comorbidities

Data of comorbidities were complete in 162 (90%) cases. High blood pressure and/or cardiac disease was reported in 75 (46.29%) cases. Nineteen (11.72%) patients suffered from diabetes mellitus. Chronic obstructive pulmonary disease (COPD) and/or asthma were diagnosed in 6 (3.70%) cases. Concomitant renal, hepatic and gastrointestinal diseases were reported in 11 (6.79%), 7 (4.32%) and 9 (5.56%) patients, respectively.

4.4. Types of bisphosphonates and routes of administration

Fifty-two individuals (28.9%) were given oral bisphosphonates alone. In this group ibandronic acid (n=19; 36.6%) and alendronic acid (n=18; 34.6%) were the most frequently used agents, followed by clodronic acid (n=9; 17.3%) and risedronic acid (n=6; 11.5%). In the vast majority of the cases, bisphosphonates were administered intravenously alone (n=106; 58.9%) or in combination with oral drugs (n=22; 12.2%). A total of 128 patients (71.1%) received intravenous bisphosphonate therapy. Intravenous zoledronic acid was associated with the highest risk of BRONJ, 110 patients (61.1%) were treated with this drug alone, or in combination with other agents (n=6; 3.3%). Altogether 116 patients (64.4% of all patients; 90.6% in the intravenously treated group) were administered intravenous zoledronic acid. Results of the Pearson Chi-square test showed a statistically significant relationship (p=0.023) between the severity of stages (Stage 1: mild versus Stage 2+3:serious) and the administration method.

4.5. Triggering factors

Presumed triggering factors were reported in 167 cases. Dental extraction was the most common predisposing event (n=121; 72.4%). A further 6 patients (3.6%) had previous other dentoalveolar surgery (implant placement, periodontal surgery). Pre-existing inflammatory diseases such as periodontal and/or periapical pathology were present in 19 cases (11.4%). Denture use was thought to be the main trigger factor in 12 cases (7.2%). BRONJ was considered of spontaneous origin in 9 cases (5.4%).

4.6. Staging of BRONJ

At the time of the first clinical examination 36 cases (20.0%) were categorized as stage 1. The majority of the patients (n=96; 53.3%) were diagnosed as stage 2. Forty-eight cases (26.7%) were classified as stage 3 with extraoral fistula, pathological fracture, involvement of the maxillary sinus or the inferior border or the ramus of the mandible. The underlying disease and its malignant or benign nature were determined in 176 cases. There were 36 benign cases, the distributions of stages were as follows: stage 1: 36.1% (n = 13), stage 2: 47.2% (n = 17), stage 3 16.7% (n = 6) respectively. From the 140 underlying malignancies, 23 (16.4%) were classified in stage 1, 77 cases (55.0%) ranked as stage 2 and 40 cases (28.6%) belonged to stage 3. At the time of the first visit, more severe stages (2-3) occured in a higher proportion of patients with malignancies compared to patients with benign conditions (stage 1: 36.1 % vs. 16.4 %, stage 2 : 47.2 % vs 55.0 %, stage 3: 16.7 % vs 28.6%) As stage worsened, the proportion of malignant cases increased significantly compared to the number of benign cases (stage 1 - 1:1.77, stage 2 - 1:4.53, stage 3 - 1:6.66) A significant difference was found between the benign and malignant groups (Pearson Chi-Square test p=0.026). Although much more women than men are affected by BRONJ; in stage 3 the number of female and male patients were almost equal, 26 (54.2%) and 22 (45.8%) respectively.

4.7. Localization of osteonecrosis

194 jaws were affected by bisphosphonate-related osteonecrosis of the jaws altogether. In 124 patients (68.9%) only the mandible, in 42 patients (23.3%) only the maxilla; in 14 cases (7.8%) both jaws were affected. Altogether 304 regions were affected by BRONJ in 180 patients; 213 regions (70.1%) in the mandible and 91 regions (29.9%) in the maxilla. The most common sites of osteonecrosis were the molar (n=98; 32.2%) and the premolar regions of the mandible (n=82; 27%), followed by the upper molar (n=36; 11.8%) and premolar

regions (n=35; 11.5%). The lower and upper front regions were affected in 33 (10.9%) and 20 (6.6%) cases, respectively. In terms of localization, the results correlate well with the results published by Otto et al.

5. Discussion

BRONJ is a relatively newly recognized condition that has generated great interest not only amongst oral and maxillofacial surgeons but also in other medical and research communities. Our study found female predominance among BRONJ patients (female 67,8 %, male: 32,2 %; male to female ratio 1 : 2.1) which is in line with the results of Otto and Schubert, but slightly higher than in Kos' and Mavrokokki's publications. Female-to-male ratio of 8:1 was published by Pazianas.

77.8% of the patients suffered from an underlying malignant disease, this proportion closely correlates with Mavrokokki's result, who referred to 72% of bone malignancies among their patients. The mean age of the benign group (68.57 years) is not significantly higher than the age of the malignant group (66.32 years). Within the malignant group, BRONJ developed at a significantly higher age in prostate cancer patients compared to the remainder of the group. BRONJ was diagnosed at a significantly younger age in breast cancer patients compared to the rest of the malignant group. Although in our investigation there were only two rheumatoid arthritis patients (mean: 39.0 years SD: 1 years); there is still a surprisingly huge age difference when it is compared to the results of Di Fede (n=18 mean: 68 years SD: 8 years).

Gabbert's study pointed out that osteonecrosis free survival in single bisphosphonate users was significantly longer in pamidronate-treated patients than in zoledronate or ibandronate users. In our study, from the intravenous group 127 of 128 patients (99.2%) were administered zoledronate and/or ibandronate and only one patient (0,8%) was diagnosed with BRONJ following pamidronate treatment. Our results also prove that the route of administration has a significant (p=0.023) association with the severity of the osteonecrosis.

According to Thumbigere-Math et al. increased cumulative doses and long-term bisphosphonate treatment are the most important risk factors for osteonecrosis, but the type of bisphosphonate may also play a role in the incidence of osteonecrosis; our results confirm these findings. According to the literature, the mandible is affected in 64 to 70.6%; the

maxilla is involved in 18.3 to 27%. BRONJ was present in both jaws in 9 to 11.1%. Our findings (mandible 70.1%, maxilla 23.3%, both jaws 7.8%) correlate well with these results. There is a characteristic distribution of osteonecrosis with a predilection for the molar and premolar region in both jaws, just as it was pointed out by Otto et al.

At the time of the diagnosis the majority of the patients (53.3%) were categorized as stage 2; 20.0% and 26.7% were classified as stage 1 and stage 3, respectively. These findings are similar to those of Schiodt et al (stage 1: 26%; stage 2: 58%; stage 3: 10%; unknown: 3%; resolved: 2%). Although much more women than men are affected by BRONJ, their number in stage 3 is nearly the same (26 and 22, respectively). The ratio of malignant cases to benign cases increased significantly (p=0,026) as the stage worsened (stage 1 - 1:1.77, stage 2 - 1:4.53, stage 3 - 1:6.66).

The evolution of cloud-based information technology has dramatically changed data collection and analysis for scientific purposes. To the best of our knowledge our study is the first one that has collected data on BRONJ patients from multiple centers with this method. Despite the many advantages offered by cloud-based technology our study also has some pitfalls. The participation has been voluntary and this has probably resulted in under-reporting; therefore our data are not informative about the incidence of BRONJ. The relatively high number of incomplete reports is surprising but this can be explained by the fact that the online questionnaire was not filled out at the time of the patient's examination and later data were not found in the documents. The number of patients reported in this study is high compared to other single-center or even multicenter studies but the average number of patients reported per center per year is less than 6 in the 4-year study period. The decreasing trend of the number of new patients reported per year probably reflects that voluntary researchers have lost their initial enthusiasm, but better patient management, early diagnosis and state-of-the-art prevention techniques might also played an important role. A sample size of 180 BRONJ cases is considered statistically significant but data were not always sufficient to reach statistically reliable conclusions when the patients were classified into groups. More patients are needed to improve the power of the study. A multicenter registry that collects systematic information on epidemiological data is essential to increase our knowledge of BRONJ. Cloud-based information collection is an ideal tool for this purpose. The online and

voluntary nature of the current study may slightly diminish the accuracy of the results but the increasing number of patients involved will improve statistical conclusions.

6. Summary of the new findings

1) We have successfully introduced a cloud-based multicentric real-time data collection method to obtain population-wide epidemiological data on a rare but serious side effect.
2) According to our best knowledge, this examination was the first descriptive epidemiological analysis on a Central European population in terms of BRONJ.
3) Prostate cancer patients (n=30; mean 71.57 years; range 61-89 years, SD 7.00 years) were significantly older (p=0.000075) than the other malignant cases (n=110; mean 64.93 years; range 37-85 years, SD 9.53 years)
4) Breast cancer patients were only slightly younger (n=66; mean 64.68 years; range 37-85 years, SD 10.16 years) but the age difference was statistically significant (p=0.045) when compared with the rest of the group (n=74; mean 67.86 years; range 48-89 years, SD 8.49 years).
5) We have demonstrated that if the stage worsened, the proportion of malignant cases increased significantly compared to the number of benign cases (stage 1 - 1:1.77, stage 2 - 1:4.53, stage 3 - 1:6.66) A significant difference has been found between the benign and malignant groups (p=0.026).
6) We have found statistically significant (p=0.023) correlation between the severity of stages (Stage 1: mild cases versus Stage 2+3: serious cases) and the administration method (oral versus intravenous).

Acknowledgements

I would like to express my gratitude to my supervisor ***Dr. László Seres***, who teached me, guided and helped my work from the beginning, and thanked him for allowing me to achieve this day.

I am grateful to ***Professor József Piffkó*** for initiating my scientific career under his guidance and with his help.

I would like to thank ***Dr Ágnes Janovszky*** for her irreplaceable and essential help preparing the thesis.

Many thanks to ***Dr. Krisztina Boda Jánosné Stéhlik*** for her irreplaceable and invaluable work in the statistical processing and evaluation of the database.

I would also like to thank ***all my colleagues*** involved in the research for their help and dedicated work.

Finally, I would like to thank my loving wife - ***Szandra Vereb-Jancsikin*** - and my whole family for the supportive background, which was required of me during the preparation of my thesis.

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