Factors influencing the prognosis of bisphosphonate induced osteonecrosis of the jaw

PhD thesis

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Introduction

Relevance of the topic

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a relatively recently identified disease, it has been known since 2003. It is similar but not identical to jaw osteonecroses known previously: both its etiology and its course are different. Established protocols to treat necrosis are not necessarily successful and healing is not always complete even when new recommendations are followed. Thus, detailed elucidation of the pathomechanism and finding efficient therapies to treat this disease received great attention during recent years.

We were the first in Hungary to diagnose bisphosphonate-related jaw osteonecrosis in 2005. Since then we have been following the literature on the disease and its treatment (Vaszilkó 2007). We have had the chance to compare our results in the treatment of BRONJ to the information published in the literature and to incorporate our findings into the established treatment protocol (Vaszilkó 2009, Vaszilkó 2011, Vaszilkó 2013). We have set up close collaboration with the Osteology Work Group of the First Department of Medicine at the Semmelweis University, which facilitated more detailed studying of the disease and a closer follow up of patients (Balla, Vaszilkó 2011).

The history of bisphosphonate

Bisphosphonates have been known since the mid 1800s. The first bisphosphonate was synthesized in 1865 in Germany, back then it was called disphosphonate (Menschutkin 1865). Commercially bisphosphonates were primarily used in the textile and oil industry and in the production of fertilizers.

However, it was not until the 1990-es that bisphosphonates were widely used as medicine. Nowadays, with the development of new and more efficient drugs, bisphosphonates are among the most popular and most potent drugs used against bone resorption (Cynthia 2013).

New drugs can reduce the risk for vertebral fractures by 40 to 70% and fractures of the hip area by 40 to 50% (Chesnut 2004, Papaioannou 2010, Dhanwal 2011).

Bisphosphonates as drugs

P-C-P is the basic structure of bisphosphonates, which resembles the P-O-P structure of natural pyrophosphate (Gyires 2007). This basic structure is responsible for the general physical properties such as poor solubility or membrane penetration capability, The central

carbon atom is flanked by two side chains, one of which is is responsible for binding to hydroxiapatite and the other for biochemical properties.

We separated two subgroups: nitrogen-containing aminobisphosphonates and non-nitrogen-containing aminobisphosphonates (Gyires 2007). The nowadays exclusively applied aminobisphosphonates cause the curl up of the osteoclast which results in the cessation of the bone resorption activity. Bisphosphonates are binding to the active remodeling bone surface due to hydroxyapatite affinity, which affect primarily the resorption areas (Masarachia 1996). So the drug is selectively localized in the bone and the higher concentration is primarily where it should mostly affect the bone resorption inhibition at the moment. The systemic concentration is minimal because they disappear from the circulation after resorption in a few hours: they are either binding to the bone or excreted by the kidneys to the urine in an unchanged form without metabolism (Lakatos 2012). It is a baseline drug in the treatment of malignant tumours metastasizes to the bones, such as the breast cancer, hormone refractory prostate cancer and kidney carcinoma (Rosen 2004, Body 2006). It is applied in tumour induced hypercalcaemia as well. It improves the patient's quality of life with reducing the pain caused by the tumour in the bone or by the metastasis (Small 2003, Heidenreich 2004). According to in vitro research bisphosphonates inhibit the ability of the cancer cells to metastasize, induce apoptosis, and inhibit the reproduction in cancer cells and the increase of the already ongoing bone lytic process (Green 2002, Santini 2003). The group of compounds is applied in the treatment of the osteoporosis caused by aging and glucocorticoids, and in diseases with excessive bone metabolism, such as Paget's disease, osteogenesis imperfecta, multiple myeloma, fibrous dysplasia, and heterotropic ossification. The most frequently applied active agents are alendronate, zoledronate, ibandronate and rizedronate.

Bisphosphonate-related osteonecrosis of the jaw

The osteonecrosis of the jaw was originally believed to be osteomyelitis and it thought to be an adverse effect of cancer therapy; the role of bisphosphonates in this disease was first mentioned only in 2003 (Wang 2003, Marx 2003). The disorder which appears after bisphosphonate treatment characterized by exposed bone surfaces – Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ) – was described as a separate disease, with individual and new name first in 2005 (Hellstein 2005). A recommendation (position paper) about the current situation of the science according to the bisphosphonate-related osteonecrosis of the

jaw was done by the American Association of Oral and Maxillofacial Surgeons (AAOMS) in 2006. The definition of BRONJ: the diagnosis of BRONJ is established if there is exposed bone in the maxillofacial region, which is not healing in 8 weeks from the recognition by the physician in patients, treated with bisphosphonates, who did not undergo radiation therapy to the head-neck region with therapeutic dose. Additional signs and symptoms can occur in the suspected or proved cases of BRONJ, such as pain, swelling, paresthesia, purulent discharge, ulceration of the soft tissues, formation of intraoral or extraoral fistula, loss of teeth or radiological lesions. According to the recommendation of the mentioned association the disease has 4 stages. Patients classified to *stage zero* do not have exposed necrotic bone surface, but they are presenting with non-specific signs or symptoms and radiological lesions. The lesion is considered to be *stage one* if the patient who is otherwise asymptomatic and does not have any complains has necrotic bone surface, without any signs of inflammation. The recommendation has therapeutic suggestion as well: in stage zero, symptomatic treatment and conservative treatment of other local factors, such as the management of the caries or parodontal diseases is suggested. In *stage one* oral antimicrobial solution are useful, for instance 0.12% of chlorhexidine. Surgery is not suggested in this stage. In *stage two* the previously applied gargling with chlorhexidine should be completed with antibiotic treatment. The most of the isolated pathogens are sensitive to antibiotics belonging to the penicillin group (amoxicillin + clavulan acid). In *stage three* debridement is the most important; this means resection or sequestrectomy - performed in antibiotic protection - which leads to the resection of the jaw in extended cases. The inflammation prior to tooth extraction and the following exposed bone surface as an open gate for pathogens localized in the oral cavity may have a role in the clinical course. Against this affect the bone and mucous membrane with reduced remodeling and blood supply – caused by BP therapy - is not able to perform an appropriate protection, so the process of bone necrosis may start. This theory was completed by new research as aminobisphosphonates have a negative effect on the epithelial cells (Landesberg 2008) and fibroblasts as well (Reid 2009), leading to a slower wound healing of the mucous membrane. Bisphosphonates have an immunomodulatory effect as well; they change the mechanism of TNFα, T-cells and machrophages due to IL-1 modulated process (Deng 2007). The accumulated bisphosphonate has a toxic effect on soft tissues and epithelium (Reid 2007, Bauss 2008). The difficulty of the diagnosis is that the X-ray shows the bone necrosis only late. Laser or hyperbaric therapy solely did not prove convincing results; the cessation of the drug was absolutely ineffective in short term according to the necrosis.

The aim of the study

The aim of our discussion in the research was to evaluate patients suffering from BRONJ and treated in details at the Department of Oro-Maxillofacial Surgery and Stomatology of the Semmelweis University, and to learn as much as we can about the pathomechanism of the bisphosphonates-related necrosis of the jaw and risk factors, based on the experience gained during the treatment, furthermore to find successful methods of the prevention and treatment.

Concrete questions to evaluate:

1. Has BRONJ a genetic background, and does it have any effect on the prognosis of the disease?
2. Does the hormonal environment have any influence on the bisphosphonate-related necrosis of the jaw: for instance does the estrogen deficiency (anti-estrogen medical treatment in our study) or the lack of parathyroid hormone have any effect on the course of the disease?
3. Can we predict the disease with bone markers from blood samples, and can we predict the prognosis?
4. How can diabetes mellitus, in the patient's past medical history, influence the course of the necrosis of the jaw?
5. Does the localiation in the oral cavity of BRONJ, and the stage at the first visit impact the successful treatment of the disease?
6. Do the active agent and the form of administration of bisphosphonates have any effect on the presence of BRONJ and the course of the disease?

Methods

Patients

In the present prospective study, clinical parameters of BRONJ patients consecutively referred to and treated between 2006-2013 at the Department of Oro-Maxillofacial Surgery and Stomatology of the Semmelweis University, Budapest, were evaluated. The criteria for BRONJ were based on the 2006 AAOMS position paper; the treatment strategy recommended by the Association was followed. Panoramic X-ray (OP) was performed in every patient to diagnose the disease, and the stage of BRONJ upon admission was determined based on the OP and clinical signs. In connection with the patient's subjective complains (pain, involvation of the inferior alveolar nerve) the treatment strategy was defined. Antibiotic treatment was performed from stage two in every case; when it was possible to collect pus, we did culture to start targeted antibiotic treatment. We preferred amoxicillin + clavulan acid products in 1 mg dose as primary antibiotic, but clindamycin (300 mg) and doxycyclin were used as well expecially in case of penicillin allergy. With the informed consent of the patients surgery was always done in stage two and three; prior to the operation antibiotic therapy was started at least one day before the intervention and it lasted for 7-10 days after the operation. In case of an unsuccessful conservative treatment lasting for at least 2-4 weeks, surgery was prformed in stage one as well. During the surgery we performed the resection from a necessarily minimal exploration, with the control of the vital parameters of the bone (bleeding of the bone, the colour of the bone); the extension of the resection affected only the necrotic zone. The wound closure was performed tension-free with local flaps and single layer suture was used. In the post-operative phase patients should have applied gargles containing hexidine at least for 2-3 times a day. Upon the dehiscence of the wound jodoform gauzestrips were placed onto the area, and changed in every three days, until either the secondary wound healing was reached or the reoperation was performed.

Evaluation of the bone metabolism

In a part of the patients, who presented at our Clinic for treatment between January 2010 and September 2013, according to the Roche E-170 Modular (Roche, Mannheim, Germany) protocol fasting blood glucose level was measured (Beckman Coulter, Brea, CA, USA), and 25-hydroxy vitamin D, PTH and ß-crosslaps were determined. The study was

approved by the Regional Committee of Science and Research Ethics, Semmelweis University (ad.8-101/2009-1018EKU), and all patients gave written informed consent.

Evaluation of the genetic background

In this study, we aimed to investigate the effect of CYP2C8 rs1934951 SNP that was previously suggested to be associated with ONJ and its relationship to a number of clinical and biochemical factors in 46 Hungarian subjects with bisphosphonate-induced ONJ. Blood samples were collected from each subjects and genomic DNA was extracted using High Pure PCR Template Purification kit (Roche Diagnostics, GmbH, Mannheim, Germany). DNA quality and quantity were determined with NanoDrop B-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Bone mineral density (BMD) was measured at the total femur and the lumbar spine (L2-L4) by dual X-ray absorptiometry (DPX-L, Lunar Corp. Madison, WI, USA). Osteoporotic state was defined according to the recent WHO criteria[9] *i.e.* T-score lower than -2.5 SD at any measured side. The study was approved by the Regional Committee of Science and Research Ethics, Semmelweis University (ad.8-101/2009-1018EKU), and all patients gave written informed consent.

The control group for the analysis of CYP2C8 rs1934951 polymorphism included 224 blood samples from healthy Hungarian unrelated patients. Exclusion criteria were history of bone, metabolic, or endocrine disease; any chronic illness; hormone replacement, steroid therapy, or any medication known to influence bone metabolism; premature menopause (before 40 years of age); and alcohol consumption greater than two units per day. Control subjects with biochemical abnormalities such as increased levels of serum alkaline phosphatase, thyrotrophin, parathyroid hormone, or reduced level of 25-OH-vitamin D (<30 ng/ml) were not included in the study. Biochemical parameters were measured by routine laboratory methods (Roche, Mannheim, Germany).

Follow-up

The patients on conservative treatment were called back and checked up at least in every week. The patients who underwent a surgery were contolled in 7-10 days after the operation, and the next check up was suggested to be in two weeks, or immediately at our Clinic if the recovered area causes complains. The type of the treatment of the disease, and the data of healing/relapsing were recorded. The patients were followed up and recurrence rate was recorded. A condition was considered to be a recurrence, when the patient had had a

minimum of 4 weeks of complete lack of clinical and radiological signs and symptoms following surgical or conservative therapy, and BRONJ symptoms occurred again.

Statistical analysis

Data were analysed with Fisher's exact test, Mann-Whitney test, ANNOVA, Kruskal-Wallis test and Chi^2-tests. We tested the effect of CYP2C8 genotypes on the localization of ONJ applying a multivariate logistic regression model. Principal Components Analysis (PCA) was applied to analyse the genetic results. P-value less than 0.05 was considered as significant. Chi^2-tests and logistic regression analysis were performed in the SPSS 15.0 and 22.0 software 22.0.

Results

In our study we included 121 patients presenting with bisphosphonate-related osteonecrosis of the jaw at the Department of Oro-Maxillofacial Surgery and Stomatology of Semmelweis University between June 2006 and November 2013. Of these, 65 patients had laboratory testing at the First Department of Internal Medicine and 46 was subjected to genetic testing and bone density measurement.

Classification of patients

- Classification of patients according to age and gender

Of the 121 patients, 92 were female (76%) and 29 male (24%). Post treatment recurrence of BRONJ was not significantly different between genders (p=0.100) when tested with Fisher's exact test. Likewise, treatment did not correlate with improvement in disease status (p=0.383) or total healing rate (p=0.405). The mean age of the patients was 65.18 years (range: 31 to 87 years). The mean age of males was 65.16 years while that of females was 65.55 years.
There was a statistically significant difference in the mean age at the development of BRONJ between disease groups (p=0.022, ANOVA test): osteoporotic patients were older at the onset of BRONJ.

- Primary diseases

Mammary tumor: 50/121 (41.3%); prostate cancer: 17/121 (14%); osteoporosis 23/121 (19%); multiple myeloma: 15/121 (12.4%); renal tumor: 6/121 (0.49%); chronic lymphoid leukemia: 1/121 (0.08%); non-Hodgkin lymphoma: 1/121 (0.08%); myelodisplastic syndrome: 1/121 (0.08%); colon tumor: 1/121 (0.08%); lung cancer: 2/121 (0.16%); urethral tumor: 2/121 (0.16%); bladder cancer: 1/121 (0.08%).

- BRONJ staging at first admission

The number of patients in each stage of BRONJ at first admission was as follows. Stage 1: 27 (22.3%); stage 2: 69 (57%); stage 3: 23 (19%); and two patients were in stage 0 at first admission. Primary disease correlated with neither the BRONJ stage at first admission

(p=0.958, chi square test), nor the highest observed stage during treatment (p=0.673, chi square test). Chi square test also showed a significant correlation between staging at first admission and treatment success rate both in terms of improvement in staging (p=0.048) and disease recurrence (p=0.029). We attempted to find a correlation between the highest stage observed and of the prognosis of BRONJ in each patient using chi square test. The correlation was close to significant (p=0.052) between more advanced stage of BRONJ and a higher rate of relapse while there was no correlation between less advanced stage and lower rate of relapse (p=0.066).

- Previous treatment

58.7% of the patients (71/121) had received zoledronate, 10.7% (13/121) alendronate, 10.7% (13/121) oral ibandronate, 5.0% (6/121) each had received intravenous (iv.) ibandronate and pamidronate, 3.3% (4/121 clodronate, and 2.5% (3/121) rizedronate. 35 patients had received more than one type of bisphosphonate simultaneously or consecutively. 29 of these 35 patients had received different bisphosphonate medications not just with different trade names but actually containing different active ingredients, several patients had even received 3 different active ingredients before.

There was no significant difference (p=0.097) between the distribution of males and females within groups having received each particular bisphosphonate substance. Female patients were more likely to have received oral bisphosphonates. There was no significant correlation between the type of bisphosphonate used and the improvement observed in the stage of BRONJ during treatment (p=0.485). There was also no significant correlation between the active bisphosphonate ingredient and necrosis relapse rate (p=0.112, chi square test). When taking separately patients receiving oral and iv. bisphosphonates, we did not observe any significant difference in the rate of BRONJ relapse, complete healing or improvement in staging, nor any statistically significant correlation (p=0.200, p=0.112 and p=0.315, respectively).

- Duration of bisphosphonate treatment prior to BRONJ

The mean total duration of bisphosphonate treatment prior to the onset of BRONJ was 37.26 (range: 1 to 185) months. 11 patients received bisphosphonates for less than a year preceding the onset of BRONJ, all of them intravenously. Of the 11 patients, 4 had metastatic prostate

cancer, 4 had metastatic mammary tumor, two had myeloma multiplex, and one had colon cancer.

The mean (±SD) number of months of treatment with bisphosphonates prior to the onset of BRONJ was 53.2±37.1 for oral formulations and 34.9±26.9 for iv. treatment. If the patient had received both, it was 38.1 months. The number of treatment months for each bisphosphonate was: 50.1 (aledronate), 53.0 (oral ibandronate), 69.0 (risedronate), 31 (pamidronate), 51.2 (iv. ibadronate), and 31.6 (zoledronate); there is no significant correlation (p=0.099). When comparing the mean duration of bisphosphonate treatment between patients of the two gender, there was no significant difference: 31.04 months for males and 39.33 months for females, p=0.252 (ANOVA test). 35 patients received oral bisphosphonates (pills) only. The remaining patients had received either parenteral only or both oral and parenteral bisphosphonates.

The duration of bisphosphonate treatment until the onset of necrosis is significantly correlated with the route of administration: 32.57 months for intravenous *vs.* 50.44 months for oral route, p=0.007 (Mann-Whitney test).

There was no significant correlation among iv. and oral routes of bisphosphonate administration and BRONJ staging at first admission: p=0.381 (chi square test), and this applies to the most advanced stage of BRONJ reached during treatment as well: p=0.537 (chi square test). The average time between the onset of BRONJ and first admission was 4.78 weeks (range: 0 to 25 weeks, SD: ±6.50 weeks). The difference between the times to the onset of BRONJ for different bisphosphonates is not statistically significant (p=0.070, Kruskal-Wallis test). Furthermore, there is no correlation between the duration of prior treatment with bisphosphonate and the time passed after the onset of BRONJ until the first admission (p=0.893).

- Location of the necrosis

Location according to jaw quadrants

The primary location of bone necrosis was on the maxilla in 29.8% of the patients (36/121), on the mandible in 63.3% (77/121) and on both jaws in 5.0% of the patients (6/121). The two patients in stage 0 were not evaluated in this analysis. The improvement in BRONJ staging in response to treatment appeared to be better on maxillae than on mandibles, even though the difference was not quite significant (p=0.051, Fischer's exact test). Likewise, the relapse rate of lesions on mandibles appeared to be higher than on maxillae but the difference was not

significant (p=0.076). The presence of necrosis in different quadrants is not correlated either with the improvement in BRONJ staging (p=0.290) or with the rate of relapse (p=0.212).

Location according to jaw sextants

18.7% (14/75) of the necrotic lesions were located in the frontal region and 81.3% (61/75) in the premolar-molar region. We assessed the correlation of necrosis location on jaw sextants and the change in BRONJ staging in response to treatment, but we found no such correlation (p=0.258, chi square test). There was no significant difference between the number of necrosis relapses located in the frontal region and of those in the molar region (p=0.539). There was also no correlation between jaw sextants and the number of BRONJ relapses (p=0.541, chi square test).

- Interventions prior to necrosis

Dental procedure or invasive intervention had recently occurred at the location of the BRONJ lesion in 82 cases (67.8%). In 85% of the patients this intervention had been tooth extraction. The intervention had occurred 0 to 25 weeks (average: 4.78 weeks) prior to the diagnosis of BRONJ, i.e. when the patient was admitted to our clinic with the suspicion of necrosis or simply with complaints. The length of time passed between the dental intervention and the diagnosis of BRONJ did not show a correlation with BRONJ relapse rate (p=0.443), rate of complete BRONJ healing (p=0.382), or the rate of improvement in staging (p=0.475).

- Steroid treatment

Steroids had been documented as part of prior treatment in 17 cases (14%), 89 patients had received no steroid, and in 15 cases prior medications could not be verified. Preceding steroid therapy did not affect the improvement in staging in response to treatment (p=0.440) or the rate of relapse (p=0.461).

- Chemotherapy

Prior chemotherapy had occurred in 89 cases (73.6%). Preceding chemotherapy did not affect the prognosis of necrosis in terms of improvement in staging in response to treatment (p=0.278) or the rate of relapse (p=0.461).

- Diabetes mellitus

25 patients had prior diabetes mellitus, 5 of whom had a measured blood glucose level higher than 7 mmol/liter. 3 of the 5 patients had necrosis relapse, whereas 2 did not. This ratio is statistically similar that observed in the group of patients with stable blood sugar, wherein 13 did and 7 did not have BRONJ relapse. For this reason, and due to the small number of patients in these diabetic subgroups, we have treated diabetic patients as one group for the purpose of statistical analysis, with no regard to actual blood glucose levels. Fisher's exact test has shown that there is a significant difference in the rate of BRONJ relapse between diabetic and non-diabetic patients (p=0.018). There is no significant correlation between diabetes mellitus and improvement in BRONJ staging in response to treatment (p=0.529). Underlying diseases did not show uneven distribution either (p=0,276, chi square test).

- Anti-angiogenic and anti-EGFR treatment

Three patients had received anti-angiogenic therapy, two thalidomide and one bevacizumab. Two patient had received anti-EGFR treatment. These numbers are too small for statistical analysis.

- Surgical intervention

We performed surgery on 106 of the 121 patients. The highest number of operations that we performed on an individual patient was 9, whereas the average number was 1.92. 98.3% of the patients (119/121) had received prior conservative medical treatment. 9 of our patients (7.4%) healed as a result of conservative treatment, in the rest of the patients healing cannot be confirmed due to death or the lack of long-term follow up. The highest improvement in BRONJ staging score observed after surgery was 3 points, whereas in the worst case the score deteriorated by 1 point; on average it improved by 1.48 points. Conservative treatment resulted in mixed responses: improvement by 3 points and deterioration by 3 points were also observed; on average an improvement by 0.56 points was seen. 69 patients (57%) underwent primary healing, regardless of the type of treatment. Wilcoxon test showed that surgery was superior to conservative treatment when comparing their effects on the changing in BRONJ staging from admission to end of treatment (p=0.0001).

- Anti estrogen therapy

Reliable data were available on 40 out of 50 mammary tumor patients who received hormone therapy. 32 (80%) received anti estrogen therapy and 8 (20%) did not. The mean age of those who received hormone therapy and of the rest of the patients was 63.27 years and 66.41 years, respectively. There was a significant difference between the BRONJ relapse rates of mammary tumor patients who did and of those who did not receive anti estrogen treatment ($p=0.010$, Fisher's exact test). The difference between the BRONJ relapse rate of mammary tumor patients who did and of those who did not receive hormone treatment is significant ($p<0.018$, Fisher's exact test). Patients who had received anti estrogen therapy had a mean BRONJ staging score of 2.00 at admission, while the rest of the patients who had not received hormone therapy had a mean score of 1.85. The most advanced staging scores for the same two groups were 2.40 and 2.32, respectively. The difference between these figures is not statistically significant. There is no statistical correlation between hormone therapy and improvement in staging score ($p=0.313$). When comparing the rate of relapse between breast cancer patients and the rest of the patients, the difference is not significant ($p=0.083$, Fisher's exact test). We assessed bone biomarkers in patients who had received anti estrogen therapy. The T-score on lumbar 2nd to 4th vertebrae was $p=0.034$ whereas it was $p=0.72$ on the femur. Bone mineral density (BMD) on lumbar 2nd to 4th vertebrae showed a significant correlation with hormone treatment ($p=0.022$), and the same correlation was observed on femur ($p=0.023$). In patients who had been treated with anti estrogens, β-crosslaps values are higher compared to the rest of the patients ($p=0.016$, Kruskall-Wallis test). In patients treated with PTH ($p=0.128$), Z-scores ($p=0,102$; $p=0,132$) showed no statistical correlation when tested by Mann-Whitney test.

Laboratory results of patients

65 patients had detailed laboratory test results available (Table 8). Mann-Whitney tests showed that: higher triglyceride levels correlate with BRONJ relapse rate ($p=0.096$), or on the improvement in BRONJ staging as a result of treatment ($p=0.064$). Total cholesterol, LDL and HDL levels also did not have a significant effect on the improvement in BRONJ staging score ($p=0.129$, $p=0.111$, and $p=0.395$, respectively). The effect of serum calcium levels on the improvement in BRONJ staging score was not quite significant ($p=0.063$, staging improved in 44 patients whereas it remained the same in 10 patients with higher serum calcium levels). Serum calcium appeared to have no effect on relapse. The difference in CTX

levels (β-crosslaps values) between patient groups was not quite significant (p=0.054, Kruskall-Wallis test).

The level of parathyroid hormone influenced prognosis in terms of neither relapses (p=0.654), nor improvement in staging score in response to treatment (p=0.208, Mann-Whitney test).

Results obtained with genetic testing

The median age of ONJ patients was 69.5 (range: 60-81) years. There was no significant difference between the ONJ and control groups in terms of age, smoking habits, calcium intake, alcohol and caffeine consumption, and physical activity.

The genotype distribution was 67.4 % normal GG (n=31), 30.4 % heterozygote AG (n=14) and 2.2 % homozygote AA (n=1) genotype in ONJ patients compared to 68.7 % (n=154), 23.6 % (n=53) and 7.6 % (n=17), respectively, in control samples. There was no difference in CYP2C8 rs1934951 genotype or allele distribution between ONJ and healthy subjects by Chi^2-test.

We found a significant effect of CYP2C8 genotype on the localization of ONJ among the affected patients. The occurrence of the mandibular ONJ was significantly higher in case of AG carriers than among subjects with GG homozygous genotype (Chi^2=5.447, p=0.02). In the multivariate logistic regression model, two variables were identified significantly affecting the risk of mandibular localization of ONJ: the length of treatment (B: 0.623, p=0.016) and CYP2C8 genotype (B: -2.947, p=0.015). In this model, the AG genotype was associated with a 19.2-fold increased risk for mandibular ONJ compared to the GG genotype.

Ten mandibular, 1 maxillar and 3 both-jaws localizations were detected among ONJ patients with AG genotype. In case of GG genotype, 17 subjects showed mandibular, 12 maxillar and 2 both-jaws localization. ONJ presence in the mandibular region (76%) was 3.3-fold increased compared to maxilla (23%) in case of AG carriers ($p \leq 0.041$). There was no significant variation of ONJ localization site in patients with GG genotype (mandible 58%: maxilla 42%, respectively). Applying multiparametric PCA test, strong positive correlation was detected between maxillar localization of ONJ and a group of variables including intravenous bisphosphonate application and serum lipid markers (triglyceride, cholesterol, HDL, LDL) (Fig. 2). Mandibular localization of ONJ was correlated positively with another set of variables including serum calcium and 25-OH-vitamin D levels, oral bisphosphonate application and the length of bisphosphonate therapy (mean 4.79 ± 3.48 years). The degree of the disease and the number of recurrences were correlated with the application of hormone-deprivation therapy for breast cancer patients. Also, different variable groups could be

ordered to each CYP2C8 rs1934951 allele (Fig. 2). The presence of A allele (AA and AG genotype) was positively associated not only with mandibular localization of ONJ but also with oral bisphosphonate use, serum calcium and 25-OH-vitamin D vitamin levels. GG genotype was related to maxillar localization of ONJ, intravenous bisphosphonate application and serum lipid markers.

Conclusion

1. There is a significant correlation between the stage at first visit and calculated treatment efficiency based on the highest stage reached during the treatment.
2. The duration of bisphosphonate treatment until the development of the necrosis has a significant correlation with the intravenous/oral medication form. The type of the active agent does not have solely statistical correlation with the duration of the bisphosphonate therapy.
3. We did not found significant result or statistical co-movement neither in the relapse rate of BRONJ nor in the complete healing or in the improvement of the stage when we compared patients on intravenous therapy with patients on oral medication.
4. The treatment of BRONJ is significantly more successful on the maxilla than on the mandible. The development of the necrosis is more likely in the molar region on both jows, than in the frontal area, but this do not influence the prognosis.
5. The level of parathyroid hormone, the previous steroid therapy and chemotherapy do not have any affect on the prognosis of BRONJ.
6. The relaps of the necrosis is significantly different in patient suffering from diabetes mellitus and in non-diabetic patients; diabetes means worse prognosis.
7. Surgery is more effective, than conservative therapy with a strong significance.
8. Hormone therapy, anti-estrogen treatment in the past medical history of patients suffering from breast cancer significantly worses the chance for necrosis healing, comparing with other underlying diseases and patients suffering from breast cancer but not receiving hormone therapy.
9. The level of calcium and triglyceride correlate with the improvement of the stage during the healing. Total cholesterol level, LDL and HDL do not affect the efficacy of the therapy. The level of CTX and ß-crosslaps do not have a homogene spread among patient groups, and they do not affect the prognosis of BRONJ.
10. With the evaluation of single nucleotide polymorphism (SNP) on cytochrome P450-2C gene (CYP2C8) we found that the representation of the necrosis on the mandible is significantly higher in patients with genotype AG and AA compared with genotype GG. There is a significant correlation between mandible localization of BRONJ and

the duration of the treatment, oral medication, serum calcium level and vitamin D level.

New results:

1. We were the first who examined the factors influencing the prognosis of necrosis of the jaw in BRONJ.

2. We were the first who recognized the importance of early treatment: the stage at first visit has a significant correlation with the success of the healing.

3. We were the first who recognized the significant correlation between the success of BRONJ treatment and the previos anti-estrogen hormone therapy, which worses the chance of necrosis healing.

4. We determined that diabetes mellitus worses significantly the relapse rate of BRONJ.

5. We were the first who recognized, during the examination of single nucleotide polymorphism (SNP) on the cytochrome P450-2C gene (CYP2C8), that the presence of allele A (genotype AA and AG) leads to a positive correlation not only with the localization of the necrosis on the mandible but also with the oral medication, level of serum calcium and vitamin D.

Publications

Publication connected to the thesis's topic:

International:

1. Balla B*, Vaszilko M*, Kósa J, Podani J, Takács I, Tóbiás B, Nagy Z, Lazáry A, Lakatos P. (2012) New approach to analyze genetic and clinical data in bisphosphonate-induced osteonecrosis of the jaw. Oral Dis, 18(6): 580-585. *coauthor **IF:2.377**

2. Cséplő K, Vaszilkó M, Sandra S, Csáki G, Sidó L, Szmrtyka Á, Suri Cs. (2009) The use of magnetic resonance therapy. Medical Corps International Forum, 3: 38-41.

3. Vaszilko M, Kovacs E, Restar L, Balla B, Cseplo K, Kosa J, Lakatos P. (2014) Potential significance of antiestrogen therapy in the development of bisphosphonate related osteonecrosis of the jaw. J Craniomaxillofac Surgery, 42(8): 1932-1936. **IF: 2,933**

Hungarian:

1. Vaszilkó M, Németh Zs, Cséplő K, Barabás J, Szabó Gy, Ujpál M. (2007) Could Bisphosphonates be blamed for the cause of not easily treatable osteonecrosis of the jaws? Dental Hirek, 3: 34-36.

2. Vaszilkó M, Barabás J, Szabó Gy, Velich N, Cséplő K, Ujpál M. (2007) Osteonecrosis of the jaws by using Bisphosphonates. Fogorvosi Sz, 100(3): 115-119.

3. Vaszilkó M., Cséplő K., Németh Zs., Barabás J., Ujpál M. (2009) Medication caused osteonecrosis of the jaws. Lege Artis Medicinae, 19(1): 47-49.

4. Vaszilkó M. (2011) Osteonecrosis of the jaws: real and unreal fears. LAM KID, 1(3): 5-14.

5. Vaszilkó M. (2013) Bisphosphonates related osteonecrosis of the jaws. Magyar Orvos, 21(5): 14-17.

6. Restár L, Újpál M, Vaszilkó M. (2013) Difficult cases of BRONJ. Dentál Hirek, 6: 32-35.

Book paragraphs:

1. Vaszilkó M, Cséplő K. The Role of Magnetotherapy in the Treatment of Bisphosphonate Related Osteonecrosis of the Jaws. In: Sandra Sándor (editor), Magnetoterápia. San-Ergonomia Kft, Budapest 2013; 335-338. ISBN: 978-963-08-6435-0

Other publications:

International:

1. Velich N, Vaszilkó M, Németh Z, Szigeti K, Bogdán S, Barabás J, Szabó G. (2007) Overall survival of oropharyngeal cancer patients treated with different treatment modalities. J Craniofac Surg, 18(1): 133-136. **IF: 0.653**

Hungarian:

1. Velich N, Vaszilkó M, Cséplo K, Szigeti K, Németh Z, Barabás J, Szabó G. (2006) Survival prospects of mesopharyngeal carcinoma patients treated primarily with intraarterial chemotherapy. A retrospective study. Orv Hetil, 147(3): 127-31.
2. Ruszin T, Vaszilkó M, Rásonyi-Kovács O, Ujpál M. (2009) A rare case of fatal outcome due to extensive facial trauma caused by dogbite. Fogorv Sz, 102(5): 187-190.
3. Vaszilkó M. (2013) New painkillers in dentistry. Dental Hírek, 3: 48-49.
4. Restár L, Újpál M, Vaszilkó M, Golopencza P, Németh Zs. (2014) Severe inflammations of dental origin in the maxillofacial area. Dental Hírek, 1: 28-30.

Book paragraphs:

1 Cséplő K, Vaszilkó M. Potentials of Magnetotherapy in Oral Surgery. In: Sandra Sándor (editor). Magnetoterápia. San-Ergonomia Kft, Budapest 2013: 315-321. ISBN: 978-963-08-6435-0