The first article that discribe the diphosphonates efects, pirophosphote analogues, was published in 1969. Later they were renamed bisphosphonates and in 1990 the mechanism of osseoues resorbtion inhibition was elucidated.

Bisphosphonates are bone antiresorptive drugs that are given both orally and intravenously. Oral bisphosphonates are used as first-line therapy in osteoporosis, and those with intravenous administration are used in oncological patients (with prostate, lung, breast or kidney neoplasm) who have osteolytic lesions such as bone metastases, are also prescribed for the alleviation of hypercalcaemia from malignancies, but also in patients with primary osteolytic bone diseases (Paget's disease, multiple myeloma) [12].

Bisphosphonates (BF) are drugs widely used in order to reduce the risk of fracture on "pathological bone" by inhibiting bone resorption by osteoclasts and increasing bone mineral density. The first generations of bisphosphonates had oral administration and did not contain nitrogen in the chemical structure. The newer generations of bisphosphonates present intravenous administration, and contain nitrogen in the chemical structure which gives them a higher relative potency. Oral bisphosphonates (Alendronate, Ibandronate) have a lower incidence in the development of osteonecrosis of the jaws compared to those administered intravenously (Pamidronate, Zolendronate).

Since 2003, cases of bisphosphonate-induced mandibular osteonecrosis (BRONJ) have been reported, especially seen in patients on intravenous administration of these drugs. Since then, the number of reported cases has gradually increased, as has the interest of specialists in finding the etiopathogenic mechanism, prevention and treatment modalities. BRONJ is clinically described as an area of the maxillary or mandibular bone exposed to mechanical injury in the oral cavity or to various dental procedures (eg dental extraction), which does not heal, the lesion lasting more than eight weeks in the patients receiving it or in the patients who have been treated with bisphosphonates, and an essential condition for this statement is the absence of irradiation of the cranio-facial region. In the specialized literature there are described cases of osteonecrosis arising after the treatment with bisphosphonates administered for a long time (over 2 years) for the underlying pathology (osteoporosis or primary and secondary bone malignancies). Of the bones of the human skeleton, most cases of osteonecrosis induced by bisphosphonates are related to the bones of the maxilla and mandible, with a higher prevalence in the jaw.

Recently, cases of osteonecrosis of the jaws were noted in patients treated with other types of antiresorptive and antiangiogenic agents, such as Denosumab, a human IgG2 monoclonal antibody with different mechanism of action and chemical structure of bisphosphonates. Through successive reviews of this pathology, the American Association of Maxillofacial and Oral Surgery (AAOMS) publishes the updating of the BRONJ nomenclature (osteonecrosis of the jaws induced by bisphosphonates) with MRONJ (the osteonecrosis of the jaws induced by the antiresorptive and antiasurgical medicine) in patients receiving another antiresorptive agent, than that of the bisphosphonate class.

MRONJ is a multifactorial pathology with insufficiently clarified etiopathogenesis. The proposed hypotheses are alteration of bone remodeling by suppression of bone resorption and inhibition of angiogenesis, repeated microtrauma, inflammation and infection of the mucosa of the oral cavity and teeth, vitamin D deficiency, as well as the drug toxicity induced by the soft tissue by the antiresorptive agents. Some specialized studies describe that invasive dento-alveolar procedures, dose and the route of intravenous administration are the most important risk factors in the development of MRONJ. Antiresorptive and anti-angiogenic drugs affect osteoclast function, implicitly with inhibition of bone resorption and remodeling. In the alveolar bone, the higher rate of bone remodeling may be an explanation for the fact that the most affected bones are the jaws. In this precarious field, performing a dental extraction precipitates the pathophysiological phenomena because this process is accompanied most often by inflammation and local infection, in addition to the rich commensal flora existing in the buccal cavity with the delay of healing the created tissue defect. Moreover, it is known that MRONJ also inhibits angiogenesis with vascular deprivation of bone and development of avascular necrosis. In the appearance of MRONJ, various risk factors such as age, sex, genetic factors, type, duration, dose and mode of administration of the antiresorptive and antiangiogenic agent, smoking, diabetes, corticosteroid therapy, neoplasms are studied. Local risk factors, such as poor oral hygiene (dental caries, periodontitis), exostoses, trauma, periodontal inflammatory diseases, dento-alveolar surgery are also considered in the etiopathogenesis of MRONJ.

The MRONJ diagnosis includes the signs, symptoms, imaging examinations and the pathological report. AAOMS has established several criteria that must be met for MRONJ: therapy with antiresorptive and antiangiogenic drugs, exposed bone surface in the oral cavity that persists for more than eight weeks, presence of intraoral or extraoral fistula, without a

history of irradiation of the head and neck, and no metastatic bone disease of the maxilla or mandible. Imaging investigations do not provide specific features for MRONJ, but they have an important value in detecting risk factors that lead to the appearance of MRONJ, detecting and evaluating the extent of osteolytic lesions and evaluating the response to the treatment. In stage 0 (called by some authors "preclinical stage") and in stage 1, panoramic and retroalveolar radiography provides information on periodontal disorders and detects changes in bone resorption. In stages 2 and 3 of MRONJ, conical beam computed tomography (CBCT), computed tomography (CT), nuclear magnetic resonance (MRI), bone scintigraphy, positron emission tomography with 2-deoxy-2- [fluorine-18] fluoro- D-glucose integrated with computed tomography (FDG PET / CT) provides useful information in managing therapeutic attitude and post-treatment evaluation of MRONJ.

Another aspect in completing the diagnosis of MRONJ is the histopathological examination. Regardless of the type of antiresorptive medication (bisphosphonate, Denosumab), the duration of treatment, the dose administered and the underlying conditions of the patients, the histopathological changes are the same: avascular necrosis, inflammation and bacterial colonization. Osteocytes necrosis is observed microscopically at 24-72 hours of anoxia, although the necrotic process begins after only 2-3 hours of oxygen deprivation of bone tissue with marked acidophilic cytoplasm and pyknotic nuclei, the final step being the loss of osteocytes when "optically empty" gaps appear in the extracellular matrix. Blood vessel obliteration produces avascular necrosis followed by inflammation and bacterial colonization. These pathological aspects were identified in both MRONJ and ORNI (osteo-radio-necrosis) and osteomyelitis. Although there are opinions in the literature that the architecture of the trabeculae is particular in MRONJ, the extracellular matrix is richer and the osteoclasts are absent, these aspects were contradicted by skeptics with the demonstration that osteoclasts also exist in MRONJ if they are meticulously searched on many microscopic fields. It is assumed that in ORNI, the vascular lumen narrows due to the marked fibrosis induced by the soft tissue radiation and in MRONJ the vascular lumen obliteration occurs by hypertrophy and cellular hyperplasia with the formation of a perivascular sleeve. It is also mentioned that the bacteria present are of the type Actinomyces israelii, an idea supported by Hansen in a study of 45 patients with MRONJ and ORNI morphologically highlighted and tested by the polymerization chain reaction (PCR). Indeed, another idea is how to place bacterial colonies in microscopic osteomyelitis differentiation of MRONJ. Marx and Tursun argue that in osteomyelitis bacterial colonization is in the bone

marrow spaces, whereas in MRONJ bacterial colonization occurs on the bone surface, a fact not confirmed by Shuster et al. with the explanation of the marked fragmentation of the biopsy fragments and the impossibility of mentioning with certainty the bone surface and the medullary spaces. The three lesions are practically impossible to differentiate only on microscopic criteria, integration in the clinical and imaging context playing a paramount role.

Aim of the study

This study summarized the epidemiological data, risk factors, diagnosis and treatment mode of MRONJ as well as the role of preventive measures in the development of MRONJ.

Material and methods

Two main elements were followed in the selection of cases: therapeutic indications (osteoporosis or neoplasms) and type of drug (BF and non-BF).

The study was retrospective and included 40 patients with osteonecrosis of the jaws induced by bisphosphonates, diagnosed and treated in the Clinic of Maxillofacial Surgery of the City Emergency Clinical Hospital of Timisoara, over a period of 4 years. Patients were selected on the basis of the AAOMS criteria and the following characteristics were fulfilled:

- patients under current administration or in the recent history of antiresorptive and antiangiogenic medications
- exposed bone surface in the oral cavity that persists for more than 8 weeks
- absence of irradiation history in the head and neck, but without metastatic bone disease of the jaw or jaw

After applying the AAOMS criteria, 2 patients were excluded from the study because they had radiotherapy in the head and neck, so that the final group is composed of 38 cases. For all patients we noted that working variables age, sex, location of osteonecrosis, risk factors (dental extraction, comorbidities), therapeutic indications of bisphosphonates (osteoporosis, neoplasia), dose and duration of administration of bisphosphonates, presence of metastases, other metastases location of the maxilla or mandible) and the history of radiotherapy and chemotherapy.

Statistical analysis was performed with Microsoft Excel and Statistical Package for the Social Science (SPSS) ver. 17.0, descriptive statistics for numerical variables. The Mann-Whitney test and the chi-square test were used to compare the data.

Surgical treatment is a therapeutic method used in all patients in the study, and the surgically resected necrotic bone is introduced into a 10% buffered formaldehyde solution and sent to the Pathological Anatomy Service

within the same hospital and undergoes several stages of treatment: fixation, macroscopic examination, descaling, tissue processing using histological technique to obtain paraffin blocks and microscopic examination. Decalcification is the process to which the bone biopsy pieces are subjected in order to remove the hydroxyapatite crystals from the mineralized bone slices to obtain histological sections without processing artifacts. The sectioning of the paraffin blocks is carried out with a Leica RM2245 semi-automatic rotary microtome, obtaining sections with a thickness of 3 μm. The staining techniques used in this work are the usual hematoxylin-eosin staining and PAS histochemical staining technique. The last step in making the histological slides for examination under optical microscopy is to mount a Canada balsam blade over the sections that are stretched on the blade and colored. The microscopic examination of the blades is performed with the Leica DM750 microscope, and the images in the paper are processed with the Leica DM Share system.

Results

MRONJ appears more frequently in men than in women, with an average age of 64.98 years. Oncological patients develop osteonecrosis more often than osteoporotic patients due to the dose of bisphosphonates (BF) administered, which is 10-12 times higher in the case of malignancies. In both men and women, the site of choice for osteonecrosis development is the mandibular bone, with cases being recorded in approximately equal proportions for the maxillary bone, or even affecting both bones.

Of the total group of 38 patients, a slight predominance is observed in women (46% men and 54% women), and the age of the group is between 47 and 81 years (mean age = 64,98; standard deviation - 8,056, with variation of 64,902).

The predominant age group was in decade 6 of life (38%), followed by decade 5 of life (32%) and decade 7 of life (30%). Within the group, there were two ages classified as extremes (47 years, respectively 81 years, both being men).

The predominance of the male sex was in the age group 60-69 years. The predominance of the female sex was in the age group 70-80 years. In the age group 50-59 years, there were no significant differences between the two sexes.

Osteonecrosis of the maxilla and mandible induced by antiresorptive and antiangiogenic medication developed in patients with neoplastic diseases (76%) was more frequent than that developed in osteoporotic patients (24%), in the studied group. There was no significant association between MRONJ, osteoporosis and neoplastic diseases with the value $p = 0.15$ ($\alpha = 0.05$, 5%).

Osteoporosis is predominantly female (87%) compared to male (13%), the $\chi 2$ test (chi-square) is statistically significant ($p = 0.006$, $\alpha = 0.01$). In contrast, the highest proportion is malignant in both sexes with a slight

predominance of the male (62% men and 38% women). Regarding the cases of MRONJ associated with other conditions (cardiovascular diseases, diabetes, rheumatoid arthritis, chronic osteitis, and hypothyroidism) were more numerous in the female sex (71% women and 29% men).

MRONJ appears in osteoporotic patients predominantly in the 7th decade of life (57%). Neoplastic patients develop MRONJ more frequently in the decade of life (50%), with 2 exceptions of extreme ages (47 years, respectively 81 years, both being men), and the share of patients with other conditions is higher in the decade 5 of life (57%).

The presence or absence of bone metastases does not influence the appearance of MRONJ. Of the examined group, 52% are neoplastic patients with bone metastases and 48% are neoplastic patients without bone metastases who have developed osteonecrosis.

The proportion of women / men (42% and 58% respectively) with neoplastic diseases without bone metastases and by age groups is 21% (group 50-59 years), 43% (group 60-69 years) and 36% (group 70-80 years). In decade 5 and 6 of life men predominated (decade 5 registering only male patients), and in decade 7 women predominated.

Within the neoplastic disease with bone metastases, MRONJ appears in approximately equal proportions (54% men and 46% women), the statistical difference being insignificant ($\chi 2$ test, $p = 0.588$)

Regarding the distribution by age group of MRONJ in neoplastic patients with bone metastases, the proportions obtained are 31% in decade 5 and 6 of life, respectively 38% in decade 7 of life, the statistical difference being insignificant ($\chi 2$ test, $p = 0772$)

Osteonecrosis of the jaws associated with the administration of bisphosphonates appears in both sexes at 2 years from the beginning of the treatment, with a slight predominance in men. For large time intervals (5 years, 10 years, respectively 13 years), only female cases were registered.

The patients of all 3 age groups studied developed osteonecrosis at 2 years of treatment with BF, and in the rest of the time intervals the number of registered cases is insignificant, compared with the number of cases registered at 2 years of treatment administration.

Gender distribution of maxillary bone damage is approximately proportional, with no significant differences between men and women. Of the total group, osteonecrosis of the mandible is equally present in both men and women (72%), osteonecrosis of the jaw appears with a slight predominance in men (22%) compared to women (21%), and the damage of both bones is recorded with a slightly higher prevalence in women (7%) than men (6%).

Osteonecrotic lesion of the mandible predominates over all three age groups, followed by the maxillary bone, with visibly lower values. Both the jaw and the mandible are affected in only 2 cases within the decade of life (with one extreme, 47-year-old man who has developed osteonecrosis on both jaw bones).

Most patients included in the study (30 cases) had MRONJ with predominantly unilateral mandibular localization, as opposed to the maxillary localization of MRONJ found in 7 patients (4 patients with right maxillary osteonecrosis and 3 patients with maxillary osteonecrosis) and one case left was bilateral. The mandibular / maxillary osteonecrotic affect had a 5: 1 ratio, with no significant differences between the location of the osteonecrosis and the sex of the patients ($\chi 2$ test, $p = 0.134$).

The distribution of MRONJ localization did not show significant differences by age groups ($\chi 2$ test, $p = 0.725$).

Patients presented the following signs and symptoms: pain in the jaw area, bone attachment exposed in the mouth cavity covered by yellow-white exudate, mobile teeth, erythema, ulcers, fistulas, abundant purulent discharge, suppuration and gingival infection. The symptoms occurred in all patients after dental extraction, the edentulous region exposed the maxillary bone with the formation of a seizure, but the history of local trauma was not a stand-alone condition in the selection of cases. In patients with infected necrotic bone attachment, the appearance of intraoral and extraoral fistulas is note.

Zometa (4 mg zoledronic acid solution) was administered to 27 patients with MRONJ (71%), intravenously, once a month, 6 patients (16%) were treated with Boniva (150 mg ibandronic acid tablets). Orally administered once a month, 4 patients (11%) treated with Fosamax (70 mg alendronic acid tablets) orally once a month and one patient (2.6%) treated with Pamired administered once a month (disodium pamidronate) 90 mg / 250 ml solution).

Patients treated with Zometa developed maxillary osteonecrosis within 3 months - 3 years with a mean of 32.11 months, the highest incidence being 2 years after starting the intravenous treatment.

Patients treated with Fosamax developed maxillary osteonecrosis at 1 year and 2 years after starting oral treatment, with an isolated case 13 years after starting treatment.

In the 6 patients treated with Boniva the period from the initiation of the treatment to the onset of osteonecrosis is between 1 year and 10 years, with an average of 40 months.

The patient treated with Pamired developed maxillary osteonecrosis 5 years after the initiation of the treatment.

The histopathological examination shows in all the patients in the studied group bone fragments with necrotic lamellae, acute inflammatory infiltrate consisting of neutrophilic granulocytes, chronic inflammatory infiltrate consisting of lymphocytes, plasmocytes and macrophages, microbial PAS positive colonies and epithelial exculcations.

The necrosis is ischemic, the ischemic lesion being microscopically revealed by the loss of osteocyte nuclei, resulting in optically empty osteocyte gaps as a result of severe ischemic episodes. It has a mosaic pattern printed by osteonecrotic areas with optically empty gaps intricate with bone areas that still contain viable osteocytes in osteoplasts. Necrotic bone trabeculae are thick, with areas of cementation, having no connection with the bone marrow,

to which are added changes of bone resorption, quantitative rich extracellular matrix, hypertrophied osteocytes, small Havers channels and medullary spaces reduced in size. These aspects may be involved in the occurrence of avascular necrosis by suppressing the source of nutrition by devitalizing the bone blades.

Regarding osteonecrosis abundance, there is no difference between osteoporotic and neoplastic patients. In both cases, the biopsy specimens examined are extremely fragmented, of small size, and the extent of osteonecrosis is subjective, being present in extremely variable proportions, with the same mosaic pattern present in all the studied cases.

Most MRONJ cases occur in neoplastic patients (72%) with solid extrous tumors (58%) and primary bone tumors (14%), followed by osteoporosis (28%).

Discussion

From the histopathological point of view, osteonecrosis of the jaws is an ischemic type avascular necrosis, having associated actinomycotic colonization. Heinsen et al. shows by PCR testing that of the species of the genus Actinomyces, Actinomyces israelii is detected in a significant percentage in both MRONJ and ORNI. The detection of Actinomyces israelii and not of other bacterial or fungal forms appears as a complication of the oncological patients due to the antitumor treatment. One possible explanation is that actinomycosis is diagnosed more often in patients who are receiving radiotherapy or who are given bisphosphonates due to the more detailed clinical follow-up, which is not the case for sporadic actinomycosis without associated neoplasia. At the level of the mandible and maxilla, Actinomyces Israeli colonies tend to occupy the bone marrow channel and adhere to the bone matrix either in the presence of inflammatory cells (predominantly neutrophilic granulocytes and plasmocytes) or in the absence of them. The histopathological aspects of osteonecrosis are the identification of the devitalized bone due to the obliteration of the vascular lumen, the appearance of inflammatory granulation tissue, inflammatory cells and actinomycotic colonies. The main role of bone biopsy, especially in oncological patients, is to confirm or deny a possible proliferation of malignant tumor cells in the bone with the clinical appearance of necrosis (thus pathologically speaking, tumor necrosis). After the histopathological exclusion of the malignancy and the description of the osteonecrosis modifications, the problem of MRONJ differentiation from ORNI is raised.

The challenge of the pathologist, in this type of bone pathology medically induced by the bisphosphonates or as a result of the different oncological treatment procedures, is to differentiate MRONJ from the infected osteoradionecrosis, considering that the majority of the oncological patients

receive treatment with bisphosphonates or have a history of radiotherapy. The morphological descriptive studies report the important role of the obliteration of the blood vessels and the colonization of the ischemic area with mycotic hyphae, interpreted as an opportunistic infection, both in the patients with MRONJ and in the patients with ORNI as a main morphological feature in the occurrence of avascular necrosis. This histopathological criterion is inconsistently observed in both types of disorders, but not identified in the patients who undergo other forms of oncological treatment. The same author, reports a year earlier by another study, that the histopathological changes in the vascular obliteration process are different. In ORNI the vascular lumen is obliterated by thickening and hyalinization of the collagen bundles from the vascular wall with numerical reduction of smooth muscle cells and fibrosis and surrounding tissue fibrosis, whereas in MRONJ the vascular lumen obliteration occurs through hypertrophy and hyperplasia, which is due to cell hyperplasia concentric, like a sleeve, narrowing and even completely obliterating the vascular lumen.

However, typical vascular histopathological changes are absent in order to make a clear difference between the two entities of osteonecrosis. Another histopathological criterion observed was the pattern of bone necrosis. In patients with MRONJ, osteonecrosis has a mosaic, non-uniform pattern, in contrast to ORNI in which the bone presents extended necrosis, sometimes being completely necrotic on the examined material. The mosaic pattern is printed by the appearance of the necrotic areas with "optically empty" osteocyte gaps interspersed with the bone blades that contain viable osteocytes in the osteoplasts. An impediment to this diagnostic feature is the amount of biopsy material received for examination or examination of a bone attachment, due to limiting the extent of bone necrosis.

The treatment options of MRONJ are surgical treatment and conservative treatment. Many published studies support conservative treatment to the detriment of surgery. AAOMS 2014 and a Japanese study recommended conservative treatment, however a disadvantage of it is that the necrotic bone does not regenerate spontaneously, with the patient's quality of life decreasing. Several recent studies suggest that surgical treatment would be more effective in resolving MRONJ. Performing the surgery as close to the time of osteonecrosis development, with the adequate resection of the necrotic areas and healing per primam would be a recommended method in the MRONJ treatment. Both low-dose and high-dose patients have a much higher complete cure rate than patients receiving conservative treatment. Several alternative therapies are described as treatment options (besides conservative

and surgical) such as: platelet rich plasma administration, parathyroid hormones, Er laser therapy, Cr: YSGG, hyperbaric oxygen therapy, bone morphogenetic protein application. However, these alternative therapies cannot be considered as unique treatment options, and a closer study is needed.

To reduce the incidence of MRONJ, preventive measures such as: complete examination of the oral cavity including radiological examination, maintenance of proper oral hygiene through patient education, periodic medical checks, removal of dental bacterial plaque and periodontal maintenance are required. Treatment of cavities or other dental problems is preferable before starting antiresorptive therapy. However, if the patient is already under treatment with antiresorptive and antiangiogenic drugs, antibiotic therapy before and after dental extraction as well as the use of plasma rich in growth factors (PRGF) is recommended. The primary prevention of MRONJ is done first by identifying the local risk factors and eliminating them by planning the dental interventions before starting the antiresorptive and antiangiogenic treatment. Both the treatment and the prevention of MRONJ require a multidisciplinary team with good collaboration between dentists, clinicians of different specialties, oncologists and maxillofacial surgeons to optimize the use of bisphosphonates and Denosumab with an adequate assessment of the risk of MRONJ.

Conclusions

- MRONJ is an important complication of patients treated with BF that occurs after minimal dentoalveolar surgery (dental extraction) but may also occur spontaneously
- patients with BF administered intravenously have a higher risk of developing MRONJ than those treated with oral BF, with osteonecrosis occurring within a shorter time from the beginning of the intravenous treatment than with the oral administration
- MRONJ treatment is managed according to symptomatology; asymptomatic patients require no interventions other than mouthwash with antibacterial solutions and clinical monitoring, and symptomatic patients receive analgesic, antibiotic, and debridement or resection surgery
- the main prophylactic measure to be followed is the optimization of the dental health, the improvement of the patient's education regarding the dental health status and the carrying out of periodic preventive dental consultations.