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Anticancer therapy has multiple, sometimes life-threatening side effects, and their influence on bone is not seen as important. Data have been published confirming the existence of side effects chemotherapy has on bone, which affect patients' quality of life. They influence a bone tissue not only in a direct way, but also when suppressing the activity of gonads. We have no information on the impact of drugs on bone belonging to the "targeted therapies". There are, however, some attempts to create antibodies that target proteins involved in bone physiology. Relatively well known is the impact of anti-cancer hormone therapy on bone metabolism. The most commonly used drugs in this type of therapy are: analogues of luteinizing hormone-releasing hormone (LHRH), selective oestrogen receptor modulators (SERMs), aromatase inhibitors and antiandrogens. A group of preparations particularly connected with this issue is the bisphosphonates, entering into interaction both with bone cells and colonizing tumour cells.

**Key words:** bone tissue, chemotherapy, targeted therapy, hormonal treatment, bisphosphonates.

# Bone tissue as the site of action of oncological drugs

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Bone tissue is of special importance in the pathophysiology and the clinical course of neoplastic diseases. Metastases to bones do not pose a direct threat to the patient's life, yet they markedly lower the quality of life and may be associated with complications leading to severe disability and, in consequence, to shortening of the patient's life. In the clinical picture of certain neoplasms, such as multiple myeloma, prostate cancer, thyroid cancer or sometimes breast cancer, the dominating symptoms are those related to bone infiltration. On the other hand, there are also effects of the oncological treatment on the bone – both those concerning the physiological bone cycle (of increasing importance with the observed prolongation of patient life) and the interactions between the neoplastic cells and bone cells. Below, we present a literature review on this subject. The issue of steroid therapy's effects on bones is not addressed here as the multitude of usages of this drug class resulted in the subject being already extensively discussed in other publications.

## Chemotherapy

Adverse effects of chemotherapy concerning bones may in a longer-term perspective lead to health complaints markedly lowering the quality of life, particularly in patients with good prognosis, even more so because this approach is often combined with different hormone and radiation therapies. As early as in 1965, a report was published presenting the results of a study on the effects of methotrexate (MTX) treatment on calcium metabolism, demonstrating elevated levels of this element in urine and stool, its lowered levels in blood serum, and indirectly its increased bone resorption [1]. The above data have been reflected in a clinical setting, in patients treated with MTX for acute myeloid leukaemia, where there have been observed pain and difficult uniting of fractures during the course of treatment and remission upon its completion [2-8]. Although the high doses of steroids have been of significance, the cytostatic agents have been deemed to play an important role here. Reduction in the bone mineral density (BMD) has been observed as well as qualitative features of osteopenia, seen in radiograms as shrinking of the cortical bone, lower Singh index, more distinct (due to thinning) trabeculae or – in contrast – lack of the trabecular structure in imaging. Similar reports have been published based on observations in patients treated with MTX for collagenoses [9-17]. Apart from clinical symptoms, there was also described in this group of patients inhibition of osteoblast activity [13], as well as a significant reduction in osteocalcin synthesis by osteoblasts [14] – seen in both of the above studies after a weekly administration of MTX at low doses. Those data are in agreement with the observed *in vitro* effects of MTX on osteoblasts, where this cytostatic agent has proven to be a potent inhibitor of osteoblast proliferation, affecting bone metabolism and regeneration [15, 16]. On the other hand, no effects of the drug on osteoblast differentiation have been found [16]. The MTX potential for directly affecting bones is indicated by its high level in the cortical and trabecular bone one day after its intramuscular administration [17]. Katz and colleagues [18] have

reported a significant reduction in the parathyroid hormone and total calcium levels in the blood in response to low MTX doses, suggesting a direct influence on secretion of this hormone. However, no significant changes in the blood levels of free calcium and osteocalcin or in the BMD as measured by double-photon absorption (DPA) have been detected. Meanwhile, osteopathy was found in children with brain tumours treated with MTX at a cumulative dose of 20 g/m$^2$ to 135 g/m$^2$, suggesting that the intracellular accumulation of this cytostatic agent and its polyglutamate derivative might have caused this [19]. The radiological and scintigraphic evaluation of bones of 87 patients with osteosarcoma [20] revealed symptoms, present in 8 of them, analogous to those accompanying leukaemia treatment. Those were as follows: osteopenia, pseudocalcification zones and multifocal nature of the process. The bones most commonly affected by the above complications were the humeral, calcaneal, pubic and tibial ones. A significant difference in age was observed between the patients with bone lesions (mean age: 9.2) and those without them (mean age: 14.9): $p < 0.001$. No direct effects of the dose size on osteopathy were seen. Yet another reported observation was the difference in BMD measurement results at 6 and 9 months after the completion of chemotherapy for osteosarcoma with doxorubicin and cisplatin in combination with a low (750 mg/m$^2$) or high (7.5 g/m$^2$) MTX dose, in comparison with the control group. A significant reduction in BMD in the high-dose group indicates a correlation between osteopenia and the dose. The absence of detectable effects of low drug doses on bones may stem from their intensive growth in patients with osteosarcoma, a neoplasm occurring more often in tall individuals. On the other hand, the presence of distinct osteopenia at high MTX doses in sites where the trabecular structure dominates is in agreement with the fact that this bone type is particularly sensitive to the effects of other osteopenia-inducing factors as well, such as oestrogen deficiency or steroid therapy [21]. Of note are the adverse effects on the bones in paediatric patients of ifosfamide (IFO) and the chemotherapy regimens including it. The renal tubule damage occurring during this drug use leads to impaired phosphate resorption, and in consequence to metabolic acidosis and to phosphate and calcium loss with urine [22]. The result of this may be the BMD reduction [23] and osteomalacia [24, 25] observed in children treated with IFO. Chemotherapy with IFO may also be associated with lower osteocalcin levels in blood serum [26]. An important factor causing osteopenia in females who underwent chemotherapy is the inhibition of ovarian activity. This is confirmed by studies conducted on patients treated for Hodgkin's lymphoma – there has been observed a statistically significant reduction in the density of trabecular and cortical bone in cases of ovarian failure, as compared with patients retaining normal ovarian function [27, 28]. The effects of chemotherapy on gonads have also been implicated in the reduction of BMD in males treated for the same condition, as suggested by the correlation between BMD and testosterone levels [29]. Impaired gonad function occurred more often in patients treated for Hodgkin's lymphoma as compared with the group with non-Hodgkin's lymphoma, due to the difference in the frequency of radiation therapy and procarbazine use [30] – however, reduced BMD is also found in females treated for non-Hodgkin's lymphoma. This is believed to be caused not only by high steroid doses but also by cytostatic agents [31]. Reduced BMD seen in patients undergoing chemotherapy for breast cancer at pre-menopausal age should also be correlated with ovarian failure. In the study by Bruning [32], chemotherapy was associated with higher incidence of premature menopause and significantly reduced BMD as compared with the control group of female patients at pre-menopausal age after mastectomy and not undergoing chemotherapy. In the ZEBRA study, an improvement in bone mineralization was not observed even after three years of completion of adjuvant chemotherapy according to the CMF (cyclophosphamide, methotrexate, 5-fluorouracil) regimen [33]. Its effects in the bones of females at pre-menopausal age treated for breast cancer have also been found in radiological structure examination [34]. The reduction in trabeculae thickness and the relative field occupied by trabeculae correlates with the clinical and experimental observations of the inhibitory effect of MTX on osteoblast proliferation and activity. The mineralization impairment is reflected in the reduction in the mean radiological bone density. The comparison of the results for a group of patients after chemotherapy with the control group of similar age and with patients with clinically manifesting senile or pre-menopausal osteoporosis indicates that this group is similar in terms of radiological structure to females at an advanced age. Thus, it is not surprising that similar symptoms are caused by high-dose chemotherapy with bone marrow or circulating progenitor cell transplantation [35-38]. Following treatment with chemotherapy only, 30% of female patients retain their ovary function [35], while when used in combination with radiation therapy (total body irradiation – TBI) only 10% of patients do so [36]. Of significance here are high doses of busulfan [37]. Castaneda and colleagues have observed that osteopenia occurs in 33% and osteoporosis in 18% of patients treated with bone marrow transplantation [38].

## Oncological hormone therapy

As mentioned above, one of the crucial mechanisms causing bone metabolism disorders in the course of oncological treatment is the inhibition of gonad function. This occurs not only during cytotoxic treatment, but it mainly accompanies the hormone therapy used in treating hormonedependent neoplasms – breast cancer and prostate cancer. In patients of both genders, sex hormones play a crucial role in the maintenance of optimal bone mass [39, 40]. Its volume depends on the balance between bone formation and bone resorption. In recent years, the effects of oestrogen on those processes have been studied in great detail [41]. The regulator of bone remodelling is a signalling pathway, the main components of which are osteoprotegerin (OPG), the receptor activator of nuclear factor κB (RANK) and its ligand (RANKL) – a protein from the tumour necrosis factor (TNF) family of which OPG is also a member [42]. The latter is produced by osteoblasts and its progeni-

tors, and it is the main activator of osteoclast differentiation and maturation, influencing their activity, function and life span [43-45]. The binding of RANK with its ligand initiates a signalling cascade in osteoclasts and leads to the formation of a mature and fully functional resorption cell. Osteoprotegerin, produced, among others, in osteoblasts, is a soluble form of RANK and is capable of binding the ligand, by which it inhibits the RANK-RANKL binding and the process of osteoclast maturation. The osteoclast maturation and activity, and, thus, also the intensity of the bone resorption process, depend on the balance between RANKL and OPG. Oestrogens stimulate the OPG synthesis in osteoblasts, which leads to lowering of the RANKL level. The result is inhibition of bone remodelling and resorption, and stimulation of bone formation. In cases of oestrogen deficiency, the RANKL level goes up and the sensitivity of RANK to its ligand increases, which leads to the stimulation and hyperactivity of osteoclasts, accompanied by an increase in bone resorption and calcium release from bones. Apart from influencing the OPGRANKL-RANK pathway, oestrogens also stimulate the synthesis of transforming growth factor β (TGF-β) and insulin-like growth factor 1 (IGF-1) [46, 47]. The effects of androgens on bone metabolism are also dependent on TGF-β and IGF-1, but they are associated to a greater extent with testosterone conversion into oestradiol and the activity of the latter. The testosterone-oestradiol conversion is effected through aromatisation, and the aromatase level as well as the oestrogen receptor function are crucial for bone metabolism in males. There have been cases reported of pathological bone mass loss in males with oestrogen receptor mutation causing its inactivation and in those with genetically determined aromatase deficiency [48, 49]. Bone remodelling caused by oncological hormone treatment is similar to that seen in the course of natural menopause, with a rapid loss of bone mass (by approx. 2-3% each year). The loss affects mainly the trabecular bone (2030% in 10 years), while to a lesser extent the cortical bone (5-10% over the same period).

## Hormone therapy in breast cancer

The main points of hormone therapy in breast cancer are the inhibition of ovary function, lowering the levels of circulating oestrogens, and the inhibition of cellular activity. To this end, analogues of luteinizing hormone-releasing hormone (LHRH), surgical oophorectomy, oestrogen receptor modulators and inhibitors of aromatase (the enzyme catalysing the conversion of androgens into oestrogens) are used. LHRH analogues are widely recognised drugs used in pre-menopausal patients. These derivatives of the natural peptide (characterised by increased activity and longer half-life as compared to the natural hormone) block the LHRH receptors on pituitary cells and thus lead to decreased gonadotropin secretion and pharmacological castration. It has been shown that after 6 months of such treatment there occurs a marked reduction in spinal BMD [50]. Tamoxifen belongs to the class of selective oestrogen receptor modulators (SERMs). In breast cancer cells, it acts as an antagonist of oestrogens, while in bones and endometrium it acts as a weak oestrogen (agonist activity). Still, it exerts a dual clinical effect, depending on the patient's hormone status. In premenopausal females, it leads to BMD loss [51], while in post-menopausal patients the use of tamoxifen results in a significant increase in the bone density, as measured in the lumbar spine region [52, 53]. On the other hand, it has not been found that the use of tamoxifen in breast cancer prophylaxis significantly lowers the risk of fractures. The NSABP P-1 study has demonstrated a 21% reduction in the risk of fractures in a group of patients aged above 50 and receiving tamoxifen as a preventive measure, in comparison with the group receiving placebo; yet this difference did not prove significant (HR 0.79, 95% CI: 0.60-1.05) [54]. Meanwhile, the IBIS-1 study, evaluating tamoxifen in breast cancer prophylaxis in groups of pre- and post-menopausal females, did not show any differences in the fracture incidence [55]. Although certain aspects of treatment with aromatase inhibitors remain to be elucidated, their use has been increasing steadily in the last decade. These preparations are divided into non-steroid (letrozole and anastrozole) and steroid ones (exemestane). Their mode of action consists in reversible (non-steroid ones) or irreversible (steroid ones) inhibition of aromatase, the enzyme catalysing the testosterone-oestradiol conversion. This leads to a lowering of oestrogen levels in circulating blood in post-menopausal females by approx. 98% [56, 57]. This mechanism is responsible for adverse effects different to those seen in the treatment with tamoxifen. Numerous clinical studies have shown that tamoxifen results in lowered levels of bone metabolism markers and in higher BMD, while aromatase inhibitors have a reverse effect here. A direct comparison of three third-generation aromatase inhibitors has revealed that they have a similar effect on the levels of bone metabolism markers [58], although some researchers suggest that the use of exemestane results in a smaller loss of bone mass as compared with the use of letrozole or anastrozole [59]. This may stem from the steroid structure of exemestane – its main metabolite, 17-hydroxyexemestane, possesses internal androgen activity, thus providing a protective effect. Additional data may be derived from the MA.27 – bone protocol study. In the studies concerning adjuvant treatment (ATAC, BIG 1-98, IES), the use of all of the above-mentioned drugs resulted in an increased risk of fractures as compared with the use of tamoxifen [60-62]. The absolute differences have not been large (0.8-4%), yet proved significant. In the ATAC study, it was found that the use of anastrozole, as compared with tamoxifen, resulted in a significantly higher incidence of osteoporosis with complications in the form of fractures (11% vs. 7.7%, $p < 0.0001$), and that the incidence of adverse effects increased with time [61]. Also, the other non-steroid aromatase inhibitor – letrozole – caused fractures more often than tamoxifen (5.8% vs. 4%, $p = 0.0006$) [60]. In studies with sequential (following prior application of tamoxifen) use of aromatase inhibitors (IES, MA.17), the differences were smaller (in MA.17 the difference was not significant), which might be related to the protective action of tamoxifen on the bone density [62, 63]. The use of letrozole following 5-year treatment with tamoxifen (MA.17) resulted in a higher incidence of osteoporosis and fractures as com-

pared with placebo [respectively: 8.1% and 6.0% for osteoporosis ($p$ = 0.003), and 5.3% and 4.6% for fractures] [64, 65]. In the IES study, it was found that the use of exemestane, as compared with tamoxifen, caused a higher incidence of osteoporosis (7.4% and 5.7%, respectively, $p$ = 0.05) and factures (3.1% and 2.3%, respectively) [62]. Also the ABCSG 8 and ARNO 95 studies demonstrated a higher percentage of fractures in the group of patients treated sequentially with an aromatase inhibitor (anastrozole), as compared with the treatment with tamoxifen only (2.4% vs. 2.1%), but the difference was not significant [66]. Summing up: the data from the abovementioned randomised trials indicate unambiguously that the use of aromatase inhibitors in adjuvant treatment is associated with a higher risk of osteoporosis and fractures. When analysing the risk of such complications in patients treated with aromatase inhibitors, the coexistence of other risk factors should be taken into account. These include: baseline T-score < –1.5, age > 65, body mass index (BMI) < 20, positive family history for hip fractures, oral steroid therapy for over 6 months, and tobacco smoking (at present or in the past). The importance of all the above factors has been confirmed in large clinical trials conducted on a population of healthy post-menopausal females [67-72].

### Hormone therapy in prostate cancer

Antiandrogen treatment has found use in all stages of prostate cancer. Its basic idea is to limit the effects of androgens on neoplastic cells, achieved by surgical orchiectomy, the use of LHRH analogues, as well as androgen receptor antagonists and drugs directly inhibiting the synthesis of adrenal gland androgens (ketoconazole and aminoglutethimide). The antiandrogen treatment usually causes a marked BMD loss, most pronounced in the first year of therapy (2.4-10%) and growing by 1-2% each year, as compared with the loss associated with age [42, 73]. The retrospective Medicare 1992-2001 analysis (4494 patients) has shown that the use of antiandrogen hormone therapy increases the risk of BMD reduction to the osteopenia/osteoporosis level and the risk of fractures to 42%, as compared with 16% in the group not subject to antiandrogen treatment [74]. The bone mass loss in the course of antiandrogen treatment is also dependent on lifestyle: bad diet, alcohol abuse, tobacco smoking or a significant reduction in exercise. The oldest drug class in use is oestrogens (mainly diethylstilbestrol, DES – a half-synthetic oestrogen derivative), yet nowadays they are losing significance due to the high risk of thromboembolic complications [75, 76]. As regards bone, oestrogens do not exhibit toxicity. This is because their use, as opposed to surgical orchiectomy or LHRH analogue use, does not have a significant lowering effect on bone density [77], and may even exhibit a protective action on bones [78]. LHRH analogues currently constitute an alternative – one widely used and preferred by patients – to surgical orchiectomy [79]. In the prospective studies evaluating the effects of castration on the bone status, usually both methods (pharmacological and surgical) have been assessed together, and it has been shown that the treatment accelerates bone remodelling and results in a BMD reduction by 4-10% in the first year of therapy [73, 77, 80-83]. The above processes progress over the course of treatment and result in a higher risk of bone complications. Several studies have demonstrated an increase in the risk of fractures in patients treated with LHRH analogues [74]. In the population of patients with prostate cancer, fractures occur often, in several or a dozen or so patients [80, 81]. There is observed a significant correlation between fractures and shorter survival [86]. However, their causes are complex – apart from osteoporosis, there coexist bone metastases and injuries, often all in one patient [84, 85]. Surgical or pharmacological castration results in the lowering of circulating testosterone levels by approx. 90%, since peripheral conversion of adrenal gland androgens into testosterone accounts for the remaining 10% [87], hence the concept of therapy consisting in blocking of the androgen receptors on neoplastic cells by the so-called antiandrogens. There are distinguished two classes of these preparations: steroid (blocking the androgen receptor and inhibiting the testosterone production – cyproterone acetate) and non-steroid ones (blocking the receptor only – flutamide, nilutamide, bicalutamide). It has been shown that flutamide acts on osteoblast colonies as an androgen receptor agonist, which in these cells leads to increased synthesis of interleukin-6 and inhibition of synthesis of cytokines involved in bone resorption [86]. This mechanism may result in a reduction of bone mass in the course of testosterone deficiency. In this context, the combined use of flutamide and LHRH (maximum androgen blockage – MAB) would have a protective effect on bones. Unfortunately, this is not always the case in practice, as it has been shown that the use of MAB results in a bone density reduction by approx. 6% after 6 months of treatment [88]. Bicalutamide used in monotherapy may result in an increased bone density, as shown by prospective studies [89]. This is likely to be caused by elevated levels of circulating oestradiol during the treatment with bicalutamide.

## Targeted therapy

The recently distinguished class of drugs defined as targeted therapy encompasses mainly monoclonal antibodies (usually directed against the extracellular receptor domains) and inhibitors of tyrosine kinase forming the intracellular portion of the membrane receptor. Adverse effects on bones in the course of treatment with the above preparations are not addressed, although some of those drugs are targeted at bones precisely. One of them is denosumab – a monoclonal antibody against RANKL, inhibiting the RANK/RANKL signalling pathway, which results in inhibited bone resorption *in vitro* and *in vivo* [90, 91]. Apart from its other uses, the drug produced promising results in studies on patients with advanced neoplastic disease affecting bones [92, 93].

Yet another class of substances belonging to targeted therapy are the inhibitors of Src tyrosine kinase which in an *in vivo* model have proven essential in forming the ruffled borders of osteoclast membrane, thus affecting their resorption potential [94].

The above preparations exhibit activity in pre-clinical studies and in early clinical trials, providing in a longer-term

perspective the possibility of using them in cases of neoplasm-induced osteolysis [95, 96].

Of interest are observations on the bone-associated effects of targeted therapy (monoclonal antibodies and tyrosine kinase inhibitors) directed against insulin-like growth factor 1 (IGF-1), related to its activity stimulating RANKL synthesis and thus bone resorption, to its significance for the integrity of cortical bone (circulating IGF-1) and trabecular bone (skeletal IGF-1), and to the maintenance of bone structure in adults. It is also known that the IGF-1 level correlates with bone density in post-menopausal females [97-102].

A special role in the pathogenesis of neoplastic diseases affecting bones is played by a protein resembling the parathyroid hormone (PTHrP), responsible for rapid calcium release from bones, hypercalcaemia and weakening [103]. Hence, understandably, attempts are made at neutralising this factor. In *in vitro* studies, humanised monoclonal antibodies against PTHrP have proven effective in counteracting hypercalcaemia caused by this factor derived from osteosarcoma, lung cancer and breast cancer cells [104-106].

## Bisphosphonates

Bisphosphonates are a class of drugs of established standing in oncology, also used in the treatment of osteoporosis and – in its labelled form – in bone scintigraphy. As pyrophosphate derivatives, bisphosphonates are classified in two main categories, depending on the presence or absence of a nitrogen atom in the molecule. Of crucial importance for their activity are radicals – particularly the presence of nitrogen in the R2 radical. Bisphosphonates are a standard of care in neoplastic diseases affecting bones [107]. The above substances are capable of inhibiting the bone metabolism by reducing the intensity of resorption. This stems from the effects they have on bone cells, particularly on osteoclasts. Bisphosphonates not only inhibit the activity of osteoclasts but also delay their maturation and accelerate apoptosis [108]. They are also capable of affecting the cell ultrastructure, mainly through disturbing the formation of ruffled membrane borders mentioned above [109, 110].

Bisphosphonates also act on osteoblasts, slowing down their proliferation, facilitating their maturation and stimulating their bone-forming activity, while by also changing the secretory potential of osteoblasts they indirectly decrease the osteoclast activity [111-113]. It is also worth mentioning that bisphosphonates induce apoptosis in the breast cancer cell line *in vitro* [114].

All the above makes this class of substances not only recommended as effective in preventing complications of neoplastic bone infiltration and the occurrence of new disease foci in bones, but also capable of reducing bone loss in patients after treatment of early breast cancer, and in addition it may prove of importance in the prevention of early breast cancer recurrence [107, 115-122].

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