Construction of a database for the evaluation and the clinical management of patients with breast cancer treated with antiestrogens and/or aromatase inhibitors

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Summary
Breast cancer, mostly exhibiting an hormone-dependent pathogenesis, is a commonly diagnosed cancer in females. It is well known that sex steroids favor the process of carcinogenesis of breast tissue and anti-hormonal therapy of breast cancer aims to decrease the action of estrogens on this tissue. For this purpose, two different compounds are prevalently used: the Selective Estrogen Receptor Modulators, preventing the cancer cell to interact with estrogens, and Aromatase Inhibitors, inhibiting the tissue conversion of androgens into estrogens. Unfortunately, latter treatments negatively impact the bone mass leading to the onset of osteoporosis. For this purpose, we propose to build a database to store and analyze information about the effects of treatment with Selective Estrogen Receptor Modulators and/or Aromatase Inhibitors on bone metabolism in patients with breast cancer referred to Our Center. We will focus on the possibility of intervening to reduce the negative effects on bone both by the identification of modifiable risk factors and administration of specific therapies, in order to create a therapeutic, diagnostic standard workup for these diseases.

KEY WORDS: breast cancer; anti-hormonal therapy; osteoporosis, fragility fractures; clinical database.

Introduction
Breast cancer (BC) (OMIM #114480) is the most commonly diagnosed cancer among women and in 2008 has been reported to account for 26% of all new cancers (1). In the United States, it has been estimated that the prevalence of BC in the life of a woman is 1:8 (2), representing the second major cause of cancer-related death. Recently, the survival rates have been demonstrated to improve (2, 3).

BCs are mostly hormone-dependent tumors, the cells of which frequently express hormone receptors for estrogens (ER) and progesterone (PgR). These sex steroids initiate and promote the process of carcinogenesis of breast tissue by increasing the rate of cell division and reducing the time available for DNA repair. The aim of anti-hormonal therapy of BC is to decrease the action of estrogens on breast tissue acting on two possible mechanisms by two different compounds: 1) the Selective Estrogen Receptor Modulators (SERMs) to prevent the cancer cell to use estrogens by interaction with ERs, modulating their response after the SERMs-receptor complex formation; 2) Aromatase Inhibitors (AIs), which inhibit the peripheral conversion of androgens into estrogens, countering the growth of cancer cells and leading to apoptosis. Recent improvements in screening, diagnosis and treatment of BC resulted in the treatment of 64% of BC cases diagnosed in the early stages of the disease (4). In such affected women, the survival rates of 98% at 5 years have been reported, allowing to continue the treatment for many years (4-6).

Unfortunately, some of these therapeutic agents, the AIs, may lead to other co-morbidities, such as an excessive bone loss that facilitates the onset of osteoporosis (7, 8). Therefore, the complications resulting from these longstanding treatments have to be adequately addressed in this population.

Estrogens and their receptors
Estrogens are a class of sex steroid hormones synthesized starting from cholesterol in ovary, adipose, adrenal and placental tissues (9). 17β-estradiol (E2) is the most abundant and active natural estrogen, which exerts its effects by binding directly to ERs (10). Its binding to ER induces a conformational change in the structure of the receptor protein, making possible either homodimerization or interaction with molecules acting as co-activators (11, 12). The transcriptional activation of genes occurs through direct interaction of the complex formed by the ligand and by homodimer coactivator proteins with the portion of DNA named estrogen response elements (EREs) located in the promoter region of the gene (11, 13-17). Therefore, ERs are members of a superfamily of inducible nuclear receptors acting as transcription factors mediating the biological effects of the steroid hormone (18). Specifically, these receptors have a conserved structure consisting of five different domains (18-20) (Figure 1).

Estrogen receptors isoforms
Two isoforms of the ERs (ER-α and ER-β) have been described (Figure 1). ER-α is expressed primarily in breast, vagina and uterine tissues, while high levels of ER-β are present in the central nervous, cardiovascular, gastrointestinal, and immune systems, as also in kidneys, lungs and bones (19). Both the isoforms show considerable sequence homology in their functional domains. ER-α and ER-β share 97% homology in the DNA binding domain (DBD) and are identical for 59% in the ligand binding domain (LBD) region (20) (Figure 1). The activity of the activation function 1 (AF-1) is regulated by growth factors that are involved in the Mitogen Activated Protein (MAP)-kinase cell “pathway” (21). The activity of activation function 2 (AF-2) region is regulated through the bin-
The understanding of these mechanisms of interaction have led to the development of the hormone replacement therapy (HRT) that provides protection to the onset of some of the diseases mentioned above (38), but at the same time explains the correlation between the intake of estrogens in post menopausal osteoporosis and the risk of developing BC and uterus cancer (37-39).

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**BC as an hormone-dependent disease**

The BC represents the first recognized hormone-dependent disease by the British physician George Beaton. In 1896, he showed that the total removal of the ovaries (oophorectomy) induced the regression of BC in pre-menopausal women (40). Lately, further studies showed that estrogens are among the main factors determining the onset and/or progression of BC, because they stimulate the proliferation of both healthy and cancer cells through the induction of proteins involved in the nucleic acids synthesis, thus resulting in the activation of genes that regulate cell division (41, 42).

These proteins, specific estrogen metabolites called catechol-estradiol-3,4-quinones (CE-3,4-Q), impair the function of the enzymes involved in transcription and/or replication of DNA, allowing the formation of DNA mutations accounting for the progression of healthy cell toward the acquisition of an hyperplastic and/or tumor phenotype (43, 44).

Therefore, it is important to consider that those compounds, used in the treatment of BC, are able to interact and modulate the transcriptional activity of ER-α in relation to estrogen binding.

**SERMs**

The ability to search for antagonists interacting with ERs has pioneered the development of compounds that modulate the activity of ER-α and ER-β. The ligands obtained by synthesis are referred to as SERMs (45). This term refers to their ability to act as estrogen agonists in certain tissues (bone, liver and cardiovascular system) and as antagonists in other body tissues (mammary glands and brain), while in uterus may play both as agonists and antagonists (46, 47).

Currently, there are several categories of SERMs, divided into four
generations, developed to make an improvement in benefits by reducing the side effects mostly associated with first generation of SERMs (20).

The first generation belongs to the class of chemical triphenylethylene derivatives and includes the prototype of SERMs, tamoxifen and its derivatives: Toremifene, Droloxifene and Ixodifene (48).

The second generation of SERMs originates from benzothiophene and includes raloxifene, the main compound, from which some derivatives were lately obtained (48).

The third generation has as a “scaffold” reference the benzothiophene and it includes Arzoxifene, while the fourth generation of SERMs, including Acolbifene, consists of benzopyran derivatives (48).

There are at least four classes of electrophilic metabolites that may induce SERMs activation: carboxylations, meldon clivins, meldon chinons α quinones. Triphenylethylen derivatives, such as tamoxifen, are hydroxylated in position α by cytochrome P450 (34, 2D6, 2C9, 1A1, 1A2 and 1B1) (49).

1st generation SERMs
Tamoxifen (Nolvadex) was, in 1970, the first orally administered drug in patients with metastatic BC (50). The first clinical trial was published in 1971 (51). Later, in the U.S. it has been studied how tamoxifen could have anticancer properties, when administered as an adjuvant, in early stage disease; the results indicated that the incidence of BC was significantly reduced with a prolongation of the survival and disease-free period after the first five years of treatment (52).

Subsequent studies have suggested that tamoxifen had beneficial effects on both the reduction of invasive BC, in patients with in situ ductal carcinoma (ISDC) (53), and prevention of BC. It was resulted important in patients at increased risk of developing BC due to the age or the presence of a positive family history or a personal history of an in situ lobular carcinoma (54).

According to what above reported, tamoxifen has been used in clinical practice as an adjuvant therapy in women with tumors positive for estrogen receptor (ER+) after surgery and/or chemotherapy.

However, significant side effects, such as increased endometrial neoplastic, thrombosis and embolic phenomena, have been reported (55, 56). Further studies have shown that a prolonged use of tamoxifen may lead to the occurrence of liver tumors, such as hepatocellular carcinoma (57), exhibiting frequent and specific mutations in the p53 tumor suppressor gene (58) and occurrence of hormone-dependent BC in rats (59). Considering all these factors, the existence of tissue-specific target genes regulation by SERMs may exist (17). It has been observed the agonist action of tamoxifen in uterine tissue could be specifically attributed to the SRC-1 co-activator, expressed here at high concentrations and much lower in other areas of the body, such as breast epithelium, in which tamoxifen perform its function as an antagonist (17). Consequently, the estrogenic activity could represent the largest contribution to the carcinogenic effects of the drug at the endometrial level (17).

The Droloxifene, with a hydroxyl in position 3, exhibits an anti-estrogenic activity in vitro, equivalent or slightly superior to tamoxifen (60). It has not reported to induce no errors in the DNA and liver tumors in rats (61).

The 4'-ido derivative of tamoxifen, known as Ixodifene, has anti-estrogenic activity fully comparable with other compounds and generating no carcinogenic effects in rats (62).

Toremifene (Feriecon) can act as an anti-estrogen in breast tissue and has also positive effects on bone density; however, it exhibits an agonist effect on endometrial cells, even fewer than reported for tamoxifen (20). Indeed, the occurrence index of the endometrial cancer in patients treated with toremifene is 1.4 versus 2.0, reported in patients undergoing treatment with tamoxifen (63). Toremifene is used only in women with advanced BC (26).

2nd generation SERMs
Great enthusiasm was created by the discovery of benzothiophene derivatives since these compounds have no estrogenic activity in the uterus whereas appear as powerful anti-estrogens in breast tissue (64, 48, 49).

Raloxifene (Evista), when used as a chemo preventive medication, reduces the significant risk of developing BC in postmenopausal women with fewer side effects of tamoxifen (65). However, it is not used as chemotherapies in the BC because it has a slower efficacy than tamoxifen in advanced stages of disease (66).

3rd generation SERMs
A member of this family is the Arzoxifene that appears to be a chemotherapy agent with fewer side effects than raloxifene (67, 68). It acts as an antagonist in the uterus (69).

Raloxifene, and its metabolite DNA, shows high binding affinity to ERs and high capacity to inhibit the estrogen-dependent growth of MCF-7 cell line (70, 71); in fact, assays on this cell line show that this drug has a greater capacity than tamoxifen to inhibit the tumor growth (70).

4th generation SERMs
The compounds belonging to this family are benzopyran derivatives and the best one known is the Acolbifene (EM-652).

This drug is a powerful anti-estrogen molecule, able to inhibit both ER-α and ER-β (71) signaling pathways and consequently the proliferation of cell lines derived from BC and cervical cancer (72-74).

Current perspectives
Currently, the standard postoperative adjuvant therapy in ER-BC, is represented by tamoxifen for a total of five years of treatment. This therapy allows a reduction in overall mortality with particularly important effects in patients exhibiting the involvement of the auxiliary lymph nodes. However, the increased risk of developing uterine cancer and thromboembolic phenomena, connected with the use of tamoxifen (58, 59), has prompted researchers to develop new therapeutic strategies leading to the development of new compounds: the AIs.

AIs
The action of these compounds consists of the inhibition of the metabolic pathways leading to the biosynthesis of estrogens in cancer cells. In particular, aromatase enzyme belongs to the P450 cytochrome family, responsible for the synthesis of estrogens starting from androgen precursors and in particular the formation of estrone and estradiol from testosterone (75).

This enzyme is present in granulosa cells of ovarian follicles, subcutaneous fat, liver and muscle. At menopause, estrogen production is mainly due to the subcutaneous fat aromatase activity. In fact, there is a direct correlation between “body mass index” and estrogen circulating levels in postmenopausal women (76).

Recent studies have identified the BC tissue as an important site for estrogen production and approximately 2/3 of BC exhibit aromatase activity and synthesize significant amounts of biologically active estrogens, so as to provide a concentration of estradiol in tumor tissue 10 times superior to the plasma values (77)

The total estrogen suppression in postmenopausal women may be reach by the inhibition or inactivation of the aromatase enzyme by AIs. These compounds have a total anti-estrogenic action, lacking of the partial agonistic activity of tamoxifen that allows the latter to have a positive effect on bone and a negative effect on the risk of uterine cancer and venous thromboembolism.

The AIs are classified into type 1 inhibitors, or steroid enzymatic inactivating drugs (steroids analogues of Androstenedione irre-
versely binding to the same site of the aromatase), and type 2 enzyme inhibitors, or nonsteroid enzymatic inactivating drugs (nonsteroid substances reversibly binding to the heme group of the aromatase enzyme) (78).

Three generations of these compounds are known:

1st generation AIs

The first AI used in the clinical practice was the aminoglutethimide, initially used as an anticonvulsant drug, followed by testonolactone not proved to be a potent inhibitor. The use of aminogluthemide for the treatment of BC has been abandoned because of the complete inhibition on adrenal steroidogenesis, determining a “chemical adrenalectomy”. In fact, it accelerates the metabolism of the estrogen sulfate, resulting in lower free plasma and urinary estrogen levels (79), and induces metabolic enzymes mediating and inhibiting the liver enzymes controlling the synthesis of cortisol, aldosterone, thyroxin, and aromatase itself (79). Therefore, administration of aminogluthemide must be accompanied by administration of glucocorticoids, hydrocortisone, and, in some patients, thyroxin (79).

2nd generation AIs

Fradazoide and formestano belong to this class of inhibitors. The fradazoide is a fairly potent inhibitor of aromatase and shows a significant reduction in toxicity when compared to aminoglutethimide. The main inhibitor of this class is represented by formestano, a structural analogue of androstenedione which shows high specificity for the enzyme, belonging to the type 1 inhibitors class (enzymatic inactivators).

This drug has a significant clinical efficacy, whose limit is represented mainly by the route of administration (intramuscular injection).

3rd generation AIs

The third-generation AIs is represented by anastrozole (Arimidex), letrozole (Femara) and exemestane (Aromasin). In pre-clinical studies, these new compounds have shown that they: a) do not affect adrenal steroidogenesis, since they do not change the basal levels of cortisol and aldosterone; b) have a high pharmacological power (greater three orders of magnitude than aminogluthemide) associated with a good tolerability; and c) can be administered orally, making these drugs very handy and suitable for a prolonged treatment (77, 78).

Letrozole and anastrozole are type 2 AIs (non-steroidal inhibitors) with a plasma half-life of approximately 48 hours (77).

On the contrary, exemestane is a type 1 AI (steroid activator) with a plasma half-life of approximately 27 hours (77).

Contraindications to the use of AIs

The use of AIs is contraindicated in: 1) pre-menopausal patients: AIs induce an increased secretion of gonadotropins, because of the reduced feedback of estrogen at hypothalamus and pituitary level; in some animal studies, the AIs in premenopausal subjects determined an increase in size and weight of the ovaries (78); 2) women with negative hormone receptor BC since they are not usually responsive to hormonal treatment (78).

Use of AIs in BC

The treatment of women with ER+ BC aims to induce deep hypopoeostrogenism. While in the past the menopause was often induced by surgery, currently the pharmacological castration is the preferred choice. In premenopausal women, the treatment consists of a gonadotropins agonist (GnRH) combined with AIs, whereas in postmenopausal women only AIs are used.

1. AIs as adjuvant treatment in post-surgery for BC

Several clinical trials have begun to test the possible role of third-generation AIs as an adjuvant treatment of BC in postmenopausal women. The first and the most important of these trials is represented by the ATAC (Arimidex and Tamoxifen Alone or in Combination) trial conducted on 9366 patients (80).

After a median follow-up of approximately 33 months, early results showed a small reduction in tumor recurrence rates (87% vs. 89%) in women taking anastrozole compared with those enrolled in the tamoxifen group.

Subsequently, the analysis of the data collected after 4 years of therapy confirmed this behavior. In fact, it has been reported both a longer disease-free survival in 86.9% of patients treated with anastrozole compared with 84.5% of those treated with tamoxifen, as also a reduction in drug-induced side effects in the anastrozole group. The tamoxifen-anastrozole association does not appear to offer additional benefits to the individually use of such compounds.

The ATAC (80) also revealed a lower incidence of contralateral occurrence of BC in patients treated with anastrozole compared with those treated with tamoxifen (0.3% vs. 1%). Although adjuvant therapy with tamoxifen remains the standard treatment in ER+ BC patients, treatment with AIs may be based on the results obtained in more recent clinical trials, representing a valid alternative in women with high thromboembolic risk or low tolerance to tamoxifen. Recently, anastrozole was approved for the adjuvant treatment of early ER+ BC in postmenopausal patients, especially when tamoxifen was contraindicated (81-84).

2. Use of AIs as neoadjuvant treatment of locally advanced BC

The reduction of the tumor mass, before surgery, through the use of endocrine therapy is an attractive option. Some randomized clinical trials on postmenopausal women with ER+ BC, larger than 3 cm. in diameter, showed that administration for a few months of anastrozole, letrozole or exemestane was able to determine a higher reduction of the tumor volume than tamoxifen (allowing in most cases the use of a conservative surgical therapy) (85).

3. Use of AIs in the treatment of metastatic BC

Clinical double-blind multicentre studies have shown that AIs of the third generation (particularly letrozole) are superior to tamoxifen as a first line endocrine treatment of advanced ER+ BC (85), because these compounds are able to determine a greater tumor reduction and disease-free period (85). In addition, third generation AIs have also proven to be superior to megestrol acetate as a second line endocrine therapy of advanced BC with a lower incidence of side effects.

4. Use of AIs in the preventive treatment of BC

Preliminary results of the ATAC study suggest that AIs, due both to their anti-estrogenic and inhibition of the development of BCS, may have an important role as a preventive drug treatment of BC (80). As mentioned previously, this important clinical trial showed a lower incidence of contralateral BC in women in the arm with anastrozole adjuvant therapy compared with those treated with tamoxifen (0.3% vs. 1%), after a follow-up of about 33 months (80).

Unfortunately, even if the preventive efficacy of AIs appears to be superior to that of tamoxifen, further clinical studies are needed to define their potential use in chemoprevention in women at high risk for BC.

Effects of hormonal therapy on bone mineral density (BMD)

**SERMs**

**Tamoxifen**

Tamoxifen binds to both ER-α and ER-β and has a partial ago-
After 36 months of treatment, women taking raloxifene (60 mg/die) showed, in comparison with patients on placebo, an increase of 2.3% of BMD at all the skeletal sites examined. Moreover, patients treated with raloxifene showed also a significant reduction in the incidence of new vertebral fractures compared with placebo group (35% for the group of women with fractures before therapy, and 50% for those without fractures at baseline) (93, 94). However, raloxifene was associated with an increased risk of thrombo-embolic events for which it is not recommended in patients who complain or at high risk of venous thrombosis (95).

**Ala**

At menopause, serum levels of estrogens decrease by about 90% (96) and this leads to an increased bone turnover and a real bone loss, which can take 5-10 years to reach a 30% trabecular bone loss and 10% cortical bone loss that determine an increase of the fracture risk (97).

The use at post-menopause of anastrozole, letrozole and exemestane lower estrogen serum levels of 81-94%/88-98% and 52-72% (98), respectively.

Observational studies have found an increased bone loss and fracture rates in women treated with AIs. In these women, compared to those treated with other drugs, a retrospective cohort study on 12,368 patients with BC, has documented significantly higher rates of reduction in bone mass (respectively, 8.7% versus 7.1%) and fracture (respectively, 13.5% versus 10.3%) (99). A study on 1043 women with BC found that patients treated with Alas had a 2.5 times higher probability of experiencing fractures compared with those treated with tamoxifen (100).

**Anastrozole**

Evidence of increased bone loss during treatment with anastrozole were evidenced in several studies. The ATAC (Arimidex, Tamoxifen, Alone or in Combination) randomized 6241 postmenopausal women with ER+BC treated for 5 years with anastrozole or tamoxifen. After 68 months of follow-up a significantly higher incidence of fractures was reported in the anastrozole-treated group versus tamoxifen (respectively, 11% versus 7.7%) (101). The results, updated after a 100 months follow-up period, described an annual rate of fractures higher in the anastrozole than in tamoxifen arm (respectively, 2.93% versus 1.90%), with similar annual rates, after completion of therapy, between the two arms (respectively, 1.56% to 1.51%) (102).

In ABCSG (Austrian Breast Cancer Study Group) 8 and ARNO (Arimidex/Novadex) 95 studies randomized 3224 women with BC treated with tamoxifen for 2 years and then undergone three years of treatment with anastrozole or continuously for 3 more years with tamoxifen. After 28 months of follow-up, the combined analysis of the two studies described a small, but significant, increase in fracture rates in women who were switched to anastrozole with respect to those who continued therapy with tamoxifen (respectively, 2.1% to 1%) (103).

A sub-protocol of the ATAC study has evaluated 308 patients with BC for two years, reporting the association between anastrozole and BMD loss, whereas tamoxifen led to a modest increase in both vertebral (respectively -4.0% to -1.9%) and femoral neck (respectively -3.2% to -1.2%) BMD (104). Preliminary results at 5 years confirmed the significant loss of BMD with anastrozole, although this loss seemed to slow down after 2 years (105).

In a prospective cohort study on 103 postmenopausal women with BC compared with 114 healthy controls revealed a significant reduction at vertebral and femoral neck BMD after 1 year of treatment with anastrozole (104). However, not even after 30 months of Arimidex/Novadex 95 follow-up study (979 randomized women) (106), or after 36 months of follow-up in the Italian Tamoxifen Arimidex trial (448 patients) (107), a significant increase in the incidence of fractures, following treatment with tamoxifen to anastrozole, was revealed. The inconsistent association between the use of anastrozole and...
fracture incidence in different studies is likely to be multifactorial in origin, and includes: 1) the possible effect of tamoxifen previously taken; 2) studies without statistical power sufficient to identify the risk of fracture; 3) different age groups; 4) different rates of baseline BMD and bone mass reduction (108).

**Letrozole**

Two controlled trials on adjuvant therapy with letrozole have documented conflicting results about the incidence of the fractures rate. The Breast International Group 1-98 study, four arms, has compared 5 years of treatment with letrozole monotherapy, letrozole followed by tamoxifen, tamoxifen alone and tamoxifen followed by letrozole. A comparison between the arms initially provided with letrozole or tamoxifen (4933 randomized patients) showed a significant increase in fractures with letrozole after 51 months of follow-up (respectively, 8.6% versus 5.8%) (109). However, the MA.17 study, letrozole or placebo after 5 years of tamoxifen (5187 randomized women), has documented a not significant increase in fracture rate with letrozole at 30 months of follow-up (respectively, 5.3% versus 4.6%) (110).

A supporting study to MA.17, which evaluated 226 patients, found a significantly greater loss of BMD with letrozole versus placebo after 2 years at lumbar spine (respectively, -5.4% to -0.7%) and neck total hip (respectively, -3.6% to -0.7%) levels (111). The not significant difference in rate fractures of MA.17 study could be due to inequalities in the Breast International Group study control arm, which provided a substance with known protective effect on bone, in addition to a relatively short follow-up period. No studies, however, had sufficient statistical power to assess the incidence of fractures (108).

**Exemestane**

Exemestane, irreversibly inhibiting the enzyme aromatase, has raised particular concern about its effects on bone metabolism. Animal studies have documented the possibility that the molecule may have androgenic properties that may decrease the degree of reduction of bone mass (112, 113).

Intergroup Exemestane Study, including 4724 postmenopausal women, provided an initial treatment for 2 or 3 years with tamoxifen and then switched to or a treatment with exemestane or staying on tamoxifen. After an average of about 65-7 months of follow-up, there was a significant increase in the incidence of fractures in patients treated with tamoxifen versus exemestane (respectively, 7.0% versus 4.9%) (114).

A bone sub-protocol concerning 226 patients, found a significant reduction in BMD with exemestane after 6 months (-2.7% at the lumbar spine and -1.4% at the femoral neck), which is then gradually slowed. Women who continued taking tamoxifen did not show any significant change in BMD (115).

Tamoxifen Exemestane Adjuvant Multinational study confirmed a significant reduction of lumbar spine BMD after 1 year of therapy with exemestane versus tamoxifen (116).

A significant loss of BMD, as a result of switching from tamoxifen to exemestane, was also observed in a small study on 70 post-menopausal women with BC (117).

A randomized placebo-controlled trial, lasting two years, on exemestane in 147 postmenopausal women with early stage BC, has documented a significant increase in BMD reduction by exemestane in the femoral neck, with no difference at the lumbar level (118). The evaluation of patients within 1 year after completion of therapy showed a stabilization, without further reductions in BMD in the exemestane arm (118).

After reviewing the literature, we can confirm that there is considerable evidence of an association between both an increased loss of bone mass and fracture incidence in women treated with AIs compared with those taking tamoxifen or placebo, but it is not clear whether differences between different AIs exist in relation to the degree of bone loss.

Direct comparison studies, currently underway, including the MA.27 (exemestane to anastrozole) and Femara Versus Anastrozole Clinical Evaluation (letrozole to anastrozole), will provide comparisons for the loss of bone mass, and other outcomes (108). Since most of the comparison was made with tamoxifen, which guarantees a certain degree of bone protection, the level of reduction in BMD observed with AIs may appear larger, lacking of a control group treated with placebo (108).

**Effects of therapy with AIs and/or SERMs on bone turnover markers**

Estrogens play an important role in maintaining the balance of bone metabolism (119). At menopause, the decline in blood levels of estradiol leads to a significant increase in bone resorption, reflected by the increase of serum bone resorption markers, such as C-telopeptide (CTX) and N-telopeptide (NTX), and the decrease of bone formation markers, such as N-terminal propeptide of procollagen type I (PnP), osteocalcin (OC), bone alkaline phosphatase (bAP) and parathyroid hormone (PTH) (120).

In ER+BC women, undergoing treatment with AIs, a significantly higher than expected increase in bone turnover, with respect their postmenopausal status, has been described. Markers of bone resorption were increased, while the bone formation ones were found to be either decreased or increased (104, 121).

In a previous study on postmenopausal women, treatment with exemestane showed a profile of action on bone metabolism slightly different from the one seen in therapy with non-steroidal AIs (121). Indeed, treatment with exemestane led to a significant increase in bone formation markers, while anastrozole or letrozole did not (122).

Unlike in postmenopausal women with ER+BC, tamoxifen leads to a normalization of bone markers, as demonstrated by a decrease in bone resorption and formation (104). Several studies have reported that tamoxifen has some beneficial effects on bone metabolism and on the risk of fractures in postmenopausal women (89, 123-125). However, the results showed that these positive effects do not last once the treatment with tamoxifen is stopped. The study by McCaig et al. showed that in patients treated with AIs there is an increase of bone turnover behind the loss of bone mass. Patients, previously receiving tamoxifen, had a significantly greater increase in bone turnover markers, such PnP, CTX, NTX, bAP and PTH compared to patients not receiving tamoxifen (126).

Even the data from the ATAC study showed that bone resorption and formation were suppressed, respectively of 30 and 15%, in patients treated with tamoxifen compared to the untreated population (104).

In BC patients, initial treatment with tamoxifen and AIs thereafter, a different effect of AIs on bone metabolism was noted compared to that one obtained on women not previously treated with SERMs.

A recent study showed that a previous tamoxifen treatment deeply increases the effects of AIs on bone metabolism, especially in the transition from suspension of tamoxifen at the beginning of AIs therapy. These results are similar to those observed in the Intergroup Exemestane Study (IES) in patients treated with tamoxifen for 2-3 years and then switched to exemestane, in which, within 6 months, there was a significant decrease in BMD from baseline at both lumbar spine and hip levels, 2.7% and 1.4% respectively (127).

Therefore, any benefit that therapy with tamoxifen had produced on bone density was lost after stopping this treatment and beginning the one with AIs. Therefore McCaig et al. concluded that in patients receiving aromatase or letrozole after tamoxifen therapy the monitoring of bone...
metabolism, as performed in patients starting first-line treatment with anastrozole or letrozole (126), is necessary. It has been noted that the AIs have been associated with an accelerated bone loss, whereas tamoxifen has been shown to offer some protection against bone loss in postmenopausal women (109, 114, 128). However, we have to remember that the AIs therapies have demonstrated a superior efficacy, compared to tamoxifen, in terms of disease-free survival and, in some cases, of overall survival. The IES study, reported that women with ER/BC treated with exemestane had a reduced risk of death by 17% compared to those treated with tamoxifen (128).

Effect of therapy with bisphosphonate on bone loss induced by hormonal treatment of BC

Bisphosphonates are used in the treatment of women with BC because they have no interaction with ERs or PgRs and because they have demonstrated to increase BMD in postmenopausal women (129).

Several clinical studies have evaluated the efficacy of bisphosphonates in preventing the loss of bone mass in BC. Preliminary data of the study with anastrozole and risedronate and of the ARI-BON study, in which women with osteoporosis treated with Al were randomized to treatment with risedronate (administered at doses of 35 mg weekly) or ibandronate (dose 150 mg monthly), documented significant reductions in BMD loss after 1 year of bisphosphonate therapy (130-132).

Small studies have shown protective effects with risedronate, pamidronate and zoledronate, in similar patients (133-136). In recent years, the ABCSG assessed the zoledronate in pre-menopausal women randomized to ovarian ablation with goserelin, LH-RH (LH-releasing hormone) agonist, resulting in a reversible ovarian suppression, plus tamoxifen or anastrozole (ABCSG-12). Among the 401 patients enrolled in the bone sub-protocol, those treated with zoledronate showed stable values of BMD, whereas there was a significant reduction of this parameter in those who received ablative endocrine therapy alone (137).

Preliminary results at five years, after 24 months from the end of treatment, indicate that these women continue to experience a loss of bone mass compared to those treated with bisphosphonates (respectively, -6.3% versus -4.0%) (138).

Some recent studies have evaluated precluding the loss of bisphosphonates in postmenopausal women treated with AIs and the largest of these trials, called Zomeri Femina Adjunt Synergy Trials (Z-FAST [United States]/ZO-FAST [Europe]), analyzed the effectiveness of intravenous zoledronic acid at the dose of 4 mg every 6 months, in women treated with adjuvant therapy with letrozole and baseline T-score ≤-2.0. In a treatment arm, the zoledronate was started simultaneously with letrozole, while in another arm it has been postponed until the recording of a reduction in BMD. The one year results of the Z-FAST study documented an average increase of 1.9% in lumbar BMD from baseline in the arm of early treatment with zoledronic acid, versus an average reduction of 2.4% in the delayed administration: total difference of 4.4% (139).

A 36 months of follow-up showed that the absolute difference between the two arms, in lumbar spine BMD, had increased to 6.3%, with a greater number of fractures in the delayed than that simultaneously treatment arm (respectively, 6.3% versus 5.6%), although the study had not sufficient power to detect significantly differences in fracture rates (140).

Results at 1 year of ZO-FAST are similar with an overall difference of 5.7% between the study arms in favor of an early administration (141).

After 24 months of follow-up, the results still show a significant difference in BMD in favor of the early treatment with zoledronate (142).

These data are also confirmed by a subsequent study that evaluated the ability of zoledronic acid to preserve BMD when started simultaneously with letrozole in patients with BC and previously treated with tamoxifen (143).

A recent study confirms that post-menopausal women with a T-score < -2.0 are at increased risk of fracture. Treatment with AIs has been shown to improve disease-free survival in women with ER/BC, but it was also associated with an increased bone loss and increased incidence of fractures than other therapies. This study has shown that concomitant therapy with intravenous zoledronic acid is associated with improvement and/or preservation of BMD in these women (144).

Aim of this project

In medicine, the need of information, accurate from an analytical point of view, credible from a clinical point of view, valid from a statistical point of view, led to the creation of systems capable of providing not only a valid epidemiological support for the planning and management of health interventions (such as activities involving the use of drugs in prevention), but also estimates of incidence, prevalence and mortality of the affected population. Preventive activities, that we have to currently play in the health’s field, should avoid the occurrence of a future adverse event, or should delay its onset. This will allow us to save on future health’s costs. Osteoporosis and/or osteoporosis, and consequently fragility fractures occurrence, in patients with BC are potentially preventable conditions and therefore careful basal assessment, followed by a continuous monitoring of therapeutic interventions, may prevent or reduce the risk of adverse events, such as the fragility fractures.

So, with this project, we propose to build a clinical database to afford, to store and analyze, on a continuate and systematic manner, information about the effects of treatment with SERMs and/or AIs on bone metabolism in patients with BC referred to Our Center. We will focus on the possibility of intervening on slowing the negative effects on bone both by the identification of modifiable risk factors and the use of specific drugs, so that to create, with the information obtained, a therapeutic, diagnostic standard workup for these diseases.

Construction of the database and discussion

The achievements of these objectives requires analysis of three critical dimensions of the data collection (material): 1) the extension of the population must be designed to get a database that is sufficiently representative of the population concerned; 2) the depth and the extent of data; 3) the time must be aimed to create a database with a time extension sufficient for a reliable assessment of the diagnostic and therapeutic pathways. Taking into account these considerations, we have built an Excel file (method) to divide the patients into two groups: 1) those treated with tamoxifen and AIs; and 2) those treated with AIs as a first-line therapy.

Then, for each group the following fields for data collection were set:

-1st field: Anamnesis
-2nd field: Oncology
-3rd field: Diagnosis of bone loss
-4th field: Therapy

1st field: Anamnesis

The first area involves the collection of the patient’s family and physiological history, from which we extrapolate the data correlated with the development of BC and osteoporosis. Our attention has focused, in particular, on some aspects of life such as social-reproductive-age, family history of BC and osteo-
porosis, age at menarche, number of pregnancies, breastfeeding, oral contraceptive use and duration of therapy, age at menopause, use and duration of hormone replacement therapy, smoking habits, body mass index (BMI), physical activity, diet intake of dairy products, history of previous fractures, presence of co-morbidities. The risk factors, correlated with both BC and osteoporosis, that we identified and listed below have, as a common denominator, their effect on the level and duration of exposure to endogenous and exogenous estrogens (Table 1):

- **Age**: over 80% of cases of BC and osteoporosis affect women over 50 years (2, 145).
- **Familiarity**: for BC about 10% of women with BC has a family member with BC, especially in cases where juvenile cases are presenting in which some genes, predisposing to the occurrence of this tumor such as BRCA1 and BRCA2, are involved. Mutations of these genes are responsible for 50% of hereditary BC forms (2).

Currently, we know that various factors, both environmental and genetics, contribute to the pathogenesis of osteoporosis. Genetic factors are represented by a pool of genes that regulate the expression of the characteristics associated with the development of the disease (mass and bone microarchitecture) being responsible for 50-80% of the interindividual variability in BMD (146-150). A major contribution to evaluate the influence of environment and genes on phenotypic variability in BMD and the development of osteoporosis have been provided by studies on mono-and dizygotic twins (146, 147, 151-154).

Even studies of family groups have confirmed the existence of such a contribution, showing a correlation between vertebral BMD in mothers and daughters, the BMD of the daughters of osteoporotic women compared with women of that age appears to be reduced, and associated with an increased risk of fracture after menopause (151, 152).

- **Age at menarche**: later menarche lesser the BC risk (2), while for osteoporosis a late menarche is associated with reduced bone formation (145).
- **Number of pregnancies** (specifying the earliest age at first pregnancy, the presence of abortion and/or voluntary termination of pregnancy). This period of reduced estrogen production has a protective effect on the development of BC. More numerous are the children greater is the protection. Such a protection seems to be preceded by a short period (several years), immediately after pregnancy, which noted an increase in the risk of BC. Therefore, having children leads to a reduction of long-term risk versus nulliparous (2). After delivery, osteoporosis can be facilitated by a diet poor in calcium during the months of pregnancy, or an inefficient hormonal regulation of calcium metabolism, which creates a negative calcium balance with easier occurrence of gestosis (152).

- **Breastfeeding and its duration**: Breastfeeding allows the cell to complete its maturation and makes it more resistant to possible neoplastic transformation (2). Osteoporosis during lactation is linked to an increased need of nutrients like calcium and vitamin D (152).

- **Use of oral contraceptives (OC) and duration**: several studies suggest a slightly increased risk of BC associated with the use of OCs, the risk appears to decrease with age and time extent from the their interruption. In fact, after 10 years from the cessation of OC, the risk of BC returns at the average of the general population (2).

- **Age at menopause**: earlier the menopause lesser the BC. A 10 years anticipation of menopause halve the risk of BC (2). For osteoporosis, it is known that later the menopause higher the estrogen levels that may prevent bone mass loss (145).

- **Hormone replacement therapy (HRT) and its duration**: An increased risk of BC incidence and mortality by the use of HRT in postmenopausal women has been reported. The risk is directly associated with duration of exposure (2). The HRT prevent bone loss at menopause (145).

### ANAMNESIS:

- **Risk factors correlated with BC and osteoporosis**:
  - **Age**
  - **Familiarity**
  - **Age at menarche**
  - **Number of pregnancies**
  - **Breastfeeding and its duration**
  - **Use of oral contraceptives (OC) and duration**
  - **Age at menopause**
  - **Hormone replacement therapy (HRT) and its duration**
  - **Smoking habits**
  - **BMI**
  - **Physical Activity**
  - **Recruitment of dairy products**
  - **Previous fractures**
  - **Presence of co-morbidity/drugs**

- **Smoking habits**: smoking increases the risk of fractures. Combined analysis of studies on 60,000 subjects in Canada, USA, Europe, Australia and Japan have shown that smoking increases the risk of fragility fractures (155). For the BC, this correlation is still controversial, with some studies indicating that smoking leads to an increased risk of BC (156).

- **BMI**: an increase in BMI in postmenopausal women relates to an increased risk of developing BC due to the production of estrogen by the adipose tissue (2). A low BMI below the value of 19 may predisposes to osteoporosis. In fact, the bone is a dynamic tissue that responds to the load and subjects with a BMD >20 tend to have higher BMD and consequently a more resistant bone structure (155). It is well known that both a low BMI and weight loss are strongly associated with either low bone mass or an increase in fracture risk, while obesity protects against osteoporosis (157).

- **Physical Activity**: Physical activity, through the reduction of body fat, has a protective role against BC either before or after menopause (2). In childhood, physical exercise favors a high peak of bone mass and is recognized as a protective factor against bone stress fracture. In case of high peak, the aging-related bone depletion will be difficult to conduct the subject below the fracture threshold (158). Even in postmenopausal women physical activity may prevent bone loss. When physical exercise is associated with HRT or calcium supplementation, the effect on bone density is strengthened. Physical activity may help to increase bone density even around 40 years of age, but it has not proved to be effective in reducing fractures in postmenopausal subjects (158). Moreover, exercise helps to reduce the risk of falls and, consequently, the risk of fracture, since it improves the sense of balance, maintaining a close relationship between joint and muscle mass (158).

- **Recruitment of dairy products**: The requirement varies according to age. An inadequate daily calcium intake during the juvenile or in certain stages of life, such as pregnancy and lactation, increases bone resorption, decreases bone formation and reduces skeletal mineralization, thus predisposing to osteoporosis. However, calcium is effective in reducing vertebral and non vertebral fractures only if and when is associated with vitamin D3 (159). Vegetarian people eating raw foods have a low BMD without signs of an increased bone turnover. Given the low calories and protein intake, they usually have a low BMI and low fat content. Such a diet is associated with low bone mass even in the presence of proper vitamin D values (155, 148).

- **Previous fractures**: Multiple studies show that patients with a previous fragility fracture are at a higher relative risk of fracture than subjects who have never experienced fractures (145).

- **Presence of co-morbidity/drugs**: Several studies have evaluated the effects of certain co-morbidities history of BC (such as
Aromatase is encoded by the CYP19 gene located on chromosome 15q21.1. A tissue-specific expression of different isoforms of aromatase is regulated at the mRNA transcription levels. These polymorphisms include the C>T variant at the 3′ untranslated region, represented by a different number of repeats (TTTA)n at intron 4. Currently, data on the effect on bone of this polymorphism are still scarce, even if a new era of pharmacogenetics is an interesting perspective to identify potential subjects suitable to receive individual treatments. A study on postmenopausal Italian women showed that the allele (TTTA)12 is the most common in non-osteoporotic women, suggesting a possible protective action (169). Moreover, women with a number of repeats >11 show a higher lumbar BMD than women with a low number of repetitions, (TTTA)8-11. These data are also confirmed by studies on male individuals (170). Furthermore, in in vitro studies, the fibroblasts phenotype of subjects with a high number of TTTA repeats show a higher aromatase activity than cells of subjects with the opposite genotype (170).

Therefore, in this field we will: 1) collect the information required to assess the effectiveness of bisphosphonates therapy, particularly zoledronic acid in BC patients treated with anti-hormonal therapy; 2) study the BMD changes induced by these drugs, bone turnover markers, the appearance of any related fractures, and a potentially different response to treatment in relation to the presence of ERs and CYP19 gene polymorphisms (Table 4).

Conclusions
The therapy with AIs in women with BC is correlated with increased loss of bone mass and fracture risk when compared to those treated with tamoxifen or placebo. The real impact of this loss on bone health will depend on the early identification of patients at risk of fracture and the application of appropriate prevention strategies. The control and measurement of the parameters needed to diagnose osteoporosis, such as family history of fractures, previous personal fractures, low BMD, physical activity, smoking habits, daily calcium and vitamin D intake, are not usually evaluated in randomized clinical trials appearing in the international literature.
Therefore, we have decided to introduce such parameters into our database. However, further studies will be necessary to document which is the most appropriate therapy for these patients. Such studies will also require more extended follow-up periods to evaluate the efficacy and toxicity of bisphosphonates before considering them as first-line treatment in BC patients. Moreover, the increasing knowledge on major genes and genetic pathways involved in the pathogenesis of osteoporosis or altering the response to therapy, will be helpful to prepare preventive strategies and appropriate treatment on the basis of the pharmacogenetics findings. A greater knowledge, due to the collection of a large amount of information obtainable from an appropriate and dedicated database, may help to identify risk factors for bone loss and the adequate therapeutic choice, providing the opportunity to build a feasible, effective and homogeneous diagnostic-therapeutic path, providing also the opportunity for a preventive action to the development of osteopenia/osteoporosis in patients with BC.

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