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Epidemiological study of aromatase inhibitors in women diagnosed with breast cancer: evaluation and management of secondary effects

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EPIDEMIOLOGICAL STUDY OF AROMATASE INHIBITORS IN WOMEN DIAGNOSED WITH BREAST CANCER: EVALUATION AND MANAGEMENT OF SECONDARY EFFECTS

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Epidemiological study of aromatase inhibitors in women diagnosed with breast cancer: evaluation and management of secondary effects

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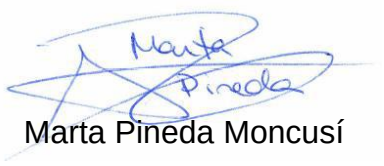

Marta Pineda Moncusí

Barcelona, 2020

“As always in life, people want a simple answer . . . and it’s always wrong.” — Susan Greenfield

AGRAÏMENTS

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ABSTRACT

Aromatase inhibitors (AI) are one of the main therapies to treat estrogen-receptor positive breast cancer. AI use is associated with several side effects that affects patient's quality of life and reduces treatment adherence. Hence, it is necessary to make further efforts in elucidating and diminishing the AI-related side effects.

In this line, this thesis provided new and additional evidence for this purpose. Starting by the importance of assessing vitamin D levels during AI treatment, especially to those who underwent to chemotherapy. We also studied the bone health evolution at the end and one-year after AI cessation, and the impact of oral bisphosphonates (BP). Moreover, we analyzed the arthralgia (VAS score) and health-related quality of life in osteoporosis (ECOS-16 score) progression during the AI treatment until one-year post-treatment. Then, fracture incidence and risk during AI therapy compared to tamoxifen (TAM) was analyzed, as well as the protective effect of BP. Finally, we studied the cardiovascular and thromboembolic risk, and overall survival benefit of AI compared to TAM.

Our research leads us to state that bone health and circulant vitamin D levels monitoring, plus calcium and vitamin D supplementation is key for the clinical management of AI patients. BP treatment is proved to diminish bone loss and fracture risk, but cannot reverse risk levels towards patients at low fracture risk. Furthermore, prior TAM treatment enhances the odds to withdraw during the first year, increases bone loss during

AI treatment, and restricts the recovery in lumbar spine location at one-year post-treatment. On the other hand, since there are no differences in cardiovascular and thromboembolic risk between AI and TAM users, but AI users have lower all-cause mortality, AI should be the preferable choice.

In summary, it is mandatory to clinical monitoring AI patients, especially those who were previously treated with TAM, including fracture risk and related risk factors assessments. These would reduce early cessation of treatment and improve patients' quality of life.

TABLE OF CONTENTS

GLOSSARY

+	Positive expression
AI	Aromatase inhibitors
BAP	Bone-specific alkaline phosphatase
BC	Breast cancer
BMD	Bone mineral density
BMI	Body mass index
BP	Bisphosphonates
CTX	C-telopeptide
DCIS	Ductal carcinoma in situ
DXA/DEXA	Dual-energy x-ray absorptiometry
E1	Estrone
E2	Estradiol
E3	Estriol
ER	Estrogen receptor
FRAX	Fracture Risk Assessment Tool
GnRH	Gonadotropin-releasing hormone
HDL	High-density lipoprotein
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratios

HR+	Hormone receptor positive
LDL	Low-density lipoprotein
NTX	N-telopeptide
OC	Osteocalcin
PINP	Amino-terminal propeptide of type I procollagen
PR	Progesterone receptor
RANKL	Receptor activator of the NF-κB ligand
RCT	Randomized control trials
SD	Standard deviations
SERM	Selective estrogen-receptor modulator
SHR	Subdistribution hazards models
TAM	Tamoxifen
TBS	Trabecular bone score

INTRODUCTION

1. BREAST ANATOMY

The female breast is a subcutaneous organ located on the upper ventral region of the torso. The anatomy of breast is complex (Fig 1). It includes the mammary glands, 15-20 lobules separated by bands of connective tissue that produce and supply milk, and the ducts that transfer milk from the lobules to the nipple. The nipple is surrounded by a pink/brown pigmented region called areola. All this structure is supported and protected by a fatty tissue that gives the breast its soft consistency. The breast also contains blood vessels, lymph vessels, and lymph nodes.

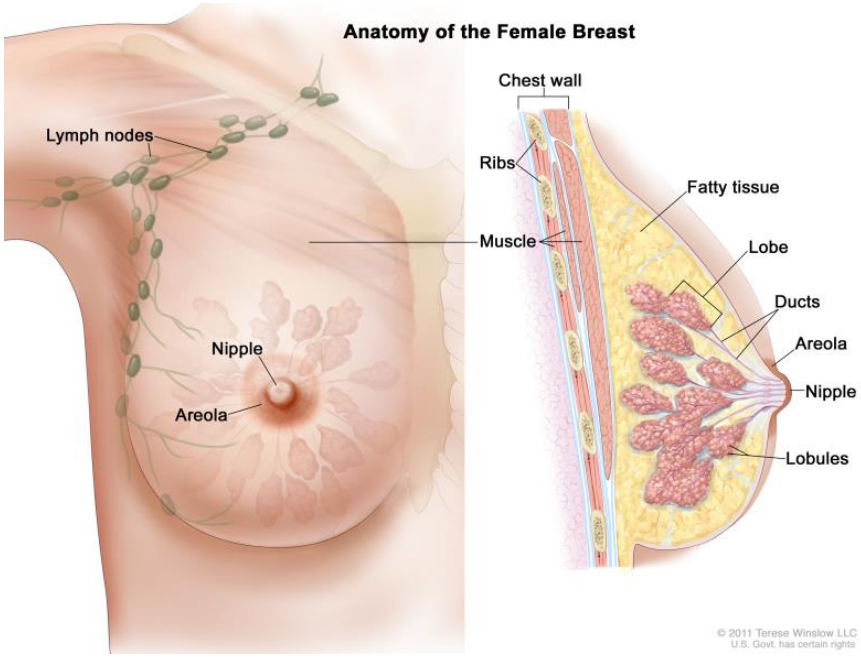

Figure 1. Anatomy of the Female Breast. External and internal anatomy of the female breast. Extracted from: Terese Winslow LLC 2011, https://www.teresewinslow.com/breast. For the

2. BREAST CANCER

Breast cancer (BC) is defined as an uncontrolled grow of breast cells, forming a tumor, that could invade surrounding tissues (invasive breast cancer) or spread to distant areas of the body (metastatic breast cancer). Most of them begin in the ducts (ductal cancers), and some start in lobules (lobular cancers), while other types are less common. Signs of BC include a lump, bloody nipple discharge, or skin changes [1]. BC mainly affects women, men can be affected but its incidence is considered rare (<1%) [2]. This study is focus on women affected by BC.

2.1 Epidemiology

BC is the most common cancer among women (Fig 2) with an estimate of more than 2 million of cases detected annually across the world. It represented the 24.2% of diagnosed cancers in women at 2018. The estimated number of deaths in 2018 was 626,679 cases, representing the 15% of female deaths caused by a cancer [3].

Early detection and new treatment strategies have improved the survival of patients. Nowadays it is considered that 70-80% of them can be cured [4].

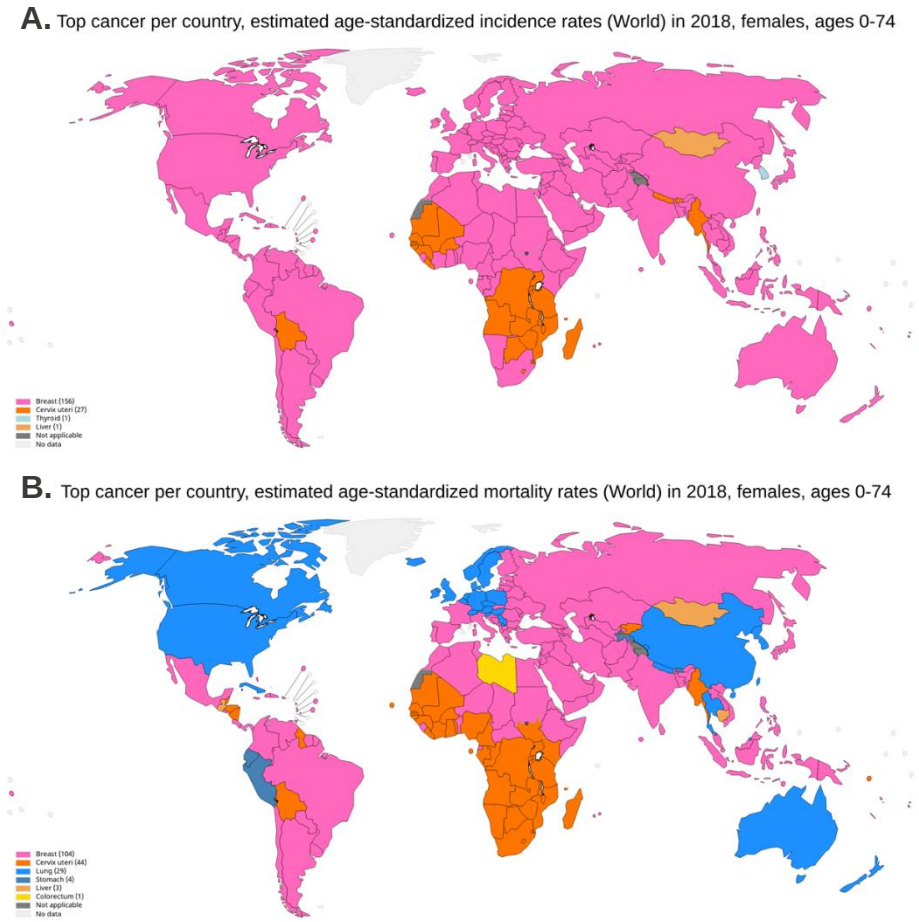

Figure 2. Worldwide image of (A) incidence and (B) mortality of the most common type of cancer in each country. Data provided by GLOBOCAN 2018 database from the International Agency for Research on Cancer's Global Cancer Observatory. Adapted from: http://gco.iarc.fr/today

2.2 General risk factors

Epidemiologic studies have identified a list of well-known risk factors of BC. Those can be sorted in two types, non-modifiable and modifiable factors. A brief summary of the most investigated risk factors in each category is described below.

2.2.1 Non-modifiable factors

2.2.1.1 Genetic factors

Genes associated with BC can be classified according to its penetrance [5].

High penetrance genes

Highly penetrant, but rare genetic variants cause the majority of hereditary BC cases. Fifteen percent of familial BC are explained by mutations, rearrangements or deletions in tumor suppression genes *BRCA1* and *BRCA2*. Female carriers have a lifetime risk of BC up to 85% [6].

Other rare but highly penetrant variants are in genes involved in tumor suppression like *PTEN* (85% lifetime risk), *TP53* (lifetime risk of 25% by age 74), *CDH1* (39% lifetime risk), and *STK11* (lifetime risk of 32% by age 60). It is estimated that this genes, in conjunction with *BRCA1* and *BRCA2*, explain until 25% of BC hereditary cases [5].

Moderate-penetrance genes

Genes involved in DNA repair that interact with *BRCA1*, *BRCA2*, and/or the BRCA pathways, and confer about a two-fold increase

in BC risk. Among them, there are *CHEK2*, *BRIP1 (BACH1)*, *ATM*, and *PALB2* genes [5]. The most common mutation is CHEK2*1100delC, observed up to 1%–2% in general population [7].

These genes are designed as moderate-penetrance because their the genetic impact might be attenuate by environmental factors **[5]**.

Low penetrance genes

Genetic variants common in the population and often located in noncoding regions of the genome, which can contribute to BC risk in a polygenic way, conferring very small risk increases. This increment of risk might be through activation of growth-promoting genes rather than inactivation of DNA repair, which is more frequent in the groups previously described. Among polymorphisms associated with increased risk of BC, there are the Pro919Ser variant in *BRIP1*, and noncoding regions in 2q35 and 8q24 [5].

2.2.1.2 Hormonal risk factors

Hormone exposure has been related to risk of sporadic BC. Sex hormones enhance cell proliferation, increasing the probability of DNA damage accumulation and promoting malignant cells growth. Among them, estrogens stand out as the greatest promoter of BC. Consequently, reproductive history and number of menstrual cycles are important determinants for developing BC [4].

Age of menarche

Risk of BC is 10% reduced each 2 years increase in age of menarche. In contrast, earlier menarche, earlier thelarche – outset of breast development during puberty –, longer period between thelarche and menarche, earlier regular periods, and shorter time between menarche and the onset of regular periods are associated with an increased BC risk [8].

Maternal age for first pregnancy and breastfeeding

Nulliparous women [9] or advanced age at first live birth [10] increment the risk of BC, while early pregnancy and high levels of estrogen during pregnancy diminish the risk [4]. Moreover, each birth decreases the BC risk by 7% [11].

Anothaisintawee et al. (2013)[12] described a 14% lower risk of BC in parous Caucasian women who ever had breastfed compared with parous Caucasian women who never breastfed, and a 28% lower risk in breastfeeding longer that 12 months compared to shorter periods. On the other hand, The Collaborative Group on Hormonal Factors in Breast Cancer (2002) reported a 4.3% reduction in BC risk by each year of breastfeeding [11]. The benefit of breastfeeding was independent of the number of births.

Age of menopause

Later menopause increases the risk of BC in 2.9% for every year older of menopause onset. Moreover, premenopausal women at identical age than postmenopausal women had an increased risk of 43% [13].

2.2.2 Modifiable risk factors

Different cultural factors, lifestyle and national awareness campaigns, can modify the epidemiological patterns of BC. Indeed, nearly 20% of BC can be attributed to modifiable risk factors [4].

2.2.2.1 Overweight and obesity

In postmenopausal women, each 5 kg/m^2 increases the BC risk by 12% [14]. In this line, obesity might increase the risk of BC in elderly nulliparous women [9]. Conversely, association in premenopausal women remains uncertain: an increase in body mass index (BMI) was associated with an increment of BC risk in Asian-Pacific women, but inverse correlation was observed in women of other regions [14].

2.2.2.2 Physical activity

In postmenopausal women, several meta-analyses have found a 12% reduction of BC risk in higher levels of physical activity compared to the lowest, including walking and occupational, recreational or household activity. On the other hand, evidence in premenopausal women is limited, suggesting a protective effect of vigorous physical activity [15].

2.2.2.3 Alcohol use

Daily consume of 10 gr of alcohol – approximately one drink – by an adult women increases BC risk in 7-10% in both, pre- and postmenopausal women [4].

2.2.2.4 Contraceptives

Both current or recent users of hormonal contraceptives have an average incremented BC risk of 20% compared to women who never used, from 9% with less than one year of use to 38% after ten years of exposure [16]. Moreover, age of first oral contraceptives use has a significant linear dose–response relationship with BC risk: every year-old increases BC incidence by 0.7% [17].

2.3 Breast cancer classification

BC can be classified or descripted in many ways. One of them is based on tumor size, lymph node status and receptor status. All of them are important factors to assign the best treatment.

2.3.1 Tumor size

The tumor size, or stage, is the extent of the breast cancer:

Stage 0: non-invasive cancer, limited in the interior of the milk duct. *E.g.* Ductal carcinoma in situ (DCIS).

Stage I-III: stage I tumors are relatively small with no or a minor spreading to the sentinel lymph node (the first lymph node affected by the cancer). Stages II and III have larger size and spreading to nearby lymph nodes. Specially stage III, that can grow into nearby tissues, and affects more adjacent lymph nodes.

Stage IV: metastatic BC that has spread outside the breast and nearby lymph nodes to other parts of the body.

Recurrent breast cancer: cancer that returns after a primary treatment. It can appear in the same breast or in the surgery scar (local), in the nearby lymph nodes (regional), or in a distant area.

2.3.2 Lymph node invasion

Lymph nodes and lymph vessels collects and transport fluids, filed with waste materials, viruses and bacteria, among others, independently of the bloodstream. When a BC is spreading, the first invaded tissue is frequently the lymph nodes under the arm (axillary lymph nodes). The first lymph node – or group of lymph nodes – affected by the primary tumor is termed the sentinel lymph node. Occasionally, lymph nodes near the clavicle or near the sternum are also invaded.

Lymph node invasion is classified by categories (N) from 0 to 3, according to the number of invaded nodes:

N0: cancer has not invaded any lymph node.

N1: cancer has extended to 1 to 3 axillary lymph nodes, and/or it can be found in internal mammary lymph nodes on sentinel lymph node biopsy.

N2: cancer has extended to 4 to 9 axillary lymph nodes, and/or it has enlarged the internal mammary lymph nodes.

N3: cancer has spread greater than 2 mm at least in one area, plus invasion of 10 or more axillary lymph nodes or invasion of

the infraclavicular nodes. Other variations are invasion of axillary lymph node bigger than 2 mm plus enlargement of the internal mammary lymph nodes, or plus supraclavicular nodes invasion.

2.3.3 Receptor status

At the time of diagnosis, the presence of receptors in tumor cells is evaluated.

2.3.3.1 Hormone receptor positive

Estrogen receptor (ER) [18,19] and progesterone receptor (PR) [20] regulate the cell proliferation and differentiation in target tissues, like breast. Tumors with positive expression (+) of ER and/or PR are denominated hormone receptor positive (HR+). This occurs approximately in 70-80% of BC diagnosis [21].

2.3.3.2 HER2-positive

Human epidermal growth factor receptor 2 (*HER2*) gene codifies for the protein HER2. Its function is to activate intracellular signaling pathways in response to extracellular signals[22].

HER2 gene amplification or HER2 protein overexpression leads to an overgrown of the tumor cells. This BC is determined as HER2+ and it is observed in 15-20% of all BC. This subtype is more aggressive and has an increased mortality than HR+ [23].

2.3.3.3 Triple negative

Tumor cells with no presence of ER, PR and HER2 are determined as triple negative. Generally, this form is more common in younger women (<40 years old), who are African-

American or who have *BRCA1* gene mutation [24]. Likewise, it has been associated with central obesity in premenopausal women[25].

Triple negative is more aggressive, its' grow and spread is faster than HR+ and HER2+, and it represents the 15-20% of all BC [26,27].

3. ESTROGEN RECEPTOR POSITIVE BREAST CANCER

Within HR+ BC, overexpression of ER is detected in 60-70% of cases [28]. Its binding with estrogens promotes cell proliferation in breast tissue, which has been associated with an increase in BC risk (as it is mentioned in *section 2.2.1.2*). Furthermore, products of the estrogen metabolism have been described as carcinogens [29].

Therefore, estrogen pathway, ER, and their implication in BC are further explained below:

3.1 Estrogen pathway

Estrogens are the main sex hormones in women. These steroids control the development and regulation of the reproductive system during women's life. Moreover, estrogens play a role in the regulation of different metabolic target tissues, including adipose tissue, skeletal muscle and liver, among others [30]. There are three forms of functional estrogens (Fig 3) [31]:

Estradiol (E2 or 17β-estradiol): The most potent and abundant estrogen during woman's reproductive years. It is indispensable for the development and growth of the mammary glands.

Estrone (E1): The second most potent estrogen. E1 can be transformed into estradiol. Its conjugate, the estrone sulfate, is inactive and it acts as an estrogen reservoir. After menopause, E1 plays a greater role in women, becoming the most common estrogen.

Estriol (E3): The less potent estrogen. It is obtained after the 16α-hydroxylation of E1 in the liver. E3 plays a larger role during pregnancy when it is produced in large quantities by the placenta.

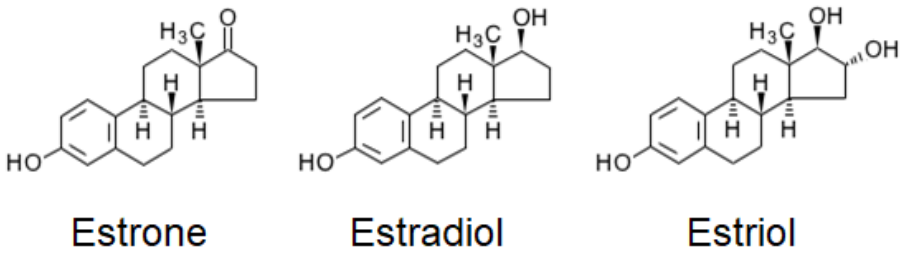

Figure 3. Chemical structure of the three types of estrogens: estrone, estradiol and estriol. Modified from: KEGG COMPOUND Database.

From puberty to menopause, the primary source of estrogens is synthetized in the ovaries. After menopause, ovaries stop the estrogen production but maintain the synthesis of its precursors: androstenedione and testosterone. These androgens are converted into estrone in peripheral tissues through aromatase

enzyme, i.e. adipose tissue, adrenal glands, bone, muscle, and skin [27,32]. (Fig 4)

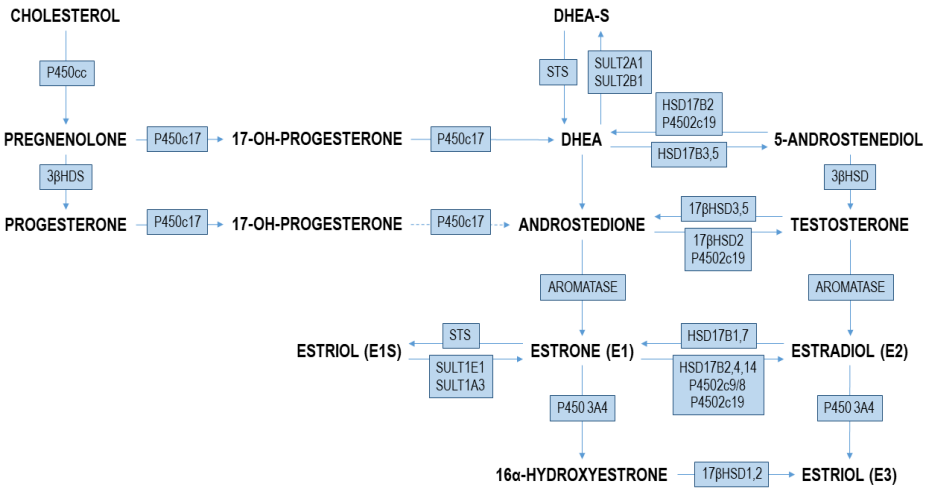

Figure 4. Main steroidogenic pathways involved in estrogen synthesis. Enzymes are shown in boxes and metabolites are emphasized in bold. Dash arrow indicates poor flux from 17-OH-progesterone (17-hydroxyprogesterone) to androstenedione via P450c17.

3.2 Estrogen receptor

ER is a nuclear hormone receptor that principally triggers the cellular proliferation and differentiation, and the regulation of apoptosis [33]. There are two isoforms, ERα [18] and ERβ [19]. ERα is an activator of estrogen effects, while dimerization of ERα-ERβ inhibits its actions [34]. Concentration of both subtypes depends on target tissue and age, but the main regulator of estrogen mechanism in breast tissues is ERα [35].

ER signaling pathway is displayed in Figure 5.

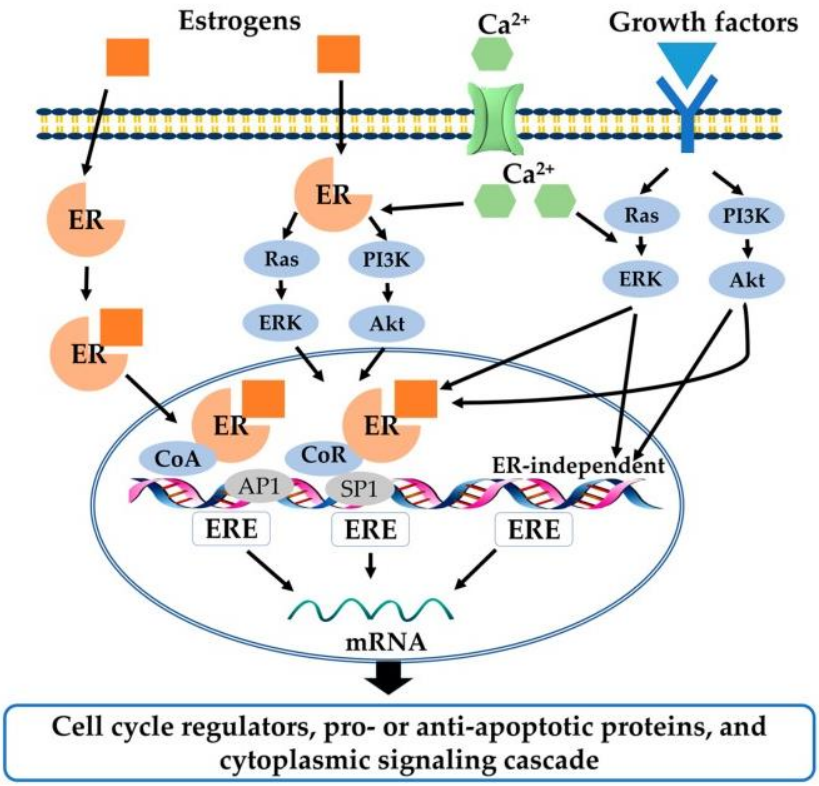

Figure 5. Signaling pathway of Estrogen Receptor. Estrogens (orange box) bind to estrogen receptor (ER). The estrogen-ER complex binds to estrogen-response element (ERE), recruiting distinct coregulatory proteins (co-activators, CoA; and co-repressors, CoR) and then, triggering the gene transcription. Conversely, an independent activation of the genomic transcription can be triggered by growth factors, through the activation of protein kinase cascades (i.e. ras; phosphatidylinositide 3-kinase, PI3K; extracellular signal–regulated kinase, ERK; and akt proteins). Modified from: Rong C, et al. Estrogen Receptor Signaling in Radiotherapy: From

Molecular Mechanisms to Clinical Studies. *Int J Mol Sci*. 2018;19(3).

3.3 Estrogen implication in breast cancer

High levels of endogenous estrogen in postmenopausal women have been associated with increased BC risk [36]. Hormone exposure is a major risk factor for sporadic breast cancer [4]. A key factor for the BC initiation is the oxidative metabolism of estrogens, which induces damage in DNA and thus, predisposition to BC (Fig 6) [29]. Moreover, proliferation of ER+ BC cells are promoted by estrogens via ER.

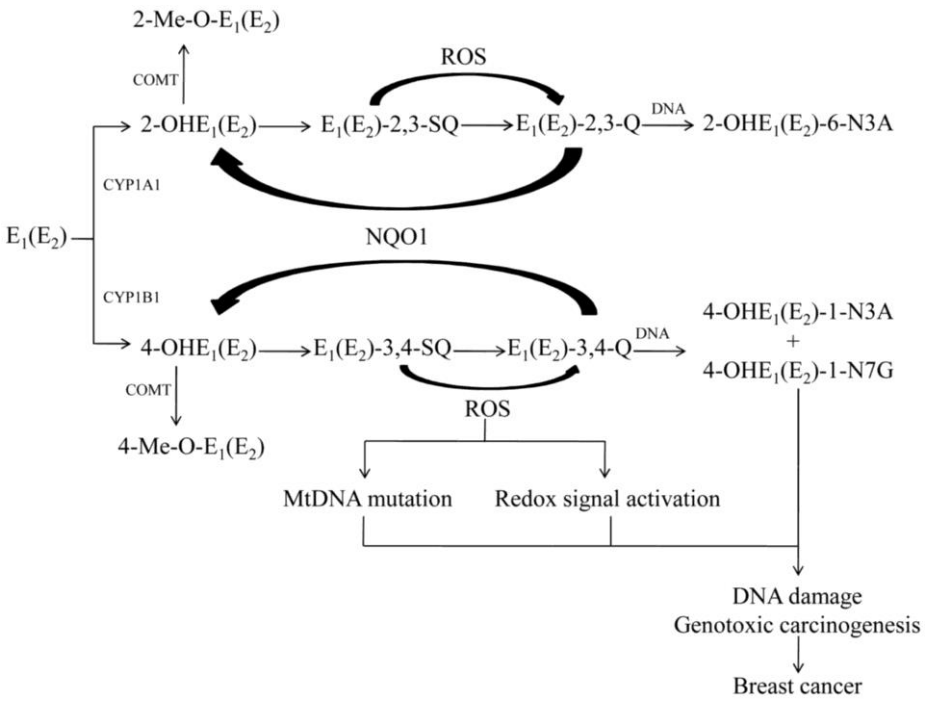

Figure 6. Metabolic pathway for estrogen carcinogenesis. The main pathway to obtain endogenous estrogens is the

formation of the 2-OH- and 4-OH-estrogens, which are known as catechol estrogens (CEs). Specially CE-3,4-quinones (CE-3,4-Q), are capable of starting the cancer process by binding to DNA and forming depurinating DNA adducts, 4-OHE1(E2)-1-N3Ade and 4-OHE1(E2)-1-N7Gua. The cleavage of these depurinating adducts generates apurinic sites in DNA that may induce mutations, and therefore, could initiate cancer. Additionally, catechol-O-methyltransferase **(**COMT) inhibition increments the amount of oxidative DNA damage and depurinating adducts. On the other hand, generation of free radicals and reactive oxygen species (ROS) by redox cycling of quinone (Q) and semiquinone (SQ) metabolites, stimulates mammary carcinogenicity progression through redox signal pathway activation, and increases genomic instability. *E1, estrone; E2, estradiol; NQO1, NAD(P)H-Quinone oxidoreductase 1; MtDNA, mitochondrial DNA; CYP, cytochrome P450.* Extracted from: Wen C. et al. Unifying mechanism in the initiation of breast cancer by metabolism of estrogen (Review). *Molecular Medicine Reports*. 2017;16(1001-1006).

4. THERAPIES FOR ESTROGEN RECEPTOR POSITIVE BREAST CANCER

Specialists recommend the treatment choice according to tumor characteristics, maximizing overall survival, disease-free survival and the quality of life.

Neoadjuvant treatments – those administered before the surgery – are advised for larger breast tumors (>2 cm), and inflammatory or locally advanced cancers. These preoperative procedures can reduce the size of tumor enough to allow the surgery. In case of early BC, neoadjuvant treatments can be suitable to treat invaded axillary nodes in order to downstage the cancer from N1 to N0 [37].

On the other hand, adjuvant treatments – those administered post-surgery – are designed to diminish patient exposure to potentially toxic therapies, and to avoid micrometastasis and recurrences [37].

This thesis is focused on therapies for ER+ BC and, in particular, on aromatase inhibitors as adjuvant therapy.

4.1 Surgery

Removal of the cancer cells in the breast is commonly a key step for BC treatment. The most commons are the breast-conserving surgery and the mastectomy [38,39].

The breast-conserving surgery is the first surgical choice. This type of surgery removes the breast tumor and a small zone around the abnormal tissue, preserving the remaining breast. Mastectomy removes the entire breast, leaving the chest muscle. Both are generally followed by radiation therapy in order to reduce the risk of recurrence [38,39].

In the same surgery or as a separate procedure, a biopsy of sentinel lymph nodes or an extirpation of axillary lymph nodes might be incorporated [38].

4.2 Radiotherapy

Radiotherapy uses high-energy X-rays to damage DNA in cancer cells, killing them or avoiding their replication.

After breast-conserving surgery, whole-breast radiotherapy is strongly recommended. It is directed to the entire breast, and it reduces the 10-year risk of any first recurrence and the 15-year risk of breast cancer-related mortality by 15% and 4%, respectively [40]. For patients with a low risk for local recurrence, a shorter treatment time is suitable (accelerated partial-breast radiotherapy) [39].

After mastectomy, it is recommended to irradiate the chest wall (post-mastectomy radiotherapy), and it often includes the regional lymph nodes that drain the breast. In node-positive patients, it diminish the 10-year risk of any recurrence by 10%, and the 20-year risk of breast cancer-related mortality by 8% [39,40].

4.3 Chemotherapy

Growth and division of cancer cells are faster than normal cells. Chemotherapy is a treatment that uses cytotoxic agents – alone or in combination – that stop the cell division, diminishing the

fast-growing cells progression or even killing them. Thus, chemotherapy affects tumor cells larger than normal cells. It can be administrated orally or intravenously, before or after the surgery. Its indication in ER+ BC depends on the risk of relapse and recurrence, and it is advisable if lymph nodes are affected [37,39].

4.4 Endocrine therapy

Endocrine therapy is an adjuvant treatment that stops estrogen production and/or action, in order to diminish the risk of promoting the grow of residual cancer cells. Patients with detectable ER expression are suitable for endocrine therapy, regardless the use of chemotherapy and/or targeted therapy.

The principal therapies for ER+ BC are tamoxifen (TAM) and aromatase inhibitors (AI). It can also be called as hormonal therapy or antiestrogenic therapy. Current recommendations distinguish between *'initial therapy'* (to complete 5 years of antiestrogenic therapy) and *'extended adjuvant therapy'* (for extending from 5, up to 10 years) (Fig 7). The extension of the treatment over the 5 years can be advisable for patients with high risk of relapse [39,41,42].

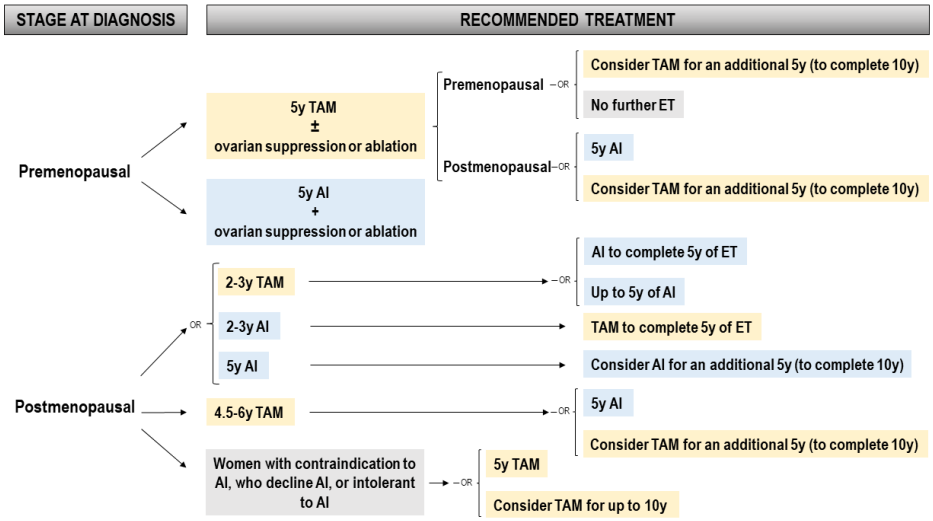

Figure 7. Endocrine treatment recommendations for estrogen receptor positive breast cancer according to NCCN guidelines. *AI, aromatase inhibitors; ET, endocrine therapy; TAM, tamoxifen; y, years.* Adapted from: NCCN Guidelines Version 1.2020 Breast Cancer.

4.4.1 Tamoxifen

TAM is a selective estrogen-receptor modulator (SERM). In breast tissue, TAM acts as a competitive inhibitor: it binds to ER, changing the receptor conformation and blocking the signal pathway (Fig 8).

TAM is the standard treatment for pre- and perimenopausal women with ER+ BC (Fig 7). Five years of TAM use in ER+ BC patients can reduce their annual BC death rate by 31% [43]. For larger reductions, ovarian suppression by gonadotropin-releasing hormone (GnRH) agonists or ovarian ablation might be

considered during TAM treatment. Data on overall survival of these patients remains immature, but SOFT trial reported an update showing an improvement of overall disease-free survival in TAM plus ovarian suppression in the premenopausal cohort who had received chemotherapy [44,45]. In case of becoming postmenopausal during the initial therapy of TAM, a switch to an AI seems to be positive [39,41].

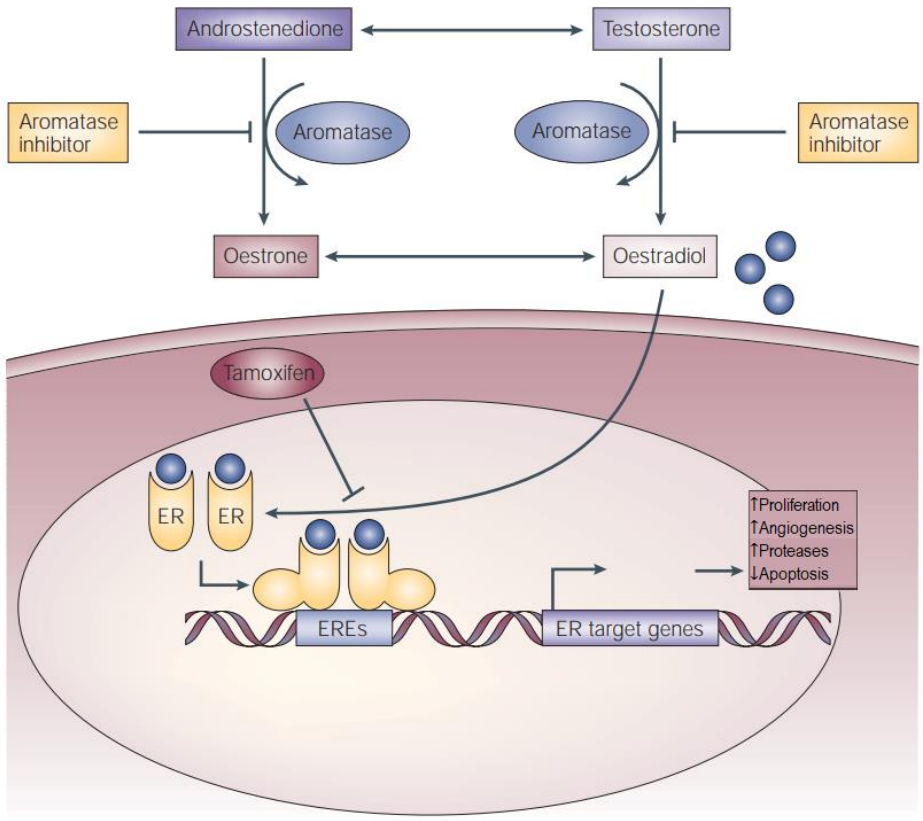

Figure 8. Schematic mechanism of action of tamoxifen and aromatase inhibitor to suppress estrogen signaling in an ER-positive cell. Tamoxifen competes against estrogens to bind estrogen receptor (ER), whereas aromatase inhibitor blocks the conversion of androgens into estrogens. Both actions impair ER

pathway, inhibiting cell grow and proliferation of estrogen-induced breast cancer cells. *EREs, estrogen-response elements (EREs).* Adapted from: Johnston SR, Dowsett M. Aromatase inhibitors for breast cancer: lessons from the laboratory. *Nature reviews Cancer*. 2003;3(11):821-831.

In contrast to breast tissue, TAM has an estrogen-like effect on bone metabolism, potentially by reducing bone resorption and turnover, and stimulating bone formation [46,47]. In postmenopausal women, TAM has been associated with maintenance of lumbar spine and femoral neck BMD [48]. Despite the long-term use in healthy premenopausal women was related to BMD reduction [49], Kim et al. described a preservation of BMD in premenopausal women diagnosed with BC [50].

On the other hand, TAM use has been associated with an increased risk of venous thromboembolism events, endometrial cancer and cataracts [51].

4.4.2 Aromatase Inhibitors

As it was described in *section 3.1*, estrogen synthesis in ovaries stops in the menopause, and then the main source of estrogen is obtained from the peripheral conversion due to the aromatase enzyme. AI block the aromatase enzyme, impeding the conversion of androstenedione and testosterone into estrogens (Fig 8). Hence, AI are not suitable for women with functional

ovaries due to their incapability to block ovarian production of estrogen [52].

The currently dispensed AI belong to the third generation, which are greater selective for the aromatase enzyme and better tolerated compared to the previous generations [53]. Inhibition of aromatase enzyme reduces over 98% of the circulating estrogen in postmenopausal women [54,55]. Among the third generation, we can distinguish two types of drugs depending on the nature of the binding to the aromatase: non-steroidal (reversible binding), which are anastrozole and letrozole; and steroidal (irreversible binding), which is exemestane [53]. Nonetheless, there are no significant differences in efficacy between them [56,57].

Several studies have found that AI use in postmenopausal women is more effective reducing the risk of BC recurrence and mortality than TAM [58-60]: compare to TAM, AI use can reduce the risk of recurrence by 30% during treatment, but not thereafter; and 10-year BC mortality rates by 15% after 5 years of monotherapy, which would correspond to 40% compared with no endocrine treatment [61]. Therefore, guidelines recommend switching from TAM to AI after reaching menopause, to complete the 5 years of antiestrogenic therapy [37,39,42]. Likewise, ovarian suppression plus AI should be considered for premenopausal women at high risk of recurrence [37,38,62].

Secondary effects of AIs are described in the following section.

5. SIDE EFFECTS OF AROMATASE INHIBITORS

Estrogen deprivation by AI use has several side effects. These affect the quality of life, treatment adherence and the associated mortality of patient [60].

5.1 Musculoskeletal events

Musculoskeletal events are the most common side effects of AI use. It is estimated to affect around 50% of patients [63], and several studies considered them the first cause of discontinuation[64,65]. Among musculoskeletal toxicities, arthralgia and bone loss induction stand out.

5.1.1 AI impact on joint pain

Arthralgia is described as pain in the joins, affecting wrists, hands, and knees. Generally, it is presented symmetrically [63]. Other common complaints or described symptoms are carpal tunnel syndrome, trigger finger, morning stiffness, myalgia, and decreased grip strength [66,67]. Development of arthralgia might occur from the first month to two years of treatment [68], but the most frequently is within the first three months [69]. A meta-analysis from *Beckwee et al. (2017)* reported prevalence of 46% [70].

The etiology and physiopathological mechanisms of AI-related arthralgia remain unknown. Although it has never been proved, the most common thought is that estrogen depletion causes the joint pain [71]. One hypothesis is that estrogens depletion might alter pain sensitivity, decreasing the pain threshold. Another

hypothesis is that estrogen drop increments the release of cytokines, exacerbating bone loss and aging, and leading to pain [72].

5.1.2 AI impact on bone

Estrogens contribute in bone health by promoting osteoblasts activity and inhibiting osteoclast resorption (Fig 9). Estrogen deficiency leads to an impairment in bone remodeling, unbalancing bone resorption and formation, which accelerates bone loss [73]. Several randomized control trials (RCT) have reported an enhanced decrease of bone mineral density (BMD) [74-76], leading to osteopenic or osteoporotic bone status.

Osteoporosis – or porous bone – is a condition where bone becomes fragile. It is characterized by bone mass reduction and deteriorated bone microarchitecture, leading to fragility fractures. Osteoporotic fractures are associated with an increment in morbidity and mortality, specially hip and vertebral fractures [77,78]. Osteopenia is the previous stage of osteoporosis, where bone mass is lower than “young normal” adult but not as much to be considered as pathologic [79]. Explanation of bone status classification is detailed in 6.2.1 section. Briefly, the World Health Organization established osteopenia and osteoporosis as having a bone densitometry T score at spine, hip, or forearm between -1 to -2.5, and ≥-2.5 standard deviations (SD), respectively [80].

Women in the menopause reduce drastically the circulating estrogen levels and AI treatment declines the remaining estrogen levels produced by peripheral tissues. AI patients have

at least 2-fold higher bone loss than healthy, age-matched postmenopausal women [81] and, compared to TAM patients, AI users have 35% more risk of fracture [82]. Likewise, bone microarchitecture is deteriorated during AI treatment [83,84].

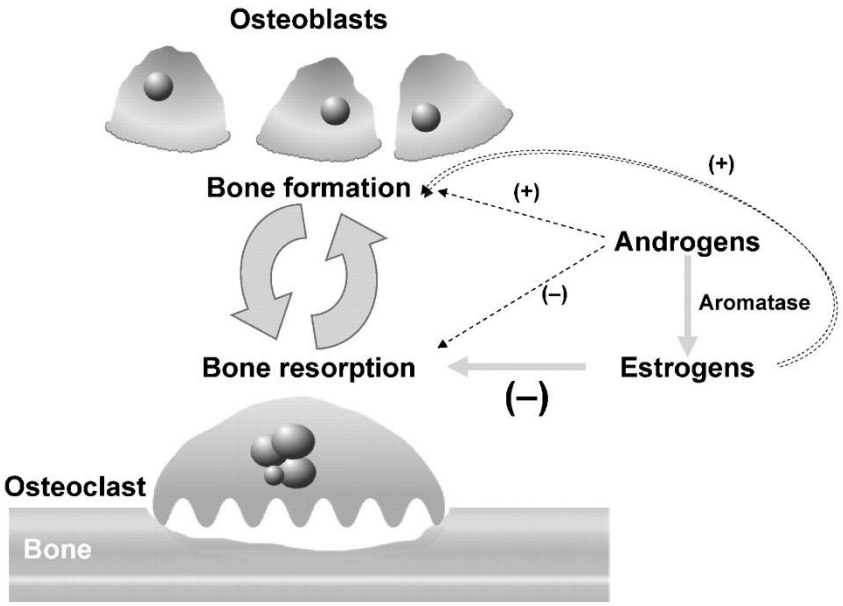

Figure 9. Estrogens action for bone health maintenance. Estrogens enhance bone formation by expanding osteoblasts lifespan and inhibiting its apoptosis, while inhibits osteoblasts function and contributes to its apoptosis. Obtained from: Angela Hirbe et al. Skeletal Complications of Breast Cancer Therapies. *Clin Cancer Res.* 2006;(12) (20) 6309s-6314s.

5.2 Cardiovascular events

Cholesterol levels are commonly used as predictors of cardiovascular events. *Bell et al. (2012)* observed an alteration of the lipid profile at three months of AI treatment: levels of high-

density lipoprotein (HDL) diminished, levels of low-density lipoprotein (LDL) increased, and therefore, LDL/HDL ratio was higher [85]. BIG 1-98 and ATAC trials reported an increased hypercholesterolemia in AI users compared to TAM users [86,87]. Conversely, no significant differences were observed between extended adjuvant letrozole therapy and placebo in MA-17 trial [88]. In the same line, results from other RCT and meta-analyses evaluating cardiovascular events are heterogeneous [89,90]. Hence, there is no clear effect of AI on cardiovascular risk.

6. MANAGEMENT OF SECONDARY EFFECTS CAUSED BY AI TREATMENT

6.1 Management of arthralgia

Arthralgia is one of the principal factors involved in AI therapy discontinuation. Hence, diminishing the impact of arthralgia in patients would decrease treatment dropout rates. Pain evaluation is complex since patient's perception can include several physiological processes. Self-report is considered the gold standard way of communication in patients with verbal capacities, while external signs of pain like crying are secondary. Likewise, assessment of pain severity should be performed before and after potentially painful interventions. Different assessment tools are reported in figure 10 [91].

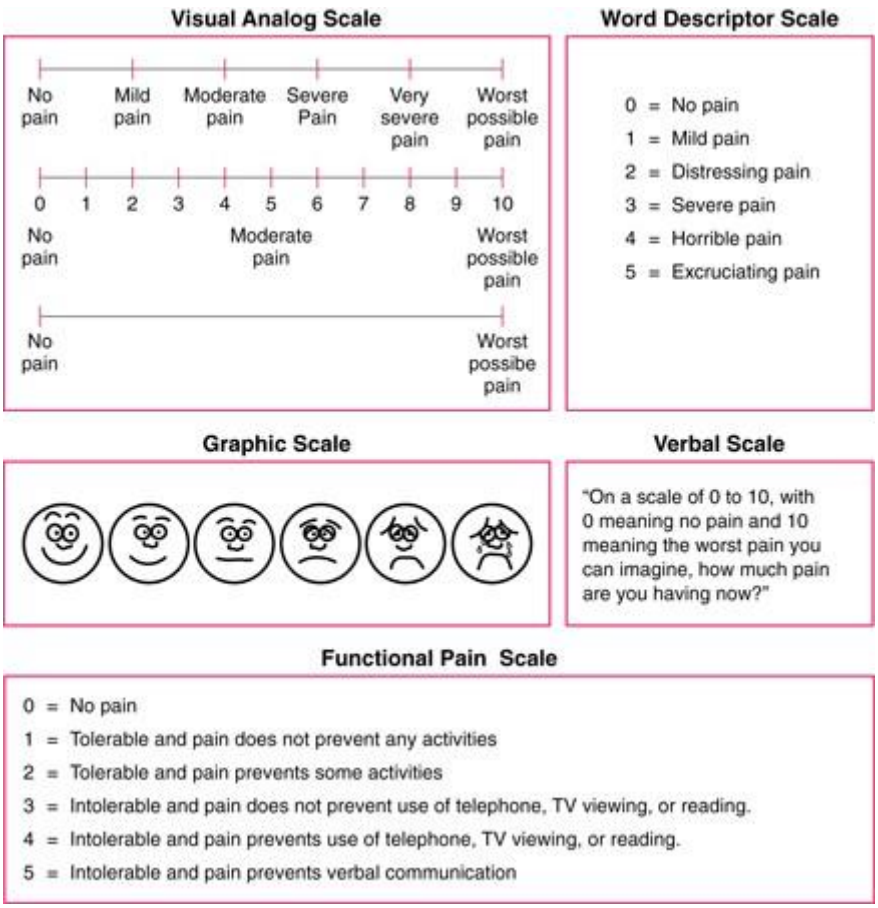

Figure 10. Examples of pain scales for quantifying pain as it is occurring. Extracted from: www.msdmanuals.com/professional/neurologic-disorders/pain/evaluation-of-pain#

Although there is no clear consensus about how manage AI-related arthralgia, guidance and education before stating AI has been described as a key factor. Physicians can recommend different lifestyle modifications that might reduce joint symptoms, including exercise and weight reduction [92,93]. Switching to another AI can be a different approach in cases of extreme pain since some patients experimented a decline in the intensity of side effects after switching [94,95]. Other interventions that have

been studied included acupuncture, diuretic therapy, corticosteroids, antidepressants, supplementation of vitamin D, and supplementation of omega-3 fatty acids. Likewise, the use of bisphosphonates (BP) has been associated with a lower incidence of arthralgias [96].

6.2 Management of bone loss

A baseline evaluation of fracture risk before treatment starting and bone status monitoring during AI therapy is strongly recommended in order to preserve and/or restore bone health. Baseline evaluation should include a detailed medical history, physical examination, laboratory assessment, and BMD assessment, to detect fracture risk factors [97]. Although there is no optimal schedule for establishing a periodic assessment during AI use, there is a treatment algorithm originally designed by *Hadji et al. (2008)* and adapted by *Rachner et al. (2018)* (Fig 11) [98,99].

6.2.1 Bone mineral density assessment and antiresorptive treatment

BMD is evaluated by bone densitometry (also called dual-energy x-ray absorptiometry, DXA or DEXA) in lumbar spine, femoral neck and total hip. Obtained BMD values are compared with young adult values using T-scores to determine bone status: a T-score equal or higher than -1 SD is considered normal, a T-score lower than -1 SD but higher than -2.5 SD is classified as osteopenia, and a T-score equal or lower than -2.5 SD is

diagnosed as osteoporosis. Patient is categorized according to T-score value [80].

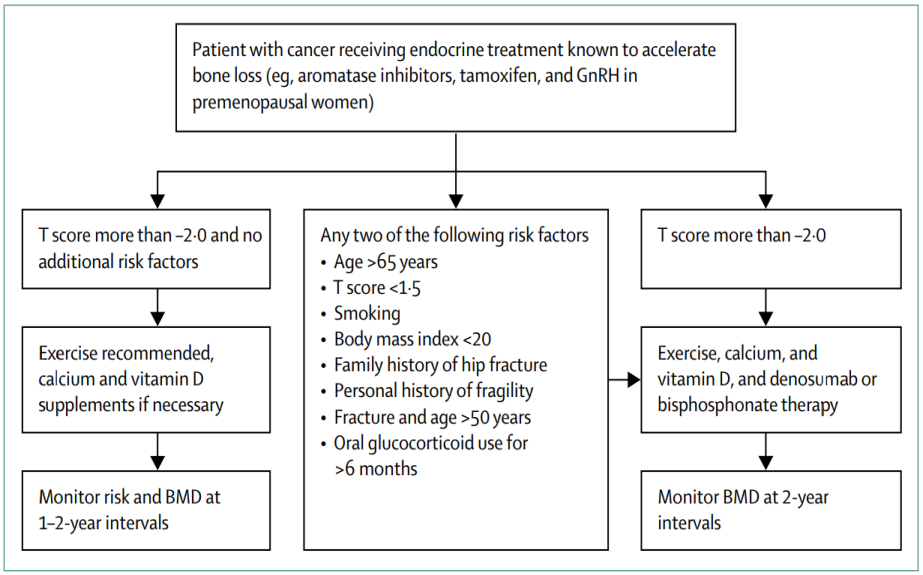

Figure 11. Proposed algorithm for managing bone health in patients with breast cancer receiving aromatase inhibitor therapy. These recommendations were based on trials results from breast cancer patients and healthy populations. Initial stratification uses the lower T-score from lumbar spine, femoral neck and total hip. If patients' bone mineral density decreases by ≥ 10% annually (using the same dual-energy x-ray absorptiometry machine), evaluation of secondary causes of bone loss as vitamin D deficiency and initiation of antiresorptive therapy is advisable. *BMD, bone mineral density; GnRH, gonadotropin-releasing hormone.* Obtained from: Rachner TD et al. Bone health during endocrine therapy for cancer. *Lancet Diabetes Endocrinol.* 2018;6(11):901–910.

Exercise and supplements of calcium and vitamin D are recommended for all AI treated patients. As well as general population, those patients diagnosed with osteoporosis should be treated with antiresorptives. Use of antiresorptive drugs is also advisable in case of women with a T-score ≤ -2 SD at any site, plus prevalent fragility fractures or one major risk factor (e.g. family history of femoral fracture, previous osteoporotic fracture, early menopause, and smoking) [100].

Antiresorptive treatments inhibit osteoclast resorption, increasing BMD and reducing fracture risk. For the management of AI-related bone loss, clinical guidelines recommend BP or, in case of BP intolerance or low adherence, denosumab [100]: BP induce mature osteoclast apoptosis, and decrease differentiation and recruitment of osteoclast precursors. Thus, cellular remodeling process is not completely stopped, but the number of mature active osteoclasts is greatly reduced [101]. On the other hand, denosumab is a monoclonal antibody against the receptor activator of the NF-κB ligand (RANKL). RANKL binding to receptor RANK in osteoclast surface promotes bone resorption, and high concentrations of this molecule promote osteoclast development [99].

6.2.2 Other risk factors assessment

Despite BMD predicts about 70% of bone strength [102], additional assessments can provide extra information that may identify other patients that would not be previously considered at high risk of fracture.

6.2.2.1 Bone microarchitecture and trabecular bone score

Deterioration of bone microarchitecture increases bone fragility and therefore fracture risk. Trabecular bone score (TBS) is a textural index that evaluates bone microarchitecture through an assessment of the pixel gray-level variations from a lumbar spine DXA image (Fig 12). This noninvasive analytical method works for any available DXA image, even if it was obtained years before [103].

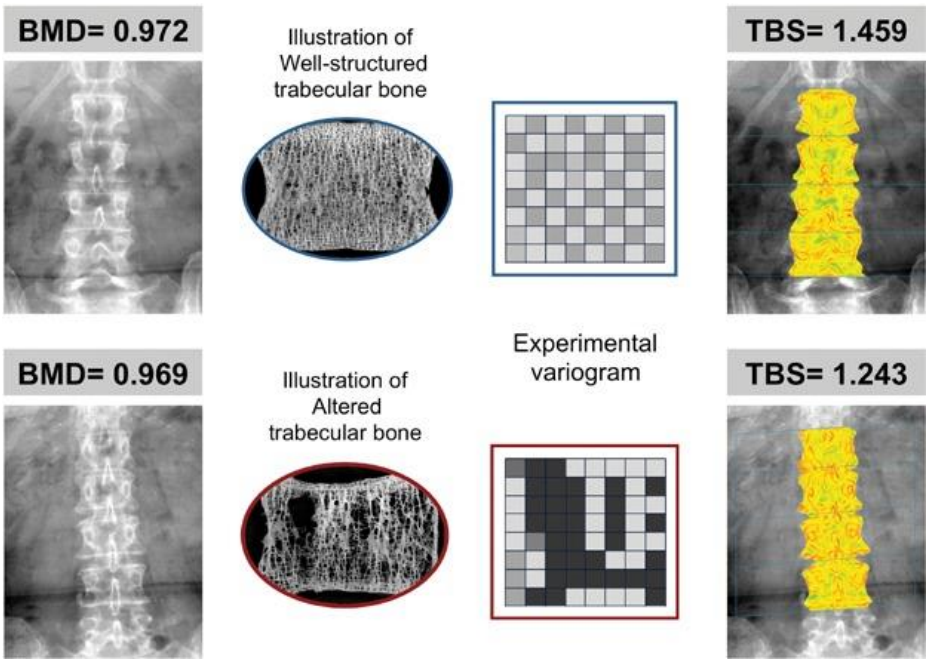

Figure 12. Representation of Trabecular Bone Score principles. The TBS software analyses the DXA scan. An algorithm evaluates the spatial organization of pixel intensity, obtaining an overall score – the trabecular bone score (TBS) –. As it is exemplified in the figure, TBS is independent from bone

mineral density (BMD): despite a very similar BMD values between the two represented patients, TBS values differs. As a principle, a high TBS value represents a dense trabecular microstructure, more numerous and connected and less sparse trabeculae; while a low value represents a porous trabecular structure, less numerous and connected, and high trabecular separation. Extracted from: Barbara C Silva et al. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image. *Journal of Bone and Mineral Research*. 2014;29(3):518-530.

By analogy with the three BMD categories, a range for postmenopausal women was proposed: TBS ≥1.350 is considered normal, between 1.200 and 1.350 is considered partially degraded, and TBS ≤1.200 is defined as degraded microarchitecture [103].

In AI users, TBS may potentially help to distinguish between patients with a threshold BMD (near to osteoporosis diagnosis) who are at high risk for fracture versus those who are not, and therefore, enhance monitoring and/or initiate antiresorptive treatment if necessary.

6.2.2.2 Bone turnover markers

The metabolic activities of osteoclasts and osteoblasts can be captured by bone remodeling markers. Estrogens decline during menopause leads to an increase of bone resorption markers such as C-telopeptide (CTX) and N-telopeptide (NTX), and a reduction of bone formation markers like bone-specific alkaline

phosphatase (BAP), osteocalcin (OC) and amino-terminal propeptide of type I procollagen (PINP) [104]. AI therapy has been associated with a greater increase in bone resorption markers than average postmenopausal values, whereas bone formation markers may either decrease or increase [105].

Ideally, change of bone turnover markers after AI therapy outset would be useful for predicting and identifying women at high-risk of bone loss. Although there is no available prediction model for AI treated women, evaluation of bone remodeling markers can help physician to control the effect of AI as well as effectiveness of antiresorptive treatment.

6.2.2.3 Fracture Risk Assessment Tool

Fracture Risk Assessment Tool (FRAX®) is a predictive computer algorithm that predicts the 10-year risk of hip fracture, and the 10-year risk of major osteoporotic (i.e. clinical spine, forearm, hip or shoulder) fracture [106]. However, FRAX might not be very suitable for AI patients since this tool was not designed for women with breast cancer, and AI therapy may be considerably underestimate [99,100].

OBJECTIVES

The main objective is to evaluate the impact of AI side effects and its risks factors in order to improve the quality of life of patients and therefore, to avoid or reduce the treatment discontinuation.

Thus, the specific objectives planned in this thesis are the following:

1. Evaluation of vitamin D levels of patients starting AI treatment in the B-ABLE cohort.

1.1. To determine the vitamin D status of postmenopausal women diagnosed with ER+ BC before starting AI treatment.

1.2. To detect factors contributing to vitamin D levels in ER+ BC patients.

2. Assessment of bone health in ER+ BC patients one year after complete AI treatment in the B-ABLE cohort.

3. Assessment of life quality and treatment discontinuation of AI-treated patients in the B-ABLE cohort.

3.1. To evaluate the evolution of joint pain and health-related quality of life during AI treatment until 1-year after AI completion in the B-ABLE cohort.

3.2. To determine the proportion of early cessation of AI treatment caused by AI intolerance in the B-ABLE cohort.

3.3. To estimate the effect of previous TAM exposure on AI discontinuation risk in the B-ABLE cohort.

4. Analysis of fracture incidence and risk during AI therapy and evaluation of the effectiveness of oral bisphosphonates in reducing fracture risk: the SIDIAP study.

4.1. To estimate the incidence of fractures during AI treatment in the SIDIAP database.

4.2. To estimate the fracture risk during AI treatment compared with TAM treatment in the SIDIAP database.

4.3. To evaluate the effectiveness of oral bisphosphonates in AI-treated patients at high risk of fracture in SIDIAP database.

5. Analysis of cardiovascular risk, thromboembolic risk, and overall survival benefit of AI compared to TAM treatment: The SIDIAP study.

REPORT OF THE DIRECTORS

Thesis title: “Epidemiological study of aromatase inhibitors in women diagnosed with estrogen receptor positive breast cancer: evaluation and management of secondary effects”

Author: Marta Pineda Moncusí

Supervisors: Dra. Natalia Garcia Giralt and Dr. Francesc Xavier Nogués Solan

Article 1

Title: Vitamin D levels in Mediterranean breast cancer patients compared with those in healthy women

Authors: Pineda-Moncusí M, Garcia-Perez MA, Rial A, Casamayor G, Cos ML, Servitja S, Tusquets I, Diez-Perez A, Cano A, Garcia-Giralt N, Nogues X.

Journal: Maturitas **Number:** 116 (2018) **Pages:** 83-88

Impact Factor (2018 JCR Science Edition): 3.654

Contribution of the PhD candidate:

Marta Pineda was the principal data manager of the databases involved in the study. She performed most of the analyses and participated in the study design. She participated in the writing, revision and final editing of the manuscript. Moreover, this study won the “*X Premio AEFA a la Calidad y a la Innovación*” award held in Bilbao (26th of October of 2018) under the name: “*Estudio de los niveles de vitamina d en mujeres con cáncer de mama comparados con mujeres sanas en población mediterráne*a”

Article 2

Title: Bone health evaluation one year after aromatase inhibitors completion

Authors: Pineda-Moncusí M, Servitja S, Casamayor G, Cos ML, Rial A, Rodriguez-Morera J, Tusquets I, Diez-Perez A, Garcia-Giralt N, Nogués X.

Journal: Bone **Number:** 117 (2018) **Pages:** 54-59

Impact Factor (2018 JCR Science Edition): 4.360

Contribution of the PhD candidate:

As in the previous article, Marta Pineda was the main author of this article. She was involved in the design of the study and she did the analyses and the assessment of bone parameters. She participated in the interpretation and discussion of the results as well as the elaboration of the manuscript.

Article 3

Title: Assessment of early therapy discontinuation and health-related quality of life in breast cancer patients treated with aromatase inhibitors: B-ABLE cohort study

Authors: Pineda-Moncusí M, Servitja S, Tusquets I, Diez-Perez A, Rial A, Cos ML, Campodarve I, Rodriguez-Morera J, Garcia-Giralt N, Nogués X.

Journal: Breast Cancer Research and Treatment **Number:** 177(1) (2019) **Pages:** 53-60

Impact Factor (2019 JCR Science Edition): 3.831

Contribution of the PhD candidate:

Marta Pineda was involved in the study design and she performed most of the statistical analyses. She participated in the discussion of the results and she wrote the article.

Article 4

Title: Increased fracture risk in women treated with aromatase inhibitors versus tamoxifen: beneficial effect of bisphosphonates

Authors: Pineda-Moncusí M, Garcia-Giralt N, Diez-Perez A, Servitja S, Tusquets I, Prieto-Alhambra D, Nogués X.

Journal: Journal of Bone and Mineral Research

Number: 35(2) (2020) **Pages:** 291-297

Impact Factor (2019 JCR Science Edition): 5.854

Contribution of the PhD candidate:

Marta Pineda did the overall data analysis and interpretation of the results; she also did the elaboration of figures and tables and drafting of the manuscript and was involved in the revision and final editing of the manuscript.

Article 5

Title: Thromboembolic, cardiovascular and overall mortality risks of aromatase inhibitors, compared to tamoxifen treatment

Authors: Pineda-Moncusí M, Garcia-Giralt N, Diez-Perez A, Tusquets I, Servitja S, Albanell J, Prieto-Alhambra D, Nogués X.

Journal: Therapeutic Advances in Medical Oncology

Number: 12 (2020) **Pages:** 1-10

Impact Factor (2019 JCR Science Edition): 6.852

Contribution of the PhD candidate:

Marta Pineda managed the SIDIAP database and she performed the epidemiological analyses and figures. She also participated in the interpretation and validation of the results; she contributed to the drafting and revision of the manuscript.

Barcelona, 7th September 2020

Supervisors' signature

Dra. Natalia Garcia Giralt

Dr. Francesc Xavier Nogués Solan

RESULTS

Article 1

Title: Vitamin D levels in Mediterranean breast cancer patients compared with those in healthy women

Summary:

To assess the vitamin D status of postmenopausal women with early estrogen-receptor-positive breast cancer and compare it to healthy postmenopausal women from the same Mediterranean region, data from 691 breast cancer patients (BC) in the B-ABLE cohort were analyzed: subsequent to recent cancer intervention (recent-BC) or after a minimum of two years from this intervention (long-term-BC). Additionally, patients were stratified by prior chemotherapy exposure (ChT+ and ChT-). Plasma concentrations of 25-hydroxyvitamin D [25(OH)D] (25(OH)D) were contrasted with data from 294 healthy women (non-BC) to estimate β-coefficients through linear regression. Age, body mass index and season of blood collection were used as confounders, and non-BC participants were used as reference group. A 23.7% of recent-BC patients had 25(OH)D deficiency, followed by 17.7% in long-term-BC group, and just 1.4% of non-BC participants. Most women were in the insufficient 25(OH)D category regardless of study group. BC patients had significantly lower 25(OH)D levels than non-BC participants (adjusted β-coefficients: -4.84 [95%CI: -6.56 to -3.12] in recent-BC, and -2.05 [95%CI: -4.96 to -0.14] in long-term-BC). Among BC patients, the lowest 25(OH)D levels were found in recent-BC (ChT+) ($p<0.001$). There were no differences between long-term-BC (ChT-), long-term-BC (ChT+) and recent-BC (ChT-).

Considering only BC patients ChT+, results showed significant reduced 25(OH)D levels in recent-BC compared to long-term-BC ($p<0.001$).

In conclusion, breast cancer patients exhibited severely reduced 25(OH)D, especially after recent chemotherapy. These 25(OH)D levels would be partially recovered at long-term but remaining much lower than in the healthy population.

Reference:

Pineda-Moncusí M, Garcia-Perez MA, Rial A, Casamayor G, Cos ML, Servitja S, Tusquets I, Diez-Perez A, Cano A, Garcia-Giralt N, Nogues X. Vitamin D levels in Mediterranean breast cancer patients compared with those in healthy women. Maturitas. 2018 Oct;116:83-88. Epub 2018 Jul 29. PubMed PMID: 30244785. doi: 10.1016/j.maturitas.2018.07.015.

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Vitamin D levels in Mediterranean breast cancer patients compared with those in healthy women

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B-ABLE cohort study

ABSTRACT

Objectives: To evaluate the vitamin D status of postmenopausal women with early estrogen-receptor-positive breast cancer and to compare it with that of healthy postmenopausal women from the same Mediterranean region.

Study design and outcome measures: Data from 691 breast cancer (BC) patients in the B-ABLE cohort were analyzed after recent cancer intervention (recent-BC) or after a minimum of two years since this intervention (long-term-BC). Patients were also stratified by previous chemotherapy exposure (ChT+ and ChT−). Plasma levels of 25-hydroxyvitamin D [25(OH)D] (25(OH)D) were compared with data from 294 healthy women (non-BC) by linear regression to estimate β-coefficients using non-BC participants as the reference group. Age, body mass index and season of blood extraction were selected as potential confounders.

Results: Of the recent-BC patients, 23.7% had 25(OH)D deficiency, compared with 17.7% of the long-term-BC group, and just 1.4% of the non-BC participants. Most of the women were located in the insufficient 25(OH)D category regardless of study group. BC patients had significantly lower 25(OH)D levels than non-BC participants (adjusted β-coefficients: −4.84 [95%CI -6.56 to -3.12] in recent-BC, and −2.05 [95%CI −4.96 to −0.14] in long-term-BC). Among BC patients, the lowest 25(OH)D levels were found in the recent-BC (ChT+) group ($p < 0.001$). No differences were found between the long-term-BC (ChT−), long-term-BC (ChT+) and recent-BC (ChT−) groups. Among the BC ChT+ patients, the recent-BC group had significantly lower 25(OH)D levels than the long-term-BC group ($p < 0.001$).

Conclusion: Severely reduced 25(OH)D levels were detected in patients with breast cancer, particularly after recent chemotherapy. These 25(OH)D levels had partially recovered over the long term, but still remained much lower than in the healthy population.

1. Introduction

Many genes are regulated by the active form of vitamin D (1,25-dihydroxyvitamin D) in tissues such as the immune system, bone, muscles, lungs, heart, and kidney, among others. Although its primary biological action, which is mediated by vitamin D receptor (VDR), is to regulate serum calcium levels and promote bone mineralization, several studies have described vitamin D as a potent non-proliferation, pro-differentiation and immunomodulation factor [1]. Its precursor, 25-hydroxyvitamin D (25(OH)D) is the circulating form of vitamin D which is considered the best marker to assess vitamin D levels [2–5].

The role of vitamin D in several physiological processes has been widely studied, but its role in breast cancer has been under discussion for years. Preclinical studies suggest a protective effect of vitamin D on

Abbreviations: VDR, vitamin D receptor; 25(OH)D, vitamin D; B-ABLE, Barcelona–Aromatase induced Bone Loss in Early breast cancer; BC, breast cancer patients; BMI, body mass index; PCA, principal component analysis; ChT, chemotherapy; ChT+, patients with chemotherapy; ChT−, patients without chemotherapy

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Table 1
Patient characteristics.

Variable	recent-BC (N = 460)	long-term-BC (N = 231)	non-BC (N = 294)
Age (median [Q1;Q3])	63.0 [57.0;67.0]	57.0 [52.0;66.0]	58.0 [53.0;62.0]
BMI (median [Q1;Q3])	28.5 [26.0;32.4]	26.8 [23.7;30.9]	26.2 [23.6;29.2]
Patients exposed to ChT (n;%)	253 (55.0%)	157 (67.7%)	0 (0%)
Serum levels of 25(OH)D (median [Q1;Q3])	15.8 [10.3;21.6]	17.5 [12.0;24.6]	22.6 [18.1;28.7]
Sorted by season of baseline blood sample collection (median [Q1;Q3](n;%))			
January–March	13.4 [8.64;20.5] (124;27.0%)	14.1 [8.90;22.0] (67;29.0%)	22.6 [18.1;28.7] (118;40.1%)
April–June	15.6 [11.9;21.7] (85;18.5%)	18.1 [12.0;23.9] (58;25.1%)	23.2 [18.8;28.2] (87;29.6%)
July–September	19.0 [11.8;24.0] (120;26.1%)	20.6 [16.4;28.8] (42;18.2%)	20.4 [17.2;28.2] (27;9.18%)
October–December	14.7 [9.11;19.7] (131;28.5%)	18.2 [13.2;27.1] (64;27.7%)	25.3 [18.0;32.2] (62;21.1%)

Abbreviations: BC, breast cancer; BMI, body mass index; ChT, chemotherapy; Q, quartile.

cancer risk and progression [6,7], but clinical and epidemiologic studies have reported controversial results [8–12]. However, the available evidences that vitamin D levels may affect breast cancer survival [13,14] are sufficient to suggest that vitamin D status should be monitored. Previous studies in the B-ABLE cohort -a prospective, clinical cohort study of women diagnosed with early breast cancer and candidates for aromatase inhibitor treatment- showed that low levels of serum 25(OH)D are associated with worsening joint pain and increased loss of bone mass [15–17]. Exploring the behavior of vitamin D in these patients could be important for preventing musculoskeletal disorders as well as other events affecting quality of life.

In the present study, 25(OH)D status has been exhaustively explored in the B-ABLE cohort and compared to healthy postmenopausal women from the same Mediterranean region.

2. Methods

2.1. Study design and participants

This observational cohort study compared serum vitamin D levels (25(OH)D) in Mediterranean postmenopausal women diagnosed with early estrogen-receptor-positive breast cancer and a control group of healthy postmenopausal women. Postmenopausal status was defined as > 55 years old with amenorrhea for > 12 months, or ≤55 years with levels of luteinizing hormone > 30 mIU/mL or follicle-stimulating hormone values > 40 mIU/mL.

Data from 691 breast cancer patients (BC) were collected in the B-ABLE cohort (Barcelona–Aromatase induced Bone Loss in Early breast cancer) [17] from January 2006 to Jun 2017 in Hospital del Mar (Barcelona, Spain), of which 460 were postmenopausal women with breast cancer included at 6 weeks post-surgery or 1 month after the last cycle of chemotherapy (recent-BC); the remaining 231 women were included once menopause started after taking tamoxifen for a minimum of 2 years and up to 5 years (long-term-BC). Data were collected for a large number of demographic and clinical variables, including age at recruitment, body mass index (BMI), and serum levels of 25(OH)D, among others. Exclusion criteria included previous history of any metabolic or endocrine diseases, alcoholism, rheumatoid arthritis, and oral corticosteroids.

Healthy postmenopausal women were recruited as a control group (non-BC, n = 294) in the Hospital Clínico Universitario de Valencia from January 2004 to October 2009. Registered variables included age, BMI, and serum levels of 25(OH)D, among others. Exclusion criteria were the same as for the B-ABLE cohort.

2.2. Variables

The main outcome of the study was the serum level of 25(OH)D, obtained from peripheral blood using competitive immunoluminometric direct assay with direct-coated magnetic microparticles (coefficient of variation < 10%) (Elecsys Vitamin D total II, model 07028148190; Cobas e801 system, Roche Diagnostics GmbH, Mannheim, Germany). Season of blood extraction was registered and 25(OH)D levels were classified as optimal (≥30 ng/ml), insufficient (< 30 ng/ml and ≥10 ng/ml), or deficient (< 10 ng/ml) [18]. Age, BMI and chemotherapy (ChT) status were also used in the statistical analysis.

2.3. Statistical methods

Differences between selected populations were assessed by Kruskal–Wallis test and Chi-square test. Differences in percentages of patient distribution in 25(OH)D categories were evaluated using the Chi-square test.

Linear regression models were performed to estimate the β-coefficients in 25(OH)D levels between study groups, using non-BC participants as reference group. Age, BMI, and season of blood extraction data were selected as confounders. Linearity, interaction, and lack of perfect multicollinearity of independent variables were tested.

A sub-analysis was conducted, stratifying the study groups according to chemotherapy (ChT) status: patients treated (ChT+) or non-treated (ChT−) with chemotherapy. No non-BC participants had been exposed to ChT.

All statistical analyses were performed with R for Windows version 3.3.3 using foreign, Hmisc, compareGroups, pgirmess, fifer, pca3d, plyr, boot, ggplot2, car, and stats packages. P < 0.05 was considered significant, and all statistical contrasts were corrected by Bonferroni test per multiple comparisons.

3. Results

3.1. Patient characteristics

A total of 985 participants were included, 691 patients from the B-ABLE cohort and 294 healthy post-menopausal women. Their characteristics are reported in Table 1. Recent-BC patients were significantly older (p < 0.001) and had higher BMI (p < 0.01) than long-term-BC and non-BC participants. Different exposure to previous ChT treatment was observed between BC groups (p < 0.001). Differences in serum levels of 25(OH)D were detected between the three groups (p < 0.001), but no significant differences were found in season of blood sample collection.

3.2. Participants distribution by vitamin D categories

Subject distribution by categories of 25(OH)D showed significant differences between the three groups (p < 0.05) (Fig. 1). The recent-BC patients had the higher percentage of 25(OH)D deficiency (23.7%), followed by 17.7% in long-term-BC group, and just 1.4% of non-BC participants. Most of the women were located in the insufficient 25(OH)D category regardless of study group.

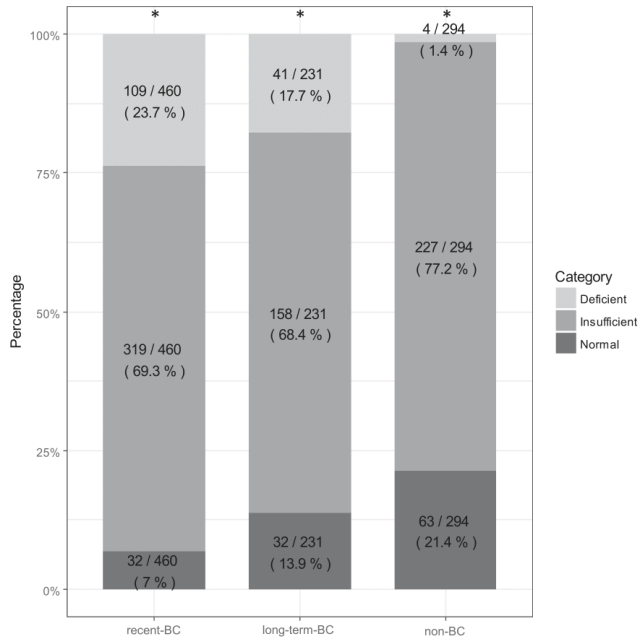

Fig. 1. Participant distribution in each study group according to vitamin D categories. Abbreviations: BC, breast cancer; Percentage, percentage of patients. * Bonferroni post hoc comparison of Chi-square test: $p < 0.001$.

3.3. Linear regression analysis of vitamin D

In the adjusted linear regression analysis using non-BC group as reference, significant differences in 25(OH)D levels were found between three groups: non-BC vs recent-BC, $p < 0.001$; non-BC vs long-term-BC, $p < 0.05$; and recent-BC vs long-term-BC patients, $p < 0.001$ (Table 2). Additionally, a significant interaction between recent-BC patients and ChT status ($p < 0.01$) suggested differences in 25(OH)D levels according to previous ChT exposure.

3.4. Subanalysis stratifying by chemotherapy exposure

In the subanalysis stratified by ChT status (Table 3), all patients with breast cancer had significantly lower 25(OH)D levels than non-BC participants. Among BC patients, the lowest 25(OH)D levels were found in recent-BC with previous ChT ($p < 0.001$). No differences were found between recent-BC (ChT−), long-term-BC (ChT−) and long-term-BC (ChT+) groups.

Patient distribution by categories of 25(OH)D, stratified by ChT status, corroborated previous regression results (Fig. 2): 25(OH)D levels of recent-BC group were significantly lower in patients with previous ChT than in those without ChT ($p < 0.001$). However, in long-term-BC patients, no 25(OH)D differences according to previous ChT were observed. Hence, considering only the 25(OH)D levels of patients with previous ChT, there were significant differences ($p < 0.001$) between patients just finalizing ChT [recent-BC (CT+)] and those after 2 to 5 years from intervention [long-term-BC (CT+)] (Fig. 2).

4. Discussion

Breast cancer patients in the B-ABLE cohort exhibited significantly lower 25(OH)D levels compared to a control group of healthy postmenopausal women from the same geographical region. The lowest 25(OH)D levels were observed in patients with recent chemotherapy.

Although our Mediterranean population could be expected to have relatively optimal 25(OH)D levels due to the region's solar patterns, a large majority of the women had insufficient 25(OH)D levels regardless of study group. Even though healthy women had higher 25(OH)D levels than BC patients, normal levels were found only in 21.4%. Vitamin D3 insufficiency and deficiency could be related to cultural factors limiting

Table 2
Values of constant α and β coefficients of 25(OH)D serum levels in recent-BC and long-term-BC patients compared with non-BC women.

Group	Unadjusted α value [95%CI]	Unadjusted β coefficient [95%CI]	Adjusted α value [95%CI]	Adjusted β coefficient [95%CI][a]
non-BC (ref)	23.76 [22.67 to 24.85]		33.27 [27.91 to 38.63]	
recent-BC		−6.88 [−8.27 to −5.49]		−4.84 [−6.56 to −3.12]
long-term-BC		−4.27 [−5.91 to −2.64]		−2.05 [−4.96 to −0.14]

Abbreviations: BC, breast cancer patients; CI, confidence interval; ref, reference category. In 95%CI, reference group is non-BC patients.
[a] Adjusted by age, body mass index, season of blood extraction and chemotherapy status.

Table 3
Subanalysis of vitamin D serum levels according to patient exposure to chemotherapy and compared with non-BC women: constant α value and β coefficients in recent-BC and long-term-BC patients.

Group	Unadjusted α value [95%CI]	Unadjusted β coefficient [95%CI]	Adjusted α value [95%CI][a]	Adjusted β coefficient [95%CI][a]
non-BC (ref)	23.76 [22.69 to 24.83]		31.62 [26.18 to 37.06]	
recent-BC (ChT-)		−4.06 [-5.72 to -2.40]		−4.21 [-5.96 to -2.45]
recent-BC (ChT+)		−9.19 [-10.76 to -7.62]		−9.24 [-10.91 to -7.58]
long-term-BC (ChT-)		−4.72 [-7.11 to -2.34]		−4.46 [-6.90 to -2.03]
long-term-BC (ChT+)		−4.06 [-5.88 to -2.25]		−4.59 [-6.40 to -2.77]

Abbreviations: BC, breast cancer patients; (ChT-), non-chemotherapy exposure; (ChT+), chemotherapy exposure; CI, confidence interval; ref, reference category. In 95%CI, reference group is non-BC patients.
[a] Adjusted by age, body mass index and season of vitamin D blood.

exposure to sunlight, including indoor occupations, population aging, or consistent use of protective clothing or sunscreen when outdoors, reducing the individual's UVR exposure [19]. Matsuoka et al. (1987) reported a 97.5% decrease of previtamin D3 production after the application of a sunscreen with a sun protection factor of only 8 [20]. Webb et al. (1988) described a severe reduction of 25(OH)D levels in latitudes above and below 35 °N and 35 °S in winter [21]. These could also explain the lower 25(OH)D values observed in our populations (latitudes: 41.38 °N in Barcelona, and 39.45 °N in Valencia).

Although cases and controls were recruited from different places, Barcelona and Valencia are very similar cities, geographically close to one another, with very similar climate, cultural behaviors and clinical health care systems. The same exclusion criteria were used for both cohorts and all analyses were adjusted by age, season of blood draw, and BMI as confounding variables.

Among women with BC, long-term participants had 25(OH)D levels significantly higher than the rest of breast cancer women; nonetheless, 17.7% of the long-term-BC group had 25(OH)D deficiency, compared to 1.4% of healthy women. This places the long-term-BC group in an intermediate 25(OH)D status between the recent-BC and non-BC groups. The overall prevalence of suboptimal levels of 25(OH)D in our study was similar to other studies of baseline 25(OH)D levels in breast cancer patients [22,23], suggesting that women with breast cancer tend to have inferior 25(OH)D levels.

Interestingly, we found an interaction between chemotherapy and 25(OH)D levels. When breast cancer women were stratified according to chemotherapy status, the lowest 25(OH)D levels were observed in patients who underwent recent chemotherapy. These results could be attributed to the photosensitivity effect of chemotherapy, affecting patients' sunlight exposure. This effect on 25(OH)D levels disappeared at long-term, when no differences according to previous chemotherapy were observed in these patients. Even though 25(OH)D levels seemed to partially recover in long-term patients, it is unclear why values comparable to the healthy population were not achieved. More time may be required to recover 25(OH)D levels, or perhaps these women have lower 25(OH)D levels due to genetic or physiological factors. Indeed, recent studies have suggested an association between 25(OH)D levels and breast cancer risk [12,24,25], overall survival [26,27], and cancer prognosis [7,28].

One limitation of our study is the time point when 25(OH)D levels were assessed. Blood analysis was done at 6 weeks post-surgery or 1 month after the last cycle of chemotherapy, not at the time of cancer diagnosis; therefore, some small variation of 25(OH)D levels could occur during this period. We cannot know the levels of 25(OH)D before breast cancer detection or before any intervention related to breast cancer treatment. Hence, we cannot analyze either the associations between 25(OH)D levels and breast cancer incidence or the effect of cancer disease *per se* on 25(OH)D levels. However, the main purpose of the present study was to report the 25(OH)D status of early breast cancer patients compared to healthy women with similar features. Additionally, our cohorts were very well-characterized and our analysis controlled for most potential confounders.

A strength of this study is that 25(OH)D deficiency in breast cancer patients was observed both in early and long-term follow-up,

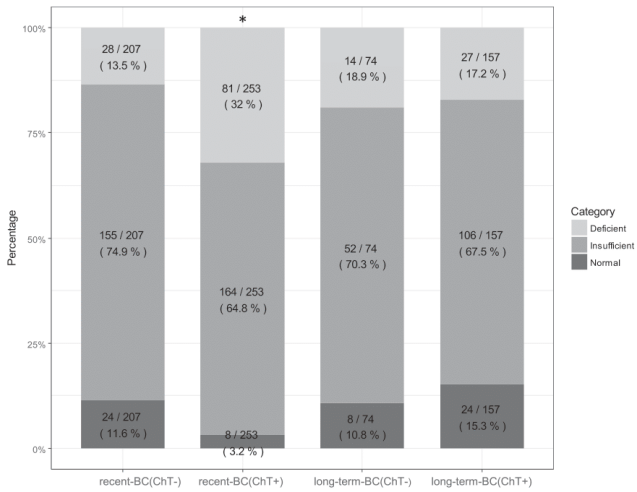

Fig. 2. Subanalysis stratifying by previous chemotherapy. Patient distribution in each study group according to vitamin D categories. Abbreviations: BC, breast cancer; ChT-, non-previous chemotherapy treatment; ChT+, previous chemotherapy treatment; Percentage, percentage of patients. * Bonferroni post hoc comparison of Chi-square test: $p < 0.05$.

underlining the consistence of this finding over time. This observation adds another evidence on the association between 25(OH)D and breast cancer as is consistently supported by numerous case-control studies and randomized controlled trials [29,30].

5. Conclusion

In summary, severely reduced 25(OH)D levels were detected in patients with breast cancer, particularly after recent chemotherapy. These 25(OH)D levels seemed to be partially recovered at long-term but remained much lower than the healthy population. Considering that 25(OH)D levels have been related to better prognosis and survival, it may be speculated that 25(OH)D supplementation might impact prognosis in all patients diagnosed of breast cancer, and especially in patients receiving chemotherapy. Intervention studies might provide an answer to that question.

Contributors

Marta Pineda-Moncusí contributed to the study concept and design, performed data analysis, and contributed to data interpretation and discussion.

Miguel Angel Garcia-Perez recruited participants and contributed to data collection.

Abora Rial recruited participants and contributed to data collection.

Guillem Casamayor recruited participants and contributed to data collection.

Maria Lourdes Cos recruited participants and contributed to data collection.

Sonia Servitja recruited participants and contributed to data collection, and contributed to data interpretation and discussion.

Ignasi Tusquets recruited participants and contributed to data collection, and contributed to data interpretation and discussion.

Adolfo Diez-Perez contributed to data interpretation and discussion.

Antonio Cano recruited participants and contributed to data collection.

Natalia Garcia-Giralt contributed to the study concept and design, performed data analysis, and contributed to data interpretation and discussion.

Xavier Nogues contributed to the study concept and design, recruited participants and contributed to data collection, and contributed to data interpretation and discussion.

All authors helped with manuscript drafting and critically reviewed the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

The study protocol was approved by the ethics committee of Parc de Salut Mar (2016/6803/I) and it was carried out in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in this research once they had read the study information sheet and any questions had been answered. The privacy rights of human subjects were always observed.

Provenance and peer review

This article has undergone peer review.

Research data (data sharing and collaboration)

There are no linked research data sets for this paper. Data will be made available on request.

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Article 2

Title: Bone health evaluation one year after aromatase inhibitors completion

Summary:

Breast cancer patients using aromatase inhibitors (AIs) experience an increased bone loss during their treatment. However, there is a scarcity of information about bone mineral density (BMD) after AI-treatment completion. Hence, we aimed to assess BMD changes one year after completing AI-therapy. Data from 864 postmenopausal women treated with AI for 5 years (5y-AI group), or for 2-3 years after taking tamoxifen therapy (pTAM-AI group), were collected. Those with osteoporosis were treated with oral bisphosphonates (BP). Changes in lumbar spine (LS), femoral neck (FN) and total hip (TH) BMD between baseline, end of treatment, and one-year post-treatment were evaluated using repeated-measures ANOVA. At the end of AI-treatment, 382 patients had available BMD values and 316 also had post-treatment BMD values. As expected, BMD levels were decreased at AI-completion in non-BP treated patients. After one year, LS BMD improved in both groups (5y-AI: +2.11% [95%CI: 1.55 to 2.68], $p < 0.001$; pTAM-AI: +1.00% [95%CI: 0.49 to 1.51], $p < 0.001$) compared with the end of AI-therapy, while FN and TH values remained stable. On the other hand, BMD values of women treated with BP were increased or maintained at the end of AI-treatment and at one-year post-treatment.

In summary, FN and TH BMD continued diminished in non-BP treated patients one year after AI-completion, while LS BMD was restored in the 5y-AI group and partly restored in the pTAM-AI group. BP treatment increased or maintained BMD values at the end of therapy and at one-year post-treatment.

Reference:

Pineda-Moncusí M, Servitja S, Casamayor G, Cos ML, Rial A, Rodriguez-Morera J, Tusquets I, Diez-Perez A, Garcia-Giralt N, Nogués X. Bone health evaluation one year after aromatase inhibitors completion. Bone. 2018 Dec;117:54-59. Epub 2018 Sep 14. PubMed PMID: 30223134. doi: 10.1016/j.bone.2018.09.010.

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Full Length Article

Bone health evaluation one year after aromatase inhibitors completion

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ABSTRACT

Introduction: Breast cancer patients treated with aromatase inhibitors (AIs) experience increased bone loss during their treatment. However, there is little information about bone mineral density (BMD) after completing AI-treatment. The present study aimed to assess BMD changes one year after AI-therapy completion.

Methods: Data were collected from 864 postmenopausal women treated with AI during 5 years (5y-AI group), or during 2–3 years after taking tamoxifen therapy (pTAM-AI group). Participants with osteoporosis were treated with oral bisphosphonates (BP). BMD changes in lumbar spine (LS), femoral neck (FN) and total hip (TH) between baseline, end of treatment, and at one year post-treatment were assessed using repeated-measures ANOVA.

Results: At the end of AI-treatment, 382 patients had available BMD values and 316 also had post-treatment BMD values. As expected, BMD levels were decreased at AI-completion in non-BP treated patients. After one year, LS BMD increased in both groups (5y-AI: +2.11% [95%CI: 1.55 to 2.68], $p < 0.001$; pTAM-AI: +1.00% [95%CI: 0.49 to 1.51], $p < 0.001$) compared with the end of AI-therapy, while values at FN and TH remained stable. On the other hand, BMD values of BP-treated patients were increased or maintained at the end of AI-treatment and also at post-treatment.

Conclusions: At one year after AI-completion, FN and TH BMD remained reduced in non-BP treated women, while LS BMD was recovered in the 5y-AI group and partially recovered in the pTAM-AI group. BP treatment increased or maintained BMD values at the end of therapy and at one year post-treatment.

1. Introduction

Aromatase inhibitor (AI) is recommended by the American Society of Clinical Oncology as the adjuvant endocrine therapy to treat estrogen receptor positive (ER+) early breast cancer in postmenopausal women. Despite its great efficiency, compared to tamoxifen (TAM) as the alternative [1–3], AI has been associated with side effects that could affect the patient's quality of life and its adherence to treatment, being arthralgia and bone loss induction among the most common [4,5].

Previous studies have described an accelerated decrease in bone mineral density (BMD) associated with AI therapy, leading to osteopenic or osteoporotic bone status, both of which are related to osteoporotic fracture [1,4]. Clinical guidelines for the management of AI-related bone loss strongly recommend a close monitoring of BMD and other risk factors to reduce the fracture risk by means of antiresorptive therapies [6].Treatment with bisphosphonates (BPs) is the current recommendation to avoid this bone loss [7–9].

Even though bone parameters have been monitored during AI treatment in many studies [10,11], there is scarce information about bone status after completion of AI treatment. A small sub-analysis in the ATAC trial, with 23 evaluated patients, showed an increase of bone mass at lumbar spine after one year of AI-completion [12]. In the MA.17R trial [13], an increase in lumbar spine (LS) and total hip (TH) BMD was reported 5–7 years post-treatment in women mainly treated with TAM followed by AI; however, half of the patients were treated with BP, concealing the results. Despite the insights on bone behavior related to AI treatment gained from these previous randomized control trials (RCTs), bone health after AI cessation has not been explored in actual clinical conditions.

In the present study, we analyzed BMD changes at the end of

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treatment and at one year after AI-completion in an observational prospective cohort (B-ABLE). In this study, the effect of previous tamoxifen and/or BP treatment was taken into account.

2. Materials and methods

2.1. Study design and participants

Caucasian postmenopausal woman diagnosed with ER+ early breast cancer and candidates for AI-treatment (letrozole, exemestane, or anastrozole) were consecutively recruited from January 2006 to January 2018 in B-ABLE cohort – a prospective, non-selected, observational, clinical cohort study – in Hospital del Mar (Barcelona, Spain). The study protocol was approved by the ethics committee of Parc de Salut Mar (2016/6803/I) and it was carried out in accordance with the Declaration of Helsinki. A written informed consent was obtained from all participants after they had read the study information sheet and any questions had been answered. The privacy rights of human subjects must always be observed.

Participants were enrolled at point of starting AI therapy, either six weeks post-surgery or one month after the last cycle of chemotherapy (5 y-AI group) or, alternatively, once starting menopause after taking TAM for two to three years (pTAM-AI group). End of treatment was considered a total of five years of hormonal adjuvant therapy, according to classic American Society of Clinical Oncology recommendations [14]. Follow-up was from the first day of AI intake to one year after AI-completion. Postmenopausal status was defined as patients > 55 years old with amenorrhea for > 12 months, or those ≤55 with levels of luteinizing hormone > 30 mIU/mL or follicle-stimulating hormone values > 40 mIU/mL. Eligible participants were excluded for previous history of any bone, metabolic or endocrine diseases, as well as alcoholism, rheumatoid arthritis, and concurrent or prior treatment with BP, oral corticosteroids, or any other bone-active drug except tamoxifen.

At the outset of the study, patients were stratified by the corresponding therapeutic regimen: 1) those with osteoporosis [T score < −2.5] or with a T score ≤ −2.0 at any site plus 1 major risk factor (i.e. family history of femoral fracture, or early menopause) or prevalent fragility fractures were treated with weekly oral risedronate or alendronate therapy (BP-treated patients) 2) all other patients were allocated to no active antiresorptive therapy (non-BP-treated patients). BMD was assessed every 12 months until one year after the end of AI therapy (post-treatment). Those who developed osteoporosis during the treatment were immediately offered oral BP treatment and were censored from the study at that point.

Additionally, all participants received supplements of calcium and 25(OH)vitD3 tablets (1000 mg and 800 IU daily, respectively), and those with baseline 25(OH)vitD deficiency (< 30 ng/mL) received an additional dose of 16,000 IU of oral calcifediol (HIDROFEROL® FAES FARMA) every 2 weeks.

2.2. Variables

2.2.1. Bone mineral density

The main outcomes of the study are the absolute and cumulative percentage change in lumbar spine (LS), femoral neck (FN) and total hip (TH) BMD from baseline to the end of treatment and at one year post-treatment.

BMD measures were obtained using a DXA densitometer QDR 4500 SL® (Hologic, Waltham, MA, USA), according to manufacturer recommendations. In our department, in vivo coefficients of variation of these techniques are 1.0% at LS, 1.60 at TH, and 1.65% at FN.

As a secondary analysis, non-BP-treated patients were categorized according to its LS-BMD shift, and their distribution was plotted.

2.2.2. Other variables

At the time of recruitment, data from large clinical variables were registered, including: age, body mass index (BMI), age of menarche and menopause, number of children, months of breastfeeding, and prior chemotherapy, among others.

2.2.3. Statistical methods

Significant differences between variables in the groups of the study were analyzed with One-way ANOVA, Kruskal-Wallis and Chi-square tests, according to variables' nature. In each group, BMD changes between baseline, end of treatment, and post-treatment were evaluated by repeated-measures ANOVA.

Statistical analysis was done with R for Windows version 3.3.3 (foreign, compareGroups, pgirmess, fifter, boot, ggplot2 and scales packages) and SPSS Statistics version 22.0. All statistical contrasts were corrected by Bonferroni test per multiple comparisons and P values lower than 0.05 were considered significant.

3. Results

3.1. Participants

From 864 participants included in the B-ABLE cohort, 382 patients completed AI treatment and had BMD values registered at this point, and 316 of those patients had BMD values at one year after AI-treatment completion (Fig. 1).

Before the end of AI therapy, 32 patients became osteoporotic and started oral BP (5 y-AI: $n = 22$, 3.8%; pTAM-AI: $n = 10$, 3.6%), who were censored from the study at this point; 122 participants were withdrawn from the study (Supplementary table 1), and 328 patients still remained in the follow-up and did not achieve the time of AI-treatment completion. Between end of treatment and one year post-treatment, 41 participants were withdrawn (Supplementary table 1), and 25 patients had not reached one year post-treatment at the end of data collection. Fracture events in participants during follow-up are reported in Supplementary table 2.

Baseline characteristics of selected patients are described in Table 1.

BP and non-BP treated patients were analyzed separately. In the BP-treated group, no significant differences were found between pTAM-AI and 5 y-AI patients. In the non-BP-treated patients, the pTAM-AI group was significantly younger ($p < 0.0001$) and more likely to be treated with chemotherapy ($p = 0.0107$) than the 5 y-AI group. Both groups did not differ in age of menarche, age of menopause, number of children, and months of breastfeeding.

3.2. BMD variation analysis

Mean percentage changes in BMD at LS, FN and TH from baseline to end of treatment and one year post-AI treatment are summarized in Fig. 2.

Absolute BMD values at the three evaluation points are reported in Table 2.

3.3. Lumbar spine BMD variation

In non-BP treated patients, LS BMD decreased significantly at the end of AI treatment in both 5 y-AI and pTAM-AI patients: −2.62% [95%CI: −3.64 to −1.60] and −3.96% [95%CI: −4.79 to −3.12], respectively; $p < 0.001$. After one year of AI-treatment completion, BMD in 5 y-AI patients significantly increased (+2.11% [95%CI: +1.55 to +2.68], $p < 0.001$), achieving values similar to baseline (−0.59% [95%CI: −1.69 to +0.50], $p = 0.732$). In contrast, baseline LS BMD values were not recovered in pTAM-AI patients (−3.01% [95%CI: −3.96 to −2.05], $p < 0.001$). However, a slight increase in BMD was detected between end of treatment and one year post-treatment (+1.00% [95%CI: +0.49 to +1.51], $p < 0.001$).

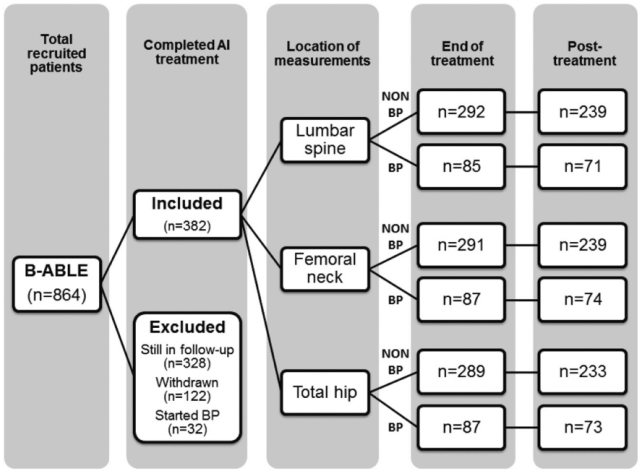

Fig. 1. Flowchart showing the number of patient records in each bone mineral density site at the end of aromatase inhibitors treatment and at post-treatment, according to antiresorptive therapy. Abbreviations: AI, aromatase inhibitors; BP, oral bisphosphonates.

In the BP-treated group, all patients had continued bone mass gains at a) the end of treatment and b) one year post-treatment: a) 5 y-AI group (+3.39% [95%CI: +1.39 to +5.40], $p = 0.005$); pTAM-AI group (+1.90% [95%CI: +0.31 to +3.48], $p = 0.145$); and b) 5 y-AI (+5.16% [95%CI: +2.91 to +7.41], $p < 0.001$); pTAM-AI (+3.23% [95%CI: +1.62 to +4.84], $p = 0.002$).

3.4. Femoral neck BMD variation

In non-BP-treated participants, FN BMD diminished in both groups (5y-AI: −3.42% [95%CI: −4.36 to −2.47]; pTAM-AI: −3.33% [95%CI: −4.15 to −2.51]; $p < 0.001$) until the end of AI treatment; these BMD values were maintained at one year post-treatment.

In contrast, BMD improved with BP therapy (5y-AI: +3.17% [95%CI: +1.37 to +4.98], $p < 0.003$; and pTAM-AI: +0.85% [95%CI: −0.73 to +2.44], $p = 0.145$) up to the end of treatment; no significant changes were detected between AI-completion and post-treatment.

3.5. Total hip BMD variation

Similar to FN BMD behavior, a significant decrease in TH BMD was detected after AI-treatment completion in non-BP-treated patients (−2.53% [95%CI: −3.40 to −1.65], $p < 0.001$ in 5y-AI group; and −3.01% [95%CI: −3.80 to −2.22], $p < 0.001$ in pTAM-AI group). The decreased TH BMD levels remained stable at one year post-treatment.

In the BP-treated patients, BMD increases were detected only in the 5y-AI group at one year post treatment (+3.89% [95%CI: +2.14 to +5.64], $p < 0.001$).

3.6. Patient distribution by LS BMD categories

As the major BMD variations were detected at the LS location in patients without BP between end of AI treatment and one year post-treatment, patient distribution according to LS BMD changes was explored (Fig. 2). A total of 65.8% of 5 y-AI and 42.4% of pTAM-AI patients experienced an intra-individual BMD gain equal to or > 1%. In 19.3% and 32.8% of patients, respectively, BMD values remained constant (Fig. 3). However, in 14.9% and 24.8%, respectively, bone mass had continued to decrease, by 1% or more, at one-year follow-up.

4. Discussion

In this prospective cohort study based on actual clinical conditions, bone health was evaluated after one year of AI-completion in women

Table 1
Baseline characteristics of patients.

Variables	Non-BP-treated patients (n = 242)		BP-treated patients (n = 74)	
	pTAM-AI group (n = 127)	5 y-AI group (n = 115)	pTAM-AI group (n = 44)	5 y-AI group (n = 30)
Mean age (years) ± SD	57.2 ± 8.65	62.8 ± 7.21	63.4 ± 9.42	60.8 ± 6.20
Mean BMI (g/cm^2) ± SD	28.5 ± 5.68	30.4 ± 4.78	27.3 ± 4.70	27.4 ± 4.45
Median age of menarche (years) [Q1;Q3]	13.0 [11.0;13.0]	12.0 [11.0;14.0]	12.0 [11.0;14.0]	13.0 [12.0;13.8]
Mean age of menopause onset (years) ± SD	48.7 ± 4.08	49.7 ± 4.40	49.5 ± 3.73	49.5 ± 3.04
Median number of children [Q1;Q3]	2.0 [1.0;2.0]	2.0 [1.0;3.0]	2.0 [1.7;3.0]	2.0 [2.0;2.7]
Median breastfeeding (months) [Q1;Q3]	3.0 [0.0;9.0]	3.0 [0.0;11.5]	3.0 [0.0;7.2]	0.0 [0.0;6.0]
Prior chemotherapy (n (%))	90 (70.9%)	58 (50.4%)	27 (61.4%)	16 (53.3%)

Abbreviations: 5 y, treated during five years; AI, aromatase inhibitors; BMI, body mass index; BP, oral bisphosphonates; pTAM, previous tamoxifen treatment; Q, quartile.

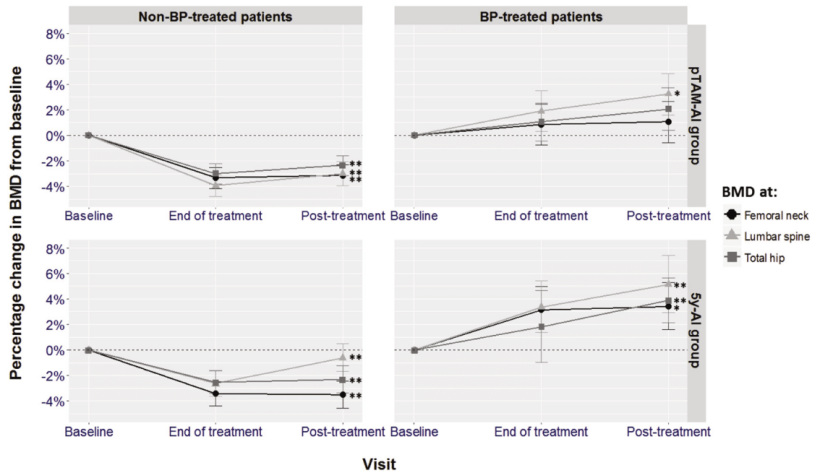

Fig. 2. Individual percent change in lumbar spine, femoral neck, and total hip bone mineral density from baseline to the end of aromatase inhibitors treatment and at post-treatment according to oral bisphosphonates and previous tamoxifen treatment. Mean ± 95%CI is reported. In ANOVA from baseline: $^{*}(p < 0.01)$; $^{**}(p < 0.001)$. Abbreviations: 5 y, treated during five years; AI, aromatase inhibitors; BMD, bone mineral density; BP, oral bisphosphonates; pTAM, previous tamoxifen treatment.

with early breast cancer, stratifying the analysis by bisphosphonates use.

Bone loss related to AI therapy was recovered at the LS location in 5 y-AI patients, but mean BMD recovery in pTAM-AI patients was only 1%. In contrast, FN and TH BMD values remained reduced at one year post-treatment even though the bone loss was stopped. In BP-treated patients, LS, FN and TH BMD levels were maintained or continued to gain at the end of follow-up.

About half of non-BP treated patients experienced a clinically significant gain (greater than or equal to 1%) in LS-BMD at one year after AI cessation, and half of this subgroup had gained > 3%. Only 15% of 5 y-AI and 25% of pTAM-AI patients continued to lose bone mass at the end of follow-up. Hence, in most patients the deleterious effect of AI in LS bone mass stopped, with a trend towards recuperation of baseline BMD after completing AI treatment. On the other hand, a lack of FN and TH BMD recovery was observed in the first year post-treatment. This could be due to the lower capacity for change at these locations [11].

Similar findings were reported in the bone sub-study of the ATAC trial [12], in which 65.2% of participants had increased their LS BMD one year after AI cessation. Similar to our study, LS BMD values increased (+2.35% [interquartile range: −5.34 to 8.19, $p = 0.04$]) and remained stable in TH BMD (+0.71% [interquartile range: −9.42 to 4.63, $p = 0.3$]). Their results, obtained from a small sample ($n = 21$ in LS; $n = 23$ in TH), were confirmed in our cohort.

In the Ma.17R trial [13], patients presented a mean gain in BMD of +4.5% in the spine and +22.4% in the hip at 5 to 7 years post-treatment. The higher increase observed in that trial could be explained by differences in length of follow-up and a lack of stratification by BP use and previous TAM treatment. In fact, one of the strengths of the present study is that we separate participants in different groups according to previous tamoxifen treatment and current BP use, which revealed differences in BMD behavior between treatment groups. In this regard, BP-treated patients improved their BMD values, as would be expected.

Bone mass loss during AI treatment is one of the most important adverse effects experienced by breast cancer patients on adjuvant endocrine therapy. In fact, the decrease of BMD has been described as the major factor of fragility fractures [15]. Moreover, some clinical trials with AI have reported an increase of fractures in both osteopenic and

Table 2
Absolute LS, FN and TH BMD values at baseline, at the end of Al treatment, and at one year post-treatment, according to BP treatment and previous TAM use.

BP treatment	Patients group	Site	N	Visit		
				Baseline	End of treatment	Post-treatment
No	pTAM-AI	LS	125	0.963 ± 0.099	0.925 ± 0.102	0.934 ± 0.106
		FN	125	0.765 ± 0.088	0.739 ± 0.087	0.740 ± 0.083
		TH	120	0.910 ± 0.095	0.882 ± 0.093	0.888 ± 0.095
	5 y-AI	LS	114	0.965 ± 0.112	0.939 ± 0.117	0.958 ± 0.121
		FN	114	0.753 ± 0.094	0.727 ± 0.092	0.725 ± 0.090
		TH	113	0.901 ± 0.093	0.878 ± 0.092	0.879 ± 0.095
Yes	pTAM-AI	LS	41	0.814 ± 0.104	0.828 ± 0.099	0.839 ± 0.103
		FN	44	0.649 ± 0.086	0.653 ± 0.083	0.654 ± 0.083
		TH	43	0.785 ± 0.103	0.792 ± 0.105	0.800 ± 0.105
	5 y-AI	LS	30	0.768 ± 0.078	0.794 ± 0.086	0.806 ± 0.079
		FN	30	0.625 ± 0.083	0.644 ± 0.085	0.646 ± 0.083
		TH	30	0.769 ± 0.096	0.783 ± 0.113	0.798 ± 0.101

Abbreviations: 5 y, treated during five years; AI, aromatase inhibitors; BMD, bone mineral density (g/cm^2 (Mean ± SD)); BP, oral bisphosphonates; FN, femoral neck; TH, total hip; LS, lumbar spine; pTAM, previous tamoxifen treatment.

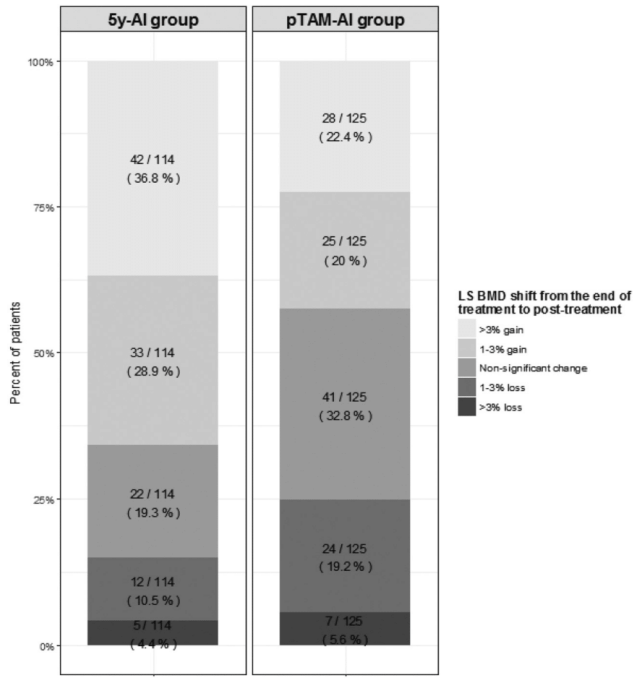

Fig. 3. Participant distribution in non-BP-treated patients according to lumbar spine bone mineral density shift between end of treatment and post-treatment. Abbreviations: 5 y, treated during five years; AI, aromatase inhibitors; BMD, bone mineral density; pTAM, previous tamoxifen treatment.

osteoporotic patients [13,16]. Even though bone mass seems to be recovered after AI cessation, bone health should be evaluated in all patients since BMD in LS remained decreasing almost in 1/4 of patients after AI treatment was ended. In these cases, BPs could be a recommended option since BP treated patients in our study showed better BMD values.

In this line, patients from our cohort are subjected to strict monitoring of 25(OH)vitD levels and calcium diet intake. All patients receive a high 25(OH)vitD supplementation from baseline. Hence, 25(OH)vitD levels improved significantly in our cohort with the proposed repletion regimen raised until the normal range reaching an average > 30 ng/mL, and persisted by the follow-up [17]. Data from previous studies strongly recommend that individuals in AI treatment, including those who are at low risk for fractures and not candidates for BP treatment, should receive calcium and 25(OH)vitD supplements [18], especially in cases where calcium intake is not enough. These supplementations could contribute to the recovery of BMD values observed after AI-completion in our study.

One limitation of our study is the limited tracking time after treatment completion, which precludes any predictions about how BMD will evolve during a longer-term follow-up, and in particular, whether FN and TH BMD will be recovered. Further research is needed to explain why patients previously treated with TAM experienced lower LS BMD recovery than patients receiving AI monotherapy. We hypothesize that the accelerated bone loss during AI treatment in pTAM-AI patients, as previously described in B-ABLE cohort [19], might delay LS BMD recovery. Likewise, we cannot know if this BMD could return to baseline levels at long term.

It is worth mentioning that although BMD measured by DXA is the gold standard surrogate for the diagnosis of osteoporosis, it is well-known that the increased risk of non-traumatic fractures is determined not only by the mineral content but also by bone quality and material properties, such as trabecular microarchitecture [10], the accumulation of microfractures, a disordered bone remodeling, bone affecting drugs, toxic habits or the influence of extra-skeletal risk factors [20].

In summary, AI-related bone loss stopped at one year after AI-completion and, in the lumbar location, BMD values were totally recovered in most patients who had received AI monotherapy and partially recovered in patients who were previously treated with TAM. However, monitoring of bone health and calcium and 25(OH)vitD supplementation is essential for the clinical management of patients after finalizing AI adjuvant therapy. Larger studies are needed to determine whether the observed BMD behavior persists beyond the one year post-treatment.

Conflicts of interest

Marta Pineda-Moncusí, Sonia Servitja, Guillem Casamayor, Maria Lourdes Cos, Abora Rial, Jaime Rodriguez-Morera, Ignasi Tusquets, Adolfo Diez-Perez, Natalia Garcia-Giralt, Xavier Nogués declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2018.09.010.

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Supplementary material

Supplementary table 1. Withdrawn causes during follow-up

Causes	Withdrawn patients before AI completion (n=122)	Withdrawn patients between end of treatment and one-year post-treatment (n=41)
AI intolerance (n (%))	33 (27.0%)	0 (0%)
BP intolerance (n (%))	3 (2.5%)	0 (0%)
Concomitant disease (n (%))	13 (10.7%)	0 (0%)
Exitus (n (%))	3 (2.5%)	0 (0%)
Extended AI-treatment (n (%))	N/A	6 (14.6%)
Metastasis (n (%))	10 (8.2%)	0 (0%)
Personal reasons (n (%))	36 (29.5%)	33 (80.5%)
Recurrence (n (%))	13 (10.7%)	1 (2.4%)
Second neoplasms (n (%))	11 (9.0%)	0 (0%)
Use of corticoids (n (%))	0 (0%)	1 (2.4%)
Abbreviations: AI, aromatase inhibitors; BP, oral bisphosphonates; N/A, not applicable.		

Supplementary table 2. Fractures recorded in the B-ABLE cohort

Fracture location	pTAM-AI group (n=13)	5y-AI group (n=29)
Clinical vertebral (n (%))	4 (30.8%)	8 (27.6%)
Femur (n (%))	1 (7.7%)	3 (10.3%)
Colles (n (%))	2 (15.4%)	8 (27.6%)
Other site (n (%))	6 (46.2%)	10 (34.5%)
Abbreviations: 5y, treated during five years; AI, aromatase inhibitors; pTAM, previous tamoxifen treatment.		

Article 3

Title: Assessment of early therapy discontinuation and health-related quality of life in breast cancer patients treated with aromatase inhibitors: B-ABLE cohort study

Summary:

Arthralgia and enhanced bone loss are the most frequent adverse events of aromatase inhibitors (AI). These diminish patients' quality of life and treatment adherence. This study assesses the early cessation of AI caused by AI intolerance, and the progression of joint pain and health-related quality of life (HRQoL) during AI treatment until 1-year after AI completion. Data of 910 women diagnosed with early breast cancer and candidates for AI were recruited in B-ABLE cohort. A survival analysis was conducted to study AI discontinuation, including Kaplan-Meier estimation and Cox regression. Patients were allocated in three different groups of study according to previous tamoxifen (TAM) exposure and length of AI treatment: TAM-2yAI, TAM-3yAI, and 5yAI. Visual Analog Scale (VAS) and ECOS-16 tests were used to evaluate joint pain and HRQoL in osteoporosis evolution, respectively, from baseline to 1-year after AI completion by repeated-measures ANOVA. Patients previously exposed to tamoxifen had greater risk of AI withdrawal compared to non-exposed (adjusted HR 5.30 [95% CI 2.23 to 12.57]). VAS and ECOS-16 scores of TAM-2yAI and TAM-3yAI groups increased during AI treatment, mainly during the first 3-12 months. After 1-year from AI completion, values tend to decrease to baseline levels. In 5yAI group, VAS and

ECOS-16 levels raised at three months, and VAS continued significantly higher at 1-year post-treatment.

To conclude, AI therapy incremented joint pain and diminished HRQoL, especially during the first year of treatment. Patients switching to AI after being treated with tamoxifen experienced greater pain and had an excess risk of discontinuation during the first 12 months of AI treatment.

Reference:

Pineda-Moncusí M, Servitja S, Tusquets I, Diez-Perez A, Rial A, Cos ML, Campodarve I, Rodriguez-Morera J, Garcia-Giralt N, Nogués X. Assessment of early therapy discontinuation and health-related quality of life in breast cancer patients treated with aromatase inhibitors: B-ABLE cohort study. Breast Cancer Res Treat. 2019 Aug;177(1):53-60. Epub 2019 May 24. PubMed PMID: 31127467. doi: 10.1007/s10549-019-05289-7.

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https://doi.org/10.1007/s10549-019-05289-7

PRECLINICAL STUDY

Assessment of early therapy discontinuation and health-related quality of life in breast cancer patients treated with aromatase inhibitors: B-ABLE cohort study

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Abstract

Purpose The most frequent adverse effects of aromatase inhibitors (AI) are arthralgia and bone loss induction. These reduce the quality of life of patients and their adherence to the treatment. This study evaluates the early AI cessation caused by AI intolerance, and the evolution of joint pain and health-related quality of life (HRQoL) during AI treatment until 1-year after AI completion.

Methods Data of 910 women diagnosed with early breast cancer and candidates for AI were recruited in B-ABLE cohort. AI discontinuation was analyzed by survival analysis, including Kaplan–Meier estimation and Cox regression. Patients were distributed in three groups of the study according to previous tamoxifen (TAM) exposure and length of AI treatment: TAM-2yAI, TAM-3yAI, and 5yAI. Evolution of joint pain and HRQoL in osteoporosis was evaluated using Visual Analog Scale (VAS) and ECOS-16 tests, respectively, from baseline to 1-year after AI completion through repeated-measures ANOVA.

Results Risk of AI discontinuation was increased in patients previously exposed to tamoxifen compared to non-exposed (adjusted HR 5.30 [95% CI 2.23 to 12.57]). VAS and ECOS-16 scores of TAM-2yAI and TAM-3yAI groups increased during AI treatment, mainly during the first 3–12 months. After 1-year from AI completion, values tend to decrease to baseline levels. In 5yAI group, VAS and ECOS-16 levels increased at three months, and VAS remained significantly higher at 1-year post-treatment.

Conclusions AI therapy increased joint pain and reduced HRQoL, mainly during the first year of treatment. Patients previously treated with tamoxifen experienced greater pain when they switched to AI therapy and had an excess risk of discontinuation during the first 12 months.

Trial registration ClinicalTrials.gov: NCT03811509. Registered 28 January 2018-Retrospectively registered, https://clinicaltrials.gov/ct2/show/NCT03811509.

Keywords Aromatase inhibitors · Tamoxifen · Breast cancer · Joint pain · Arthralgia · Health-related quality of life · B-ABLE cohort

Introduction

Aromatase inhibitors (AI) are the recommended therapy for early estrogen-receptor-positive breast cancer [1]. The introduction of AIs has improved the overall survival to 80% [2, 3]. However, this therapy has been related to several side effects that reduce the quality of life of these patients and their adherence to AI treatment [4]. The most frequent adverse effects are arthralgia—defined as joint pain—and bone loss induction [5–7].

Joint pain etiology due to AIs remains unknown, but has been related to estrogen depletion [8]. Additionally,

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decreased estrogen production induces bone loss, and therefore increases the risk of fragility fracture [9], morbidity, and mortality [10]. Validated tools for pain and health impairment assessment include Visual Analog Scale (VAS) and health-related quality of life (HRQoL) in osteoporosis (ECOS-16) [11–13]. The VAS has also been widely used in breast cancer patients, including the Barcelona–Aromatase induced Bone Loss in Early breast cancer (B-ABLE) cohort [14, 15], whereas ECOS-16 has not been validated in these patients.

The B-ABLE cohort is a prospective, clinical cohort study of women diagnosed with early breast cancer and candidates for AI treatment that aims to improve the quality of life in patients with breast cancer (ClinicalTrials.gov Identifier: NCT03811509) [16]. Previous findings in the B-ABLE cohort have described a worsening of joint pain in 50% of patients at 3 and 12 months after starting AI treatment, and an increased bone loss in up to 45% of patients after 2 years of AI treatment [15, 17].

The main objective of this study was to evaluate early cessation of AI due to patient intolerance in the B-ABLE cohort. Additionally, evolution of joint pain and HRQoL during AI treatment, and up to 1-year post-treatment, was assessed.

Materials and methods

Study design

The B-ABLE cohort is a prospective, interventional, clinical cohort study of postmenopausal women diagnosed with early estrogen-receptor-positive breast cancer and candidates for AI (letrozole or exemestane) [16, 18]. Patients were recruited from January 2006 to June 2018 in Hospital del Mar (Barcelona, Spain). End of treatment was considered a total of 5 years of hormonal adjuvant therapy, according to American Society of Clinical Oncology recommendations [19].

Participants

Participants were included upon starting AI therapy, either 6 weeks post-surgery or 1 month after the last cycle of chemotherapy (AI patients) for a 5-year treatment program (5yAI group); or alternatively, after taking tamoxifen (TAM-AI-patients) for 2–3 years and initiating AI to complete 5 years of anti-estrogen therapy (TAM-2yAI and TAM-3yAI groups) (Fig. 1). Postmenopausal status was defined as patients > 55 years old with amenorrhea for > 12 months, or those ≤ 55 with luteinizing hormone levels > 30 mIU/mL or follicle-stimulating hormone values > 40 mIU/mL.

Data for a number of demographic and clinical variables were collected, including age at recruitment, body mass index (BMI), and bone mineral density (BMD) among others. Exclusion criteria included previous history of any metabolic, endocrine, or bone diseases, as well as alcoholism, rheumatoid arthritis, and concurrent or prior treatment with bisphosphonates (BP), oral corticosteroids, or any other bone-active drug except tamoxifen. Those who developed osteoporosis during the treatment were immediately prescribed oral BP treatment and censored from the study.

Additionally, all participants received supplements of calcium and 25(OH)vitD3 tablets (1000 mg and 800 IU daily, respectively), and those with baseline 25-hydroxyvitamin D deficiency (< 30 ng/mL) received an additional dose of 16,000 IU of oral calcifediol (HIDROFEROL® FAES FARMA) every 2 weeks.

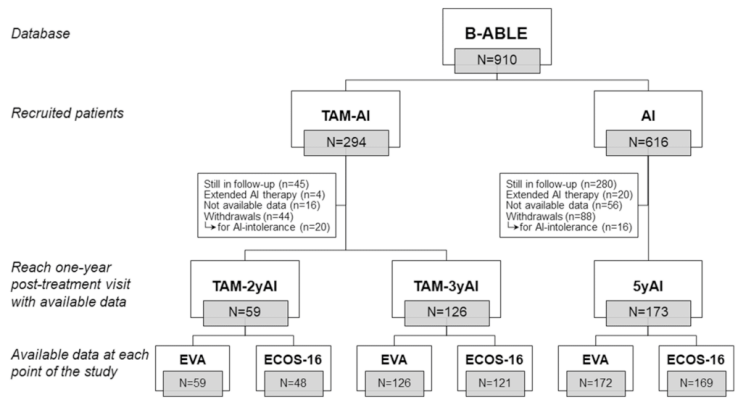

Fig. 1 Flowchart of patients from B-ABLE cohort included in the study. *2y* 2 years, *3y* 3 years, *5y* 5 years, *AI* aromatase inhibitor, *ECOS-16* health-related quality of life questionnaire in osteoporosis, *TAM* tamoxifen, *VAS* visual analogic scale

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Study outcomes

Treatment discontinuation due to AI intolerance

Participants who decided to discontinue AI treatment due to an intolerable increase in joint pain were designated as AI-intolerant.

VAS

Visual analogic scale (VAS) was used to score the intensity of self-reported joint pain at baseline (before starting AI therapy), at 3 months and every 12 months until 1 year after concluding AI therapy. Score ranged from 0 (no pain) to 10 (maximum pain). The question associated to the VAS reads as follows (translated from Catalan and Spanish by the authors): ''please, score the intensity of the pain you feel in your peripheral joints (knee, wrist, fingers/toes, elbow, shoulder, etc.), excluding spine/back pain and pain at the operated area'' [14].

ECOS-16

The ECOS-16 questionnaire is a short version of the combination of the Osteoporosis Quality Of Life Questionnaire and the Quality of Life Questionnaire of the European Foundation for Osteoporosis [12].

ECOS-16 was used to score the assessment of health-related quality of life (HRQoL) in osteoporosis at baseline (before starting AI therapy), 3 months and every 12 months until 1 year after concluding AI therapy. Score ranged from 12 (best possible health status) to 75 (worst possible health status).

Statistical methods

Cumulative hazard plots and Cox proportional hazards models by Kaplan–Meier estimation were carried out. Hazard ratios (HR) are reported with 95% confidence intervals [95% CI], using patients non-exposed to tamoxifen (AI patients) as reference group. Additionally, proportionality assumption was tested. Survival analysis was adjusted by age, body mass index, BP use, and baseline VAS and ECOS-16 scores. Survival analysis of AI-intolerants according to previous tamoxifen exposure was analyzed in all B-ABLE participants.

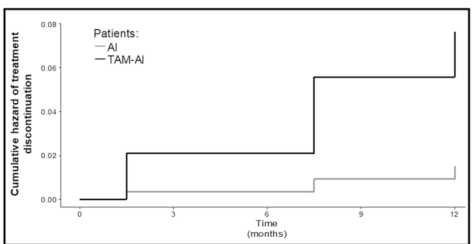
Baseline differences between groups of participants who completed AI treatment were assessed by one-way ANOVA. VAS and ECOS-16 changes in each group were analyzed by repeated-measures ANOVA from baseline to each appointment, until 1 year after AI therapy conclusion. Interaction of BP with study outcomes was tested.

Statistical analysis was carried out using R for Windows version 3.3.3 (foreign, compare Groups, plyr, and ggplot2 packages) and SPSS Statistics version 22.0. *P* values lower than 0.05 were considered significant, and all statistical contrasts were corrected by Bonferroni test for multiple comparisons.

Results

AI discontinuation

Of 910 patients recruited in the B-ABLE cohort, 36 interrupted their treatment due to AI intolerance, of which 20 patients (55.6%) had previous tamoxifen exposure (TAM-AI patients) and 16 (44.4%) were not exposed (AI patients). In survival analysis, proportionality assumption was significant ($p < 0.05$). For this reason, data were censored at 12 months of follow-up (Fig. 2). Unadjusted Cox analysis estimated a discontinuation HR of 5.10 [95% CI 2.25 to 11.58] in the TAM-AI group, compared with AI patients. After adjustment, HR was 5.30 [95% CI 2.23 to 12.57] ($p < 0.01$). Moreover, in the adjustment, higher baseline VAS levels were associated with AI intolerance (HR; 1.26 [95% CI 1.06 to 1.49], $p < 0.05$).



Number of patients at risk (n):						
Group of patients	AI	616	583	519	503	503
	TAM-AI	294	282	262	245	245
	Time (months)	0	3	6	9	12
Cumulative number of events (n):						
Group of patients	AI	0	2	2	5	8
	TAM-AI	0	6	6	15	20
	Time (months)	0	3	6	9	12

Fig. 2 Cumulative hazard plot of treatment discontinuation due to AI intolerance. Kaplan–Meier curve shows early AI cessation due to extreme pain, in terms of cumulative hazards. *AI* patients treated with aromatase inhibitors, *TAM-AI* patients previously treated with tamoxifen who switched to AI therapy

Table 1 Baseline characteristics of selected participants

	TAM-2yAI ($n=59$)	TAM-3yAI ($n=126$)	5yAI ($n=173$)	p value
Age (mean ± SD)	60.3 ± 9.81	58.1 ± 8.71[a]	62.8 ± 6.99	< 0.001
BMI (mean ± SD)	28.4 ± 5.73	27.9 ± 5.14[b]	29.7 ± 4.85	0.013
BP [*n* (%)]	19 (32.2%)	27 (21.4%)	35 (20.2%)	0.152
VAS (mean ± SD)	2.45 ± 2.31	2.39 ± 2.46	2.19 ± 2.28	0.681
ECOS-16 (mean ± SD)	27.4 ± 10.9	26.2 ± 13.6	24.3 ± 10.7	0.160

2y 2 years, *3y* 3 years, *5y* 5 years, *AI* aromatase inhibitor, *BMI* body mass index, *BP* bisphosphonates, *VAS* visual analogic scale, *ECOS-16* evaluation of health-related quality of life in osteoporosis, *TAM* tamoxifen

In one-way ANOVA, differences in post hoc comparisons are annotated as [a](p value < 0.001) and [b](p value < 0.05), compared with 5yAI group

Table 2 Absolute VAS values at each appointment, from baseline to one-year post-treatment, in each group

	TAM-2yAI ($n=59$) Mean ± SD	TAM-3yAI ($n=126$) Mean ± SD	5yAI ($n=172$) Mean ± SD
Baseline	2.45 ± 2.31	2.39 ± 2.46	2.16 ± 2.24
3 months	3.91 ± 2.84	3.29 ± 2.83	3.01 ± 2.68
12 months	4.27 ± 2.96	3.75 ± 2.65	3.26 ± 2.76
24 months	3.58 ± 2.97	3.28 ± 2.65	3.32 ± 2.68
36 months	n/a	3.37 ± 2.86	3.10 ± 2.79
48 months	n/a	n/a	3.22 ± 2.77
60 months	n/a	n/a	3.14 ± 2.88
Post-treatment	2.97 ± 2.58	2.92 ± 3.22	2.99 ± 2.89

2y 2 years, *3y* 3 years, *5y* 5 years, *AI* aromatase inhibitor, *n/a* non-applicable, *SD* standard deviation, *TAM* tamoxifen

Pain evolution during AI treatment

Currently, 358 participants had completed AI treatment and VAS and/or ECOS-16 values recorded for all appointments, including the post-treatment visit at 1-year follow-up. Baseline characteristics of patients are detailed in Table 1. The TAM-3yAI group was younger and had lower BMI, compared to the 5yAI group ($p < 0.001$ and $p < 0.05$, respectively). The groups did not differ in baseline VAS and ECOS-16 scores nor in the proportion of patients treated with BP.

VAS score

Absolute VAS values during follow-up are reported in Table 2. Mean values of absolute changes in VAS from baseline to post-treatment are summarized in Fig. 3. Repeated-measures ANOVA in each group showed significant differences in VAS progression during follow-up ($p < 0.001$). No significant interaction effect was found between VAS and BP ($p > 0.05$).

TAM-2yAI patients

In Fig. 3a, a significant increase of joint pain was observed at 3 months (1.46 [95% CI 0.74 to 2.17], $p < 0.01$), 12 months (1.82 [95% CI 1.03 to 2.61], $p < 0.001$), and 24 months (1.14 [95% CI 0.37 to 1.91], $p < 0.05$), compared to baseline. At 1-year post-treatment, VAS levels were comparable to baseline values (0.53 [95% CI − 0.20 to 1.25], $p = 1.00$).

TAM-3yAI patients

Likewise, TAM-3yAI patients (Fig. 3b) reported a significant increase of joint pain at 3 months (0.91 [95% CI 0.50 to 1.32], $p < 0.001$), 12 months (1.36 [95% CI 0.90 to 1.82], $p < 0.001$), 24 months (0.88 [95% CI 0.48 to 1.31], $p < 0.001$), and 36 months (0.98 [95% CI 0.51 to 1.44], $p < 0.001$), compared to baseline values. At 1-year post-treatment, joint pain was comparable to baseline VAS values (0.53 [95% CI 0.05 to 1.01], $p = 0.46$).

5yAI patients

As shown in Fig. 3c, joint pain was significantly increased at each appointment during AI treatment ($p < 0.001$ for all), compared to baseline VAS values (3 months: 0.85 [95% CI 0.52 to 1.18; 12 months: 1.10 [95% CI 0.74 to 1.47]; 24 months: 1.16 [95% CI 0.80 to 1.52]; 36 months 0.95 [95% CI 0.54 to 1.35]; 48 months: 1.07 [95% CI 0.66 to 1.47]; 60 months 0.98 [95% CI 0.60 to 1.35]) and also at 1 year post-treatment ($p < 0.01$): VAS 0.83 [95% CI 0.42 to 1.25].

A sub-analysis of patients reporting a clinically relevant change during the follow-up (VAS change ≥ 2 points from baseline) showed the greatest increases in joint pain during the first 3–12 months: 36 patients (61.02%) in the TAM-2yAI group had a mean VAS change of 2.81 [95% CI 1.98 to 5.26] at 3 months and 3.48 [95% CI 2.58 to 6.11] at 12 months; in the TAM-3yAI group, 81 patients (64.29%) had a mean change of 1.73 [95% CI 1.23 to 4.02] at 3 months and 2.57 [95% CI 2.06 to 4.88] at 12 months; and 117 patients (68.02%) in the 5yAI group had a mean change of 1.37 [95% CI 0.93 to 3.73] at 3 months and 1.92 [95% CI 1.48 to 4.37] 12 months.

ECOS-16 score

Absolute ECOS-16 values during follow-up are reported in Table 3 and the mean values of absolute changes from baseline to post-treatment are summarized in Fig. 4. Repeated-measures ANOVA showed significant

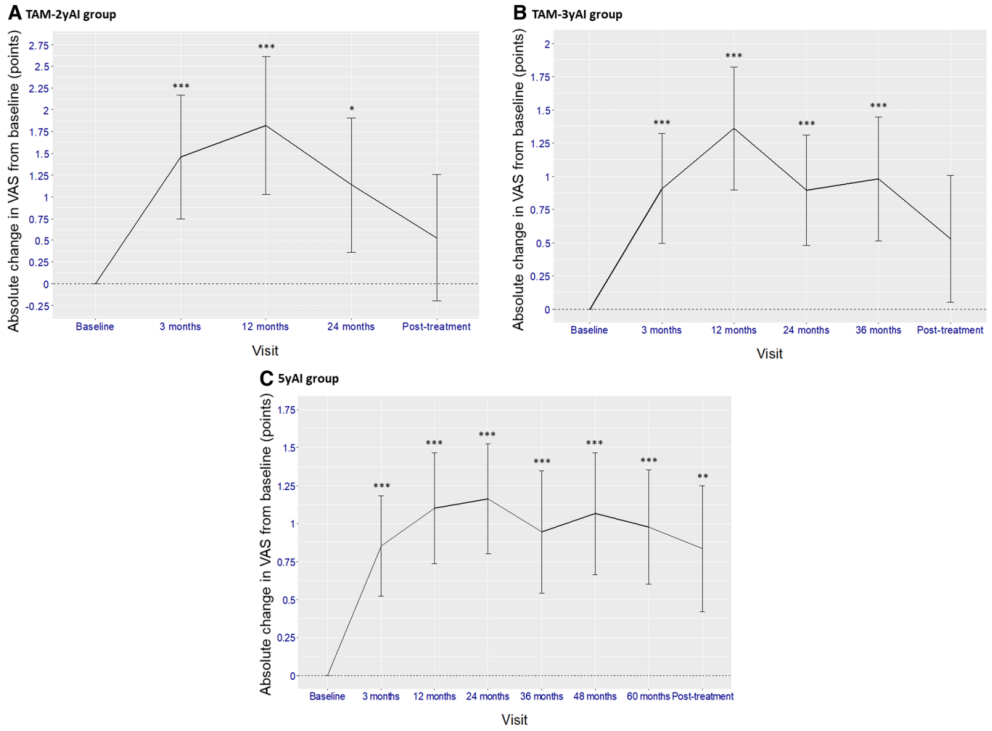


Fig. 3 Individual absolute change in VAS score during follow-up in each group of patients. **a** TAM-2yAI, **b** TAM-3yAI, and **c** 5yAI. Mean ± 95% CI is reported. Post hoc comparisons from baseline in repeated-measures ANOVA: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. *2y* 2 years, *3y* 3 years, *5y* 5 years, *AI* aromatase inhibitor, *TAM* tamoxifen

Table 3 Absolute ECOS-16 values at each appointment, from baseline to one-year post-treatment, in each group

	TAM-2yAI ($n = 48$) Mean ± SD	TAM-3yAI ($n = 121$) Mean ± SD	5yAI ($n = 169$) Mean ± SD
Baseline	26.79 ± 11.45	25.55 ± 13.39	24.28 ± 10.63
3 months	28.08 ± 12.95	27.80 ± 13.60	28.85 ± 14.33
12 months	32.06 ± 15.64	27.29 ± 13.33	29.50 ± 13.79
24 months	30.75 ± 14.01	27.69 ± 14.94	29.92 ± 14.33
36 months	n/a	28.45 ± 15.20	30.91 ± 15.20
48 months	n/a	n/a	31.01 ± 15.83
60 months	n/a	n/a	30.78 ± 14.98
Post-treatment	29.48 ± 15.07	27.24 ± 15.45	30.03 ± 14.86

2y 2 years, *3y* 3 years, *5y* 5 years, *AI* aromatase inhibitor, *n/a* non-applicable, *SD* standard deviation, *TAM* tamoxifen

differences in ECOS-16 during follow-up in each group (TAM-2yAI: $p < 0.05$; TAM-3yAI: $p < 0.05$; and 5yAI: $p < 0.001$). No significant interaction effect was found between ECOS-16 and BP ($p > 0.05$).

TAM-2yAI patients

ECOS-16 score (Fig. 4a) was increased after starting AI treatment with the worst values detected after 12 months of treatment (5.27 [95% CI 1.87 to 16.97], $p < 0.05$).

TAM-3yAI patients

Similarly, in TAM-3yAI patients (Fig. 4b), ECOS-16 increased from baseline, with the greatest increase at 36 months (2.90 [95% CI 1.02 to 13.33], $p < 0.05$).

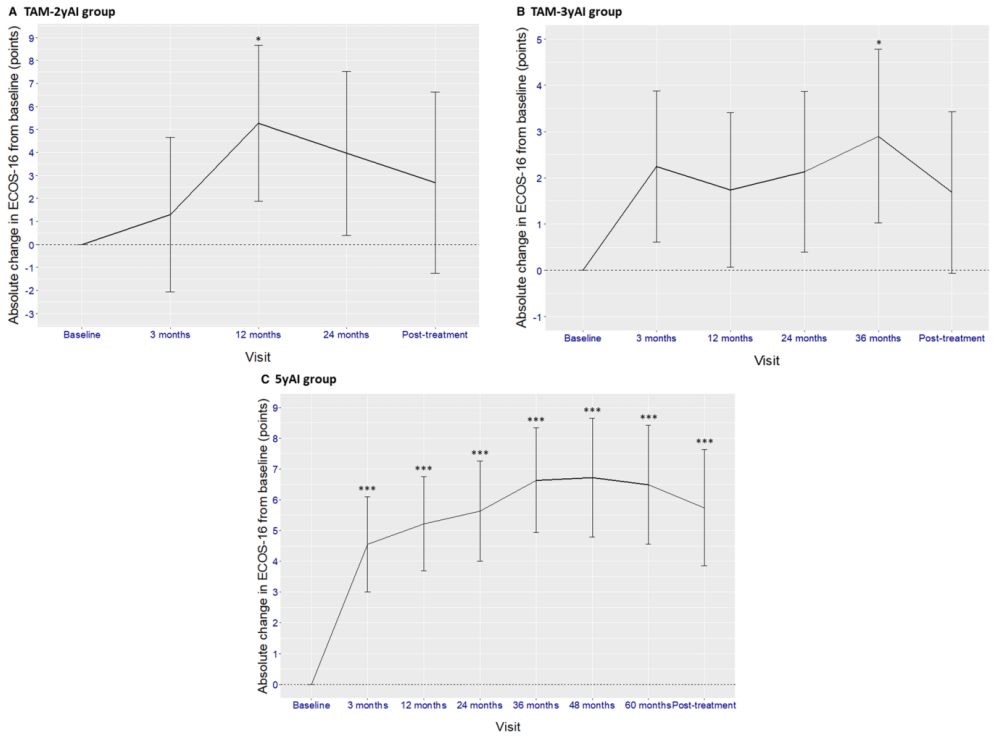


Fig. 4 Individual absolute change in ECOS-16 score during follow-up in each group of patients. **a** TAM-2yAI, **b** TAM-3yAI, and **c** 5yAI. Mean ± 95% CI is reported. Post hoc comparisons from baseline in repeated-measures ANOVA: $*p<0.05$, $**p<0.01$, $***p<0.001$. *2y* 2 years, *3y* 3 years, *5y* 5 years, *AI* aromatase inhibitor, *TAM* tamoxifen

5yAI patients

Significant increases ($p<0.001$) of ECOS-16 were observed at each follow-up visit, compared to baseline values (3 months: 4.56 [95% CI 3.02 to 14.73]; 12 months: 5.22 [95% CI 3.69 to 15.31]; 24 months: 5.64 [95% CI 4.01 to 16.35]; 36 months: 6.34 [95% CI 4.93 to 17.88]; 48 months: 6.72 [95% CI 4.79 to 19.43]; 60 months: 6.49 [95% CI 4.56 to 19.20]; and 1-year post-treatment: 5.75 [95% CI 3.85 to 18.20]).

Discussion

In this prospective study, a 3.96% of breast cancer patients treated with AI and recruited in the B-ABLE cohort discontinued treatment due to AI intolerance. Patients with previous exposure to tamoxifen therapy had a 430% increased risk of discontinuation during the first 12 months, compared to AI monotherapy. VAS and ECOS-16 scores increased rapidly in the first 3-12 months of treatment, and then stabilized. About 65% of all participants experienced arthralgia, according to VAS scores.

According to clinical trials and other published studies, up to 30% of patients discontinued AI due to its toxic effects [4], and 24.3% of patients discontinued due to musculoskeletal symptoms during the first 2 years, with a median of 6.1 months [20]. In contrast, discontinuation for AI intolerance was lower (3.96%) in the B-ABLE cohort, with an overall discontinuation during the treatment period of 14.51%. This difference might be explained by the vitamin D supplements prescribed, which diminished arthralgia levels [14], and the close monitoring of patients in the B-ABLE cohort, compared to usual care.

Furthermore, the present study considered previous tamoxifen therapy in evaluating pain reported by AI-treated patients. Hence, patients were distributed in three groups according to length of AI treatment, based on tamoxifen

exposure: TAM-2yAI, TAM-3yAI, and 5yAI. VAS and ECOS-16 scores were recorded from baseline until 1-year post-treatment.

In TAM-2yAI and TAM-3yAI patients, VAS and ECOS-16 scores increased during AI treatment, mainly during the first 3–12 months. These levels were maintained and even slightly reduced until the end of AI treatment. At 1-year post-completion, values tended to decrease to baseline levels.

The 5yAI patients showed a rapid increase in VAS and ECOS-16 levels at 3 months of AI therapy; from this point on, values stabilized or increased slightly until end of treatment. In contrast to patients exposed to tamoxifen, joint pain in 5yAI patients decreased slightly after completing AI treatment but remained significantly higher at 1-year post-treatment.

Despite increased joint pain and the worsening of HRQoL that persisted after AI completion in 5yAI patients, their absolute VAS levels were lower than patients previously treated with tamoxifen. These results are in accordance with Kadakia et al., who reported that previous tamoxifen users have a greater worsening in musculoskeletal symptoms and a significantly worse VAS score at 3 months of treatment, compared to non-users [4]. These higher joint pain levels observed in previous tamoxifen users might be associated with the early therapy discontinuation due to AI intolerance detected in TAM-AI patients of B-ABLE.

Other researchers have observed a decline in self-reported pain during the first year of treatment in patients using zoledronic acid [21]. However, oral BP therapy was not associated with AI intolerance, VAS, or ECOS-16 values in the B-ABLE cohort. Further research is needed to confirm or discard a potential role of BP in modulating joint pain in AI-treated patients.

A limitation of the study is that our cohort, recruited from the population served by our hospital, is likely more closely monitored than patients not included in a study cohort. Moreover, all patients in the B-ABLE received vitamin D supplements which may have contributed to decrease pain scores and the incidence of AI discontinuation in our study, compared to general clinical practice. Another limitation is the potential subjectivity of VAS and ECOS-16 outcomes, as pain is essentially a subjective perception influenced by a complex interaction of behavioral, environmental, biological, and social factors. However, the daily experience of toxic effects produced by therapies is comprehensively captured by self-reported pain assessment [22]. Likewise, pain has a high concordance with HRQoL [23, 24].

In conclusion, AI therapy increased joint pain and reduced HRQoL, measured by VAS and ECOS-16 scores. respectively, mainly during the first year of treatment. At 1-year post-treatment, both values returned to baseline levels in patients previously treated with tamoxifen, while patients treated with AI monotherapy for 5 years maintained higher levels of joint pain, compared to baseline. On the other hand, breast cancer patients previously treated with tamoxifen experienced greater pain when they switched to AI therapy and therefore had an excess risk of discontinuation during the first 12 months, compared to patients not exposed to tamoxifen. Strictly monitoring AI patients, especially previous tamoxifen users, might reduce the incidence of AI-treatment discontinuation.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval The ethics committee of Parc de Salut Mar (2016/6803/I) approved the study protocol, which was carried out in accordance with the 1964 Declaration of Helsinki and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study after they had read the study information sheet and any questions had been answered. The privacy rights of human subjects are carefully protected in our institution.

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Article 4

Title: Increased fracture risk in women treated with aromatase inhibitors versus tamoxifen: beneficial effect of bisphosphonates

Summary:

Aromatase inhibitors (AI) are associated with enhanced bone loss and an increased risk of osteoporotic fractures. To lessen fracture risk in these patients, oral bisphosphonates (BP) are currently recommended. This study aimed to evaluate the risk of fracture in breast cancer patients receiving AI, compared to tamoxifen users, and to evaluate the efficacy of BP in reducing fracture risk. Thus, we conducted an observational cohort study using data obtained from primary care records in a population database. Women diagnosed with breast cancer between 2006 and 2015 and treated with tamoxifen or AI (n = 36,472) were stratified according to low (without osteoporosis diagnosis nor BP exposure) or high (with osteoporosis and/or treated with BP) fracture risk. Cox models were used to estimate fracture hazard ratios (HR [95% CI]) from the propensity score-matched patients. Sensitivity analyses account for competing risk of death were performed (subdistribution hazard ratio [SHR] [95% CI]). In postmenopausal women, fracture risk of AI users displayed a HR 1.40 [95% CI: 1.05 to 1.87] and SHR 1.48 [95% CI: 1.11 to 1.98], compared to tamoxifen. Analyzing AI users at high risk of fracture, BP-treated patients had an HR 0.73 [95% CI: 0.51 to 1.04] and SHR 0.69 [95% CI: 0.48 to 0.98] compared to non-BP treated.

In summary, postmenopausal women during AI therapy had >40% excess risk of fracture compared to tamoxifen in real-life conditions, corroborating previous randomized controlled trials results. In high-risk patients, BP users had a significant lower fracture incidence during AI treatment than non-BP users. Monitoring fracture risk and related risk factors in AI patients is advisable.

Reference:

Pineda-Moncusí M, Garcia-Giralt N, Diez-Perez A, Servitja S, Tusquets I, Prieto-Alhambra D, Nogués X. Increased Fracture Risk in Women Treated With Aromatase Inhibitors Versus Tamoxifen: Beneficial Effect of Bisphosphonates. J Bone Miner Res. 2020 Feb;35(2):291-297. Epub 2019 Oct 31. PubMed PMID: 31596961. doi: 10.1002/jbmr.3886.

ORIGINAL ARTICLE

JBMR

Increased Fracture Risk in Women Treated With Aromatase Inhibitors Versus Tamoxifen: Beneficial Effect of Bisphosphonates

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ABSTRACT

Aromatase inhibitors have been associated with accelerated bone loss and an increased risk of osteoporotic fractures. Currently, bisphosphonates are recommended to reduce fracture risk in these patients. The aim of this study is to evaluate the fracture risk in breast cancer patients receiving aromatase inhibitors, compared to tamoxifen users, and to assess the effectiveness of oral bisphosphonates in reducing fracture risk. We performed an observational cohort study up to 10 years of follow-up. Data were extracted from primary care records in a population database. Women diagnosed with breast cancer between 2006 and 2015 and treated with tamoxifen or aromatase inhibitors (n = 36,472) were stratified according to low (without osteoporosis diagnosis nor bisphosphonates exposure) or high (with osteoporosis and/or treated with bisphosphonates) fracture risk. Cox models were used to calculate hazard ratios (HR [95% CI]) of fracture from the propensity score–matched patients. Sensitivity analyses account for competing risk of death were performed (subdistribution hazard ratio [SHR] [95% CI]). In postmenopausal women, fracture risk in aromatase inhibitor users showed an HR 1.40 [95% CI,1.05 to 1.87] and SHR 1.48 [95% CI, 1.11 to 1.98], compared to tamoxifen. Observing aromatase inhibitors patients at high risk of fracture, bisphosphonate-treated patients had an HR 0.73 [95% CI, 0.51 to 1.04] and SHR 0.69 [95% CI, 0.48 to 0.98] compared to nontreated. In conclusion, fracture risk in postmenopausal women during aromatase inhibitor treatment, in real-life conditions, was >40% compared to tamoxifen, corroborating previous randomized controlled trials results. In high-risk patients, bisphosphonate users had lower significant fracture incidence during aromatase inhibitor therapy than nonbisphosphonate users. Monitoring fracture risk and related risk factors in aromatase inhibitor patients is advisable. © 2019 American Society for Bone and Mineral Research.

KEY WORDS: AROMATASE INHIBITORS; ESTROGENS AND SERMS; FRACTURE PREVENTION; FRACTURE RISK ASSESSMENT; GENERAL POPULATION STUDIES

Introduction

First-line therapies for women with diagnosis of hormone receptor-positive breast cancer are aromatase inhibitors (AIs) and tamoxifen (TAM). Their effectiveness in reducing the risk of recurrence and mortality in breast cancer patients is well known.[1,2] However, these two adjuvant treatments have also been associated with side effects that may negatively affect the patient's quality of life, treatment adherence, and the associated mortality.[3]

In AI treatment, one of the most common side effects is accelerated bone loss, which is associated with an increased risk of osteoporotic fractures.[4] A Danish cohort study reported a higher risk of fracture occurrence related to AIs, compared to endocrine-untreated patients, whereas TAM had a protective effect on bone mass in postmenopausal women with breast

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cancer.[5] In a 2018 report on a population-based, retrospective cohort study, Neuner and colleagues[6] further corroborate this finding. They describe an increased risk for nonvertebral fractures in patients treated with AI, compared to TAM.

The current recommendation to reduce the fracture risk in these patients is to improve bone mineral density (BMD) using antiresorptive treatment, mainly bisphosphonates (BPs) or, in cases of low adherence or BP intolerance, denosumab.[7–9] Several phase III trials and population-based cohort studies have shown the efficacy of BP in preventing the bone loss induced by AIs.[9–11] A meta-analysis of 26 randomized clinical trials (RCTs), including both intravenous and oral BP administration, reported a small reduction of fracture risk in BP-treated patients with breast cancer.[12] However, these trials analyzed the BP effect in oncological outcomes, not for fractures. Thus, a wide range of fracture incidence was reported in these studies, perhaps due to underreporting, limiting the results interpretation. Furthermore, there is a lack of data from real clinical practice about the influence of oral BPs on fracture risk in AI-treated patients.

The aim of this study was to evaluate the fracture risk in patients with breast cancer receiving AI, compared to TAM-treated patients in a large population database of real-world practice in primary care centers. Additionally, effectiveness of oral BPs in reducing fracture risk was assessed in this population.

Subjects and Methods

Data sources

More than 7 million patient records are anonymously collected from more than 370 primary care teams of Catalonia in the System for the Development of Research in Primary Care (SIDIAP) database, covering >80% of the total Catalan population (http://www.sidiap.org). Available information includes sociodemographic data, lifestyle risk factors (alcohol use, obesity, smoking, etc.), comorbidities, and prescriptions dispensed. Data are collected by health professionals, using International Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) codes and structured forms designed for the gathering of clinical variables (smoking, BMI, etc.). Data on death, provided by the universal health insurance database of Catalonia (in Catalan, Registre Central de Persones Assegurades), and migration out of the catchment area are also registered in the SIDIAP database.

Study design and participants

This observational cohort study included women with a first diagnosis of breast cancer and treated with TAM or AIs who were registered in the SIDIAP database from January 2006 to December 2015. This study was approved by the Idiap Jordi Gol Research Ethics Committee and by the SIDIAP Database Scientific Committee.

Pharmacy dispensing records (pharmacy invoicing) include the anatomical therapeutic chemical (ATC) classification of the therapeutic regimen: L02BA01 for TAM, L02BG for AIs (L02BG03 for anastrozole, L02BG04 for letrozole, and L02BG06 for exemestane), M05BA for BP (etidronic acid, M05BA01; clodronic acid, M05BA02; alendronic acid, M05BA04; tiludronic acid, M05BA05; ibandronic acid, M05BA06; and risedronic acid, M05BA07), and M05BB03 for a combination of alendronic acid and cholecalciferol.

Patient diagnoses were registered by primary care professionals using ICD-10 codes. In case of osteoporosis, it was complemented by available *T*-score values (patients with values equal or lower than −2.5 SD were classified as osteoporotic).

Exclusion criteria were previous history of cancer (except nonmelanoma skin cancers), Cushing's syndrome, rickets, osteomalacia or Paget's disease, switching therapy (TAM followed by AI or vice versa), and use of bone-active drugs other than BP during adjuvant treatment (ie, strontium ranelate, M05BX03; raloxifene, G03XC01; and bazedoxifene, G03XC02). Participants with less than 6-month follow-up were also excluded.

Classification of low and high risk of fracture

Selected records were dichotomized according to AI or TAM exposure, then stratified into four groups according to risk of fracture: (i) low-risk AI-treated patients (AI-lowRF), ie, patients without evidence of osteoporosis diagnosis and without BP exposure; (ii) high-risk AI-treated patients (AI-highRF), ie, patients with a diagnosis of osteoporosis (according to WHO criteria) and/or BP users; (iii) low-risk TAM-treated patients (TAM-lowRF), ie, patients without evidence of osteoporosis diagnosis and without BP exposure; (iv) high-risk TAM patients (TAM-highRF), ie, patients with a diagnosis of osteoporosis and/or BP users.

Follow-up

Participants were followed from therapy initiation (first TAM, TAM-plus-BP, AI, or AI-plus-BP prescription dispensed) until the earliest of three endpoints: (i) adjuvant hormone treatment or BP treatment cessation (defined by a refill gap of 6 months or more with no dispensation of the index therapy) plus 1 month washout (for carryover effects); (ii) study outcome(s) date, as recorded in electronic medical records; or (iii) death, migration out of catchment area, or current end-date of SIDIAP data availability (December 31, 2015).

Variables

Outcomes

The study evaluated two outcomes: first fracture diagnosis of participants during AI versus TAM treatment, and first fracture diagnosis according to BP exposure within high-risk groups. Fracture locations included hip or proximal femur, vertebra, proximal humerus, and wrist or forearm. Fracture diagnosis was registered using the ICD-10 code based on clinical criteria.

Confounders

Using established clinical knowledge, a prespecified list of variables was extracted from SIDIAP and used as confounders. These confounding factors fell into three clusters:

(i) Sociodemographics: age (at treatment initiation), BMI, and socioeconomic status (assessed by MEDEA, a validated deprivation index).[13]

(ii) Lifestyle factors: smoking (current/former >1 year/never-smoker/ex-smoker) and weekly alcohol consumption, categorized by the Catalan Health Care System as none/low (mean of 0 g), moderate (not exceeding 170 g); high/alcoholic (170 g alcohol or more per week).

(iii) Past medical history: Charlson comorbidity index (measured at treatment initiation date); any previous history of fracture, rheumatoid arthritis, hyperthyroidism, liver cirrhosis, or chronic kidney disease; diagnosis of osteoporosis previous to adjuvant therapy

outset; concomitant use of sedative-hypnotic drugs at cohort entry; and previous use of systemic glucocorticoids.

Sociodemographic and lifestyle factors were included at the closest date to treatment initiation until the previous 12 months.

Statistical analysis

Differences in baseline characteristics between TAM and AI participants were described using mean ± SD and median (interquartile range) for quantitative variables with normal and non-normal distribution, respectively; *n* (%) per treatment group were used for categorical variables.

Incidence rates of fractures during TAM or AI treatment were assessed using the ERIC Notebook person-time methodology.[14]

To account for missing confounder data (BMI, smoking, alcohol drinking), multiple imputation by chained equations was carried out, obtaining 10 imputed datasets that were analyzed separately and results combined using Rubin rules. Imputed variables were evaluated by comparing them with their original values to validate its prediction. Drug-use cohorts were matched using propensity score matching (PSM) to minimize confounding by indication when comparing treatment groups. Propensity scores (PSs) represent the probability of receiving a given treatment, conditioned by baseline characteristics. PS was estimated using logistic regression models, where treatment exposure group was the outcome and the previously listed confounders were the adjustment variables. Matching was conducted using a 5:1 ratio ("the biggest group: the lowest group"), and nearest-neighbor method to select for the most similar PS. Standardized mean difference <0.1 in PS in each matched group was verified.

Survival analysis was performed, including Kaplan-Meier to estimate cumulative probability plots and Cox proportional hazards model to estimate hazard ratios (HRs) according to the exposure treatment. Proportional hazard assumption was verified in each model. Additionally, Fine and Gray models (sensitivity analyses accounting for a competing risk of death) were fitted to estimate subdistribution hazard ratios (SHRs) of the outcomes. HR and SHR are reported with 95% confidence intervals (95% CIs).

Menopause status in TAM users was unknown. To minimize the imbalance of premenopausal and postmenopausal effect between AI and TAM groups, a subset of participants older than 55 years was selected to compare fracture risk of TAM versus AI users.

All statistical analysis was performed with R for Windows version 3.3.3 using Hmisc, compareGroups, survival, survminer, ggplot2, mice, MatchIt, and dplyr packages.

Results

A total of 36,472 women treated with AI and/or TAM in the period 2006–2015 were screened and 22,591 (61.94%) were eligible for this study (7539 TAM and 15,052 AI) (Fig. 1). Median follow-up (months [Q1, Q3]) in each group was 27.0 [15.00, 48.0] in TAM and 29.0 [15.00, 50.0] in AI groups. Baseline characteristics of participants are shown in Table 1. AI users were older, had higher BMI, and were more likely to have chronic kidney disease, osteoporosis, and a previous fracture history. Additionally, AI users had greater exposure to BP, systemic corticosteroids, and sedative-hypnotic drugs, but were less likely to be current smokers than TAM users.

Fracture incidence

During the study, 658 (2.91%) patients had a fracture during the adjuvant treatment. Incidence rates (per 1000 person-years) of fractures in all participants are reported in Table 2. The highest incidence rate was found in AI users, mainly in those classified at high risk of fracture. Cumulative incidence function plot of fracture events is illustrated in Fig. 2.

In the subset of participants older than 55 years, age, BMI, any previous fracture, and glucocorticoids intake did not differ between AI and TAM users. In this subset, 581 (3.86%) fractures were reported out of a total of 15,038 patients. Incidence rates

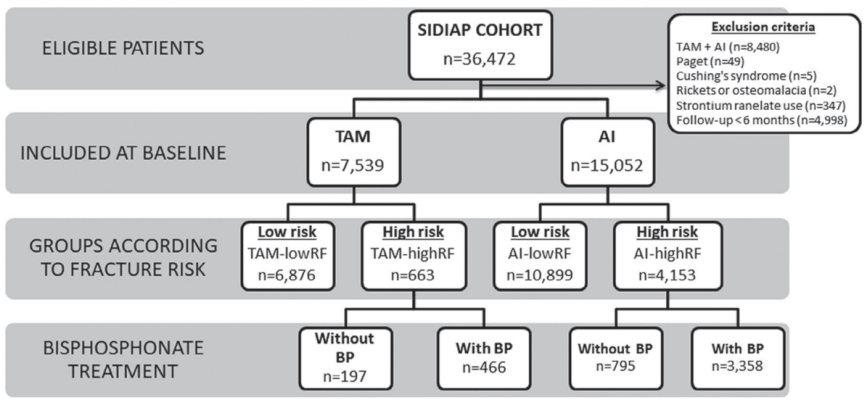

Fig. 1. Flowchart of SIDIAP cohort study. Patients at low risk are those without osteoporosis diagnosis and without BPs. Patients at high risk are those with diagnosis of osteoporosis and/or candidates to BP treatment. AI = aromatase inhibitor; BP = bisphosphonate; highRF = high risk of fracture; lowRF = low risk of fracture; TAM = tamoxifen.

Table 1. Baseline Characteristics of Patients

Variable	AI (*n* = 15,052)	TAM (*n* = 7539)
Age (years), mean ± SD	67.30 ± 11.20	52.3 ± 13.60
BMI (kg/m^2), mean ± SD	29.80 ± 5.32	28.2 ± 5.57
Missing BMI, *n* (%)	11,031 (73.29)	6153 (81.62)
Charlson comorbidity index, *n* (%)		
0	1896 (12.60)	824 (10.90)
1	594 (3.95)	139 (1.84)
2	8159 (54.20)	5467 (72.50)
3	2896 (19.20)	825 (10.90)
≥4	1507 (10.00)	284 (3.77)
Smoking, *n* (%)		
Never smokers	8387 (55.70)	2853 (37.80)
Current smokers	1072 (7.12)	1249 (16.60)
Ex-smokers	808 (5.37)	653 (8.66)
Missing	4785 (31.80)	2784 (36.90)
Risk of alcoholism, *n* (%)		
None/low	2006 (13.33)	693 (9.19)
Moderate	331 (2.20)	184 (2.44)
High/alcoholic	14 (0.09)	5 (0.07)
Missing	12,701 (84.38)	6657 (88.30)
Bisphosphonates use, *n* (%)	3450 (22.90)	480 (6.37)
Previous fracture, *n* (%)	603 (4.01)	152 (2.02)
Previous use of systemic glucocorticoids, *n* (%)	215 (1.43)	62 (0.82)
Rheumatoid arthritis, *n* (%)	117 (0.78)	43 (0.57)
Chronic kidney disease, *n* (%)	513 (3.41)	75 (0.99)
Osteoporosis, *n* (%)	1859 (12.40)	371 (4.92)
Hypnotics/sedative, *n* (%)	8843 (58.70)	3621 (48.00)

AI = aromatase inhibitor; BMI = body mass index; TAM = tamoxifen.

Table 2. Fracture Incidence in All Participants

Exposure group	Fracture *n* (%)	Incidence rate (95% CI) (cases/1000 py)
TAM-lowRF	76/6876 (1.11)	4.10 (3.26–5.11)
TAM-highRF	15/663 (2.26)	13.24 (7.69–21.34)
AI-lowRF	401/10,899 (3.67)	12.32 (11.15–13.57)
AI-highRF	166/4153 (4.00)	20.06 (17.18–23.30)

AI = aromatase inhibitors; CI = confidence interval; py = person-years; lowRF = patients at low risk of fracture; highRF = patients at high risk of fracture; TAM = tamoxifen.

are described in Table 3. As is expected, patients identified as having high risk of fracture, whether treated with TAM or AI, had the highest fracture rates.

Fracture risk analysis

TAM versus AI users

Fracture risk of AI users compared to TAM users was evaluated in patients older than 55 years. From 10 imputed datasets, PSM selected a mean ± SD of 2236.4 ± 3.37 TAM and 10,394.6 ± 275.39 AI users (Table 4). Cox analysis showed an increased fracture risk of 40% (HR 1.40; 95% CI, 1.05 to 1.87) in AI users

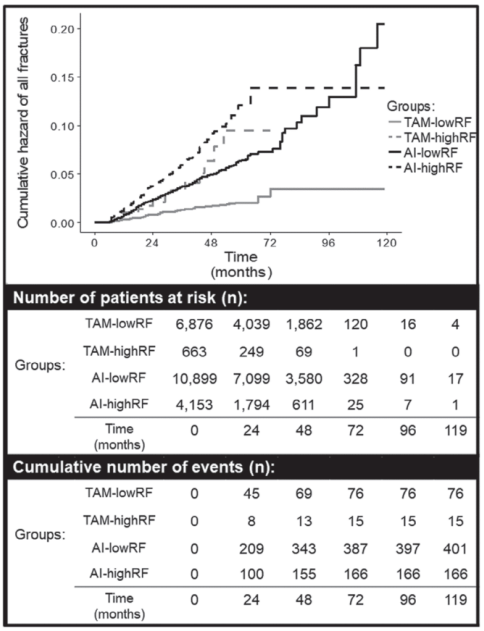

Fig. 2. Cumulative hazard plot of fracture events in study groups according to risk of fracture. Graphs show Kaplan-Meier curves representing the outcome of the study in terms of cumulative hazards. AI = aromatase inhibitor; highRF = high risk of fracture; lowRF = low risk of fracture; TAM = tamoxifen.

compared to TAM users. After competing risk adjustment, fracture risk in AI increased to 48% (SHR 1.48; 95% CI, 1.11 to 1.98).

Considering only patients at low risk of fracture, PSM selected a mean ± SD of 1737.7 ± 1.25 patients in TAM-lowRF and 7895.9 ± 85.02 patients in AI-lowRF groups. Characteristics of selected participants are reported at Supplemental Table 1. Similar results of survival analysis were obtained: AI-lowRF users had an increased fracture risk of 40% compared with TAM-lowRF users (HR 1.40; 95% CI, 0.99 to 1.96); this risk increased to 48% after competing risk adjustment (SHR 1.48; 95% CI, 1.05 to 2.08).

After matching patients at high risk of fracture treated with AI or TAM (see Supplemental Table 2), no significant differences were observed in fracture risk between both groups (HR 1.36; 95% CI, 0.75 to 2.46; SHR 1.44;95% CI, 0.80 to 2.59). However, we cannot rule out a lack of statistical power due to the reduced sample size in the TAM-highRF group (*n* = 478).

BP effect analysis

Within AI-highRF patients (see characteristics in Table 5), the incidence rate was lower in BP-treated patients than in patients without BP exposure: 18.57 (95% CI, 14.85 to 22.29) versus 26.21 (95% CI, 19.00 to 33.43), respectively. Cox analysis showed a fracture reduction trend in BP users compared to non-users that was confirmed after competing risk analysis (HR 0.73;95% CI, 0.51 to 1.04; SHR 0.69; 95% CI, 0.48 to 0.98).

Table 3. Fracture Incidence in Women Older Than 55 Years

Exposure group	Fracture *n* (%)	Incidence rate (95% CI) (cases/1000 py)
TAM-lowRF	38/1741 (2.18)	9.02 (6.48–12.26)
TAM-highRF	15/502 (2.99)	16.57 (9.63–26.72)
AI-lowRF	368/9076 (4.05)	13.55 (12.22–14.99)
AI-highRF	160/3719 (4.30)	21.35 (18.23–24.85)

AI = aromatase inhibitors; CI = confidence interval; py = person-years; lowRF = patients at low risk of fracture; highRF = patients at high risk of fracture; TAM = tamoxifen.

Table 4. Baseline Characteristics of >55-year-old Matched Patients From AI and TAM Users

Variable	TAM (*n* = 2236.4)	AI (*n* = 10,394.6)
Age (years), mean ± SD	69.80 ± 10.10	70.00 ± 9.27
BMI (kg/m^2), mean ± SD	27.20 ± 6.65	27.00 ± 6.68
Charlson comorbidity index, *n* (%)		
0	287.9 (12.9)	1354.3 (13.0)
1	96.6 (4.32)	452.9 (4.36)
2	1238.4 (55.4)	5623.4 (54.1)
3	410.1 (18.3)	1975.3 (19.0)
≥4	203.4 (9.09)	988.7 (9.51)
Smoking, *n* (%)		
Never smokers	1931.3 (86.4)	8967.7 (86.3)
Current smokers	156.2 (6.98)	739.3 (7.11)
Ex-smokers	148.9 (6.66)	687.6 (6.61)
Risk of alcoholism, *n* (%)		
None/low	1557.3 (69.6)	7281.4 (70.0)
Moderate	675.5 (30.2)	3097.3 (29.8)
High/alcoholic	3.6 (0.16)	15.9 (0.15)
Bisphosphonate use, *n* (%)	335.5 (15.0)	2475.5 (23.8)
Previous fracture, *n* (%)	94.7 (4.23)	435.6 (4.19)
Previous use of systemic glucocorticoids, *n* (%)	29.8 (1.33)	139.8 (1.34)
Rheumatoid arthritis, *n* (%)	20.7 (0.93)	86.2 (0.83)
Chronic kidney disease, *n* (%)	58.9 (2.63)	296.9 (2.86)
Osteoporosis, *n* (%)	317.2 (14.2)	1421.3 (13.7)
Hypnotics/sedative, *n* (%)	1248.8 (55.8)	5954.1 (57.3)

All values are the mean of the 10 imputed datasets. AI-lowRF = aromatase inhibitors at low risk of fracture; BMI = body mass index; TAM-lowRF = tamoxifen patients at low risk of fracture.

After stratifying according to different oral BPs, risedronic acid and alendronic acid plus cholecalciferol raised as the most effective BPs (Supplemental Tables 3 and 4, and Supplemental Fig. 1).

In TAM-highRF patients (see characteristics in Table 6), incidence rates were 10.20 (95% CI, 0.20 to 20.20) in patients without BP exposure, and 11.87 (95% CI, 1.07 to 22.67) in patients with BP exposure. No significant differences were detected in Cox analysis (TAM-highRF patients with BP: HR 1.36; 95% CI, 0.30 to 6.20; SHR 1.13 95% CI, 0.25 to 5.08, compared with non-BP).

Discussion

In this massive real-world cohort study of women diagnosed with hormone receptor–positive early breast cancer, the fracture

Table 5. Baseline Characteristics of Matched Patients Within AI-highRF Group: BP-Treated Versus Non-BP–Treated Patients

Variable	AI-highRF	
	Non-BP–treated (*n* = 764.9)	BP-treated (*n* = 2,741.1)
Age (years), mean ± SD	72.1 ± 9.82	69.4 ± 9.34
BMI (kg/m^2), mean ± SD	24.3 ± 3.74	24.4 ± 3.89
Charlson comorbidity index, *n* (%)		
0	79.9 (10.4)	335.7 (12.2)
1	35.9 (4.69)	112.7 (4.11)
2	392 (51.2)	1,554.2 (56.7)
3	161.7 (21.1)	513.1 (18.7)
≥4	225.4 (8.22)	225.4 (8.22)
Smoking, *n* (%)		
Never smokers	656.2 (85.8)	2,305.2 (84.1)
Current smokers	61.8 (8.08)	263.1 (9.60)
Ex-smokers	46.9 (6.13)	172.8 (6.30)
Previous fracture, *n* (%)	58.5 (7.65)	164.4 (6.00)
Previous use of systemic corticosteroids, *n* (%)	14.6 (1.91)	37.7 (1.38)
Rheumatoid arthritis, *n* (%)	9 (1.18)	27.2 (0.99)
Chronic kidney failure, *n* (%)	32.1 (4.20)	65.5 (2.39)
Hypnotics/sedative, *n* (%)	480.8 (62.9)	1,726.1 (63.0)

All values are the mean of the 10 imputed datasets. AI-highRF = aromatase inhibitors patients at high risk of fracture; BMI = body mass index; BP = bisphosphonate.

Table 6. Baseline Characteristics of Matched Patients Within TAM-highRF Groups: BP-Treated Versus Non-BP–Treated Patients

Variable	TAM-highRF	
	Non-BP–treated (*n* = 158.7)	BP-treated (*n* = 254.4)
Age (years), mean ± SD	67.2 ± 11.5	66.6 ± 12.1
BMI (kg/m^2), mean ± SD	24.2 ± 3.95	23.9 ± 3.93
Charlson comorbidity index, *n* (%)		
0	18.7 (11.8)	32.3 (12.7)
1	7 (4.41)	11.6 (4.56)
2	96.7 (60.9)	153.3 (60.3)
3	27.8 (17.5)	45.7 (18.0)
≥4	8.5 (5.36)	11.5 (4.52)
Smoking, *n* (%)		
Never smokers	138 (87.0)	219.2 (86.2)
Current smokers	12 (7.56)	19.7 (7.74)
Ex-smokers	8.7 (5.48)	15.5 (6.09)
Previous fracture, *n* (%)	14.4 (9.07)	20.3 (7.98)
Previous use of systemic corticosteroids, *n* (%)	1.3 (0.82)	3.5 (1.38)
Rheumatoid arthritis, *n* (%)	1.4 (0.88)	2.4 (0.94)
Chronic kidney failure, *n* (%)	1.6 (1.01)	1.8 (0.71)
Hypnotics/sedative, *n* (%)	87 (54.8)	144.5 (56.8)

All values are the mean of the 10 imputed datasets. BMI = body mass index; BP = bisphosphonate; TAM-highRF = tamoxifen patients at high risk of fracture.

risk was assessed according to adjuvant therapy. It is well known that a number of risk factors (age, menopausal status, BMD, history of fractures, etc.) are involved in the individual's propensity to fragility fracture. Classification of patients according to fracture risk levels at baseline (osteoporotic diagnosis and/or on anti-osteoporotic treatment) allowed a more accurate analysis. To minimize the potential bias of menopause effect, women older than 55 years were selected to assess the differences in fracture risk between AI and TAM users. In this subset of postmenopausal women, AI users showed about 40% increased fracture risk, compared to TAM users. Similar results were obtained in the subset of patients at low risk of fracture. In the subgroup of AI-highRF patients, lower fracture incidence was detected in BP-treated patients who had a fracture risk reduction of 30% compared to non-BP users. On the other hand, no significant differences were detected within TAM-highRF patients. To the best of our knowledge, this is the first study assessing BPs effect on breast cancer patients at high risk of fracture, observing BP-users versus non-BP users in a real-world, noncontrolled population.

The difference in risk detected between AI and TAM therapies was in line with previous studies. A recent meta-analysis by Tseng and colleagues[15] reported a 35% higher fracture risk associated with AI therapy compared to TAM ($p < 0.01$) and two cohort studies found an increased risk of fractures associated with AI therapy in postmenopausal participants.[5,16]

The protective effect of BP on fracture risk by increasing BMD, even in women treated with AI, is well known.[9–11] However, these studies are based on strictly controlled cohorts and RCTs, not on data from real-life primary care. In our cohort study, BP use reduced fracture risk by 30% in patients at high risk of fracture. Our results are in line with reported risk reductions of 30% to 40% in a general population treated with oral BP.[17]

Although similar fracture risk was observed in the TAM-highRF patients despite BP treatment, we cannot rule out a lack of statistical power due to the reduced sample size in the TAM-highRF group.

Overall, AI patients experienced more fractures than TAM users, especially AI users at high risk. In these AI patients, strict monitoring is recommended to identify patients at high risk of fracture during AI therapy for rapid BPs prescribing.

One limitation of the study was that data of severity and grade of breast cancer were not accessible. However, TAM and AI monotherapies are recommended and mainly used for hormone receptor–positive early breast cancer.[18] Likewise, available data could not distinguish between osteoporotic fracture and high-energy impact fracture. However, a random distribution of impact fracture across patient groups would be expected. On the other hand, SIDIAP does not contain BMD data and the direct effect of BP on this parameter could not be assessed. In this line, osteoporosis diagnosis was registered using ICD-10 codes by the grand practitioner, which is based on BMD assessment, plus available *T*-scores in SIDIAP data. However, we cannot discard a misclassification of osteoporotic patients due to the lack of an accurate diagnosis. As 29.5% to 46.5% of vertebral fractures are not identified,[19] the risk of all fractures associated with AI use in our cohort could be underestimated.

The strength of this study is that results are based on a large population database that comprises anonymized electronic medical records of more than 7 million patients in primary care (>80% of the population of Catalonia). The Catalan healthcare system is universal in coverage; general practitioners act as gatekeepers to the system and are responsible for long-term prescriptions. A recent study by Gray and colleagues[20] validates the use of PSM in a real-world cohort to estimate a treatment effect. Additionally, the SIDIAP database has been successfully used to assess fracture risk after oral BP treatment, a study that validated this database for real-world epidemiology studies.[21] To improve the validity of the study results, patients with a follow-up shorter than 6 months were excluded, diminishing the probability of including events unrelated to the purpose of the study.

In summary, in real-life conditions fracture risk was increased by more than 40% during AI treatment, compared to TAM therapy, in women older than 55 years; this corroborated previous RCT results. In patients at high risk, BP users had lower significant fracture incidence during AI adjuvant therapy than non-users of BP. Monitoring fracture risk and related risk factors in AI patients is advisable in order to improve the quality of life of these patients. Furthermore, it is convenient to provide antiresorptive treatment according to clinical guidelines recommendations.

Disclosures

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Authors' roles: Study design: MPM, NGG, ADP, DPA, and XN. Data collection: SS, IT, and XN. Data analysis: MPM, DPA, and NGG. Interpretation of data: All authors. Drafting of the manuscript: MPM and NGG. Critical revision of the manuscript for important intellectual content: ADP, SS, IT, DPA, XN. Approving final version of manuscript: All authors. MPM takes responsibility for the integrity of the data analysis.

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Supplemental data

Supplemental Table 1. Baseline characteristics of >55-year-old matched patients from AI-lowRF and TAM-lowRF groups

Variable	TAM-lowRF N=1,737.7	AI-lowRF N=7,895.9
Mean age (years) ± (SD)	69.60 ± 10.20	69.80 ± 9.48
Mean BMI (kg/m^2) ± (SD)	25.20 ± 4.56	25.20 ± 4.57
Charlson co-morbidity index (n(%)):		
0	223.9 (12.90%)	1,030.7 (13.10%)
1	72 (4.14%)	335.6 (4.25%)
2	964.3 (55.50%)	4,207.5 (53.30%)
3	316.9 (18.20%)	1,538.2 (19.50%)
4 or >4	160.6 (9.24%)	783.9 (9.93%)
Smoke (n(%)):		
Never Smokers	1,496.7 (86.10%)	6,777.3 (85.80%)
Current Smokers	116.2 (6.69%)	546.9 (6.93%)
Ex-smokers	124.8 (7.18%)	571.7 (7.24%)
Alcoholism, n (%):		
None/Low	1,514.2 (87.10%)	6,867.3 (87.00%)
Moderate	214.6 (12.30%)	989.8 (12.50%)
High/Alcoholic	8.9 (0.51%)	38.8 (0.49%)
Previous fracture (n(%))	51 (2.93%)	238.7 (3.02%)
Previous use of systemic glucocorticoids (n(%))	19 (1.09%)	89.2 (1.13%)
Rheumatoid arthritis (n(%))	13.8 (0.79%)	47.1 (0.60%)
Chronic kidney disease (n(%))	51 (2.93%)	246.7 (3.12%)
Hypnotics/sedative (n(%))	953.8 (54.90%)	4,450.1 (56.40%)

All values are the mean of the ten imputed datasets. Abbreviations: AI-lowRF, aromatase inhibitors patients at low risk of fracture; BMI, body mass index; TAM-lowRF, tamoxifen patients at low risk of fracture.

Supplemental Table 2. Baseline characteristics of >55-year-old matched patients from AI-highRF and TAM- highRF groups

Variable	TAM-highRF N=478.1	AI-highRF N=2,131.9
Mean age (years) ± (SD)	70.6 ± 9.36	70.6 ± 8.78
Mean BMI (kg/m^2) ± (SD)	27.6 ± 6.52	27.4 ± 6.63
Charlson co-morbidity index (n(%)):		
0	61.4 (12.8%)	279.7 (13.1%)
1	23.5 (4.92%)	98.8 (4.63%)
2	264.9 (55.4%)	1,173.7 (55.1%)
3	89.2 (18.7%)	401.6 (18.8%)
4 or >4	39.1 (8.18%)	178.1 (8.35%)
Smoke (n(%)):		
Never Smokers	419.4 (87.7%)	1,864.5 (87.5%)
Current Smokers	33.4 (6.99%)	156.2 (7.33%)
Ex-smokers	25.3 (5.29%)	111.2 (5.22%)
Alcoholism, n (%):		
None/Low	338.7 (70.8%)	1,523.5 (71.5%)
Moderate	139 (29.1%)	603.7 (28.3%)
High/Alcoholic	0.4 (0.08%)	4.7 (0.22%)
Bisphosphonates use (n(%))	306.7 (64.1%)	1,697.1 (79.6%)
Previous fracture (n(%))	39.1 (8.18%)	157.8 (7.40%)
Previous use of systemic glucocorticoids (n(%))	8.5 (1.78%)	29.5 (1.38%)
Rheumatoid arthritis (n(%))	5.5 (1.15%)	21.5 (1.01%)
Chronic kidney disease (n(%))	7.6 (1.59%)	40.9 (1.92%)
Osteoporosis (n(%))	307.3 (64.3%)	1,026.4 (48.1%)
Hypnotics/sedative (n(%))	282.8 (59.2%)	1,285.1 (60.3%)

All values are the mean of the ten imputed datasets.
Abbreviations: AI-highRF, aromatase inhibitors patients at high risk of fracture; BMI, body mass index; TAM- highRF, tamoxifen patients at high risk of fracture.

Supplemental table 3. Incidence rate from AI-highRF group according to different oral BPs use

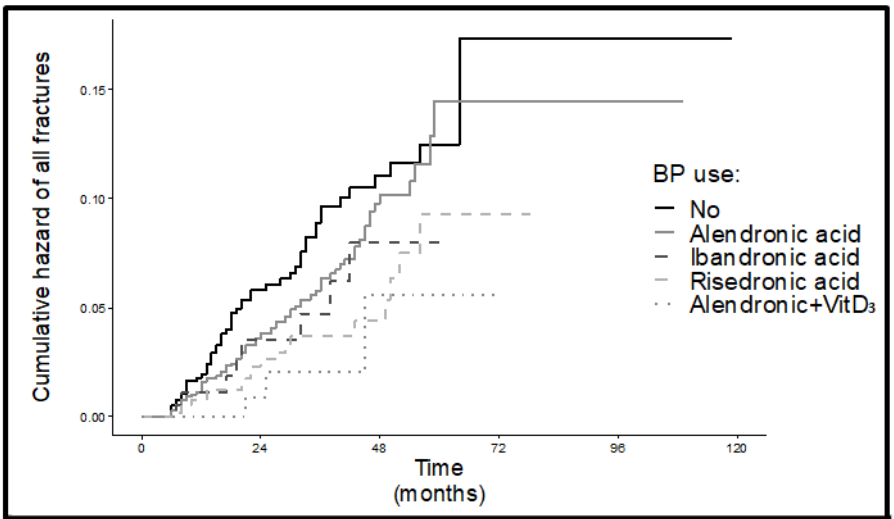
BP used	FX	Incidence rate [95%CI] (cases/1,000py)
Without BP	53/795 (6.67%)	26.64 [19.47 to 33.82]
Alendronic acid	80/2,150 (3.72%)	20.31 [15.86 to 24.76]
Ibandronic acid	8/251 (3.19%)	16.71 [5.13 to 28.28]
Risedronic acid	19/713 (2.66%)	13.13 [7.22 to 19.03]
Alendronic acid plus cholecalciferol	3/234 (1.28%)	7.35 [-0.97 to 15.68]

Abbreviations: BP, bisphosphonates; CI, confidence interval; FX, fracture; py, person-years.

Supplemental table 4. Risk of fracture in AI-highRF patients using different oral BPs compared to AI-highRF without BPs

BP used	HR [95%CI]	SHR [95%CI]
Alendronic acid	0.84 [0.58 to 1.23]	0.80 [0.55 to 1.16]
Ibandronic acid	0.64 [0.27 to 1.52]	0.60 [0.25 to 1.43]
Risedronic acid	0.47 [0.25 to 0.86]	0.43 [0.23 to 0.80]
Alendronic acid plus cholecalciferol	0.35 [0.10 to 1.21]	0.32 [0.09 to 1.09]

Abbreviations: BP, bisphosphonates; CI, confidence interval; HR, Hazard ratio; SHR, sub-distribution HR.



Number of patients at risk (n):

BP use	No	795	412	178	12	6	1
	Alendronic acid	2,150	858	264	12	1	0
	Ibandronic acid	251	100	37	0	0	0
	Risedronic acid	713	327	110	1	0	0
	Alendronic+VitD$_3$	234	95	23	0	0	0
	Time (months)	0	24	48	72	96	119

Cumulative number of events (n):

BP use	No	0	35	50	53	53	53
	Alendronic acid	0	47	76	80	80	80
	Ibandronic acid	0	5	8	8	8	8
	Risedronic acid	0	11	15	19	19	19
	Alendronic+VitD$_3$	0	1	3	3	3	3
	Time (months)	0	24	48	72	96	119

Supplemental figure 1 Cumulative hazard plot of fracture events within AI-highRF patients according to risk its BP use. Graphs show Kaplan-Meier curves representing the outcome of the study in terms of cumulative hazards. Abbreviations: BP, bisphosphonate: VitD3, cholecalciferol supplements.

Article 5

Title: Thromboembolic, cardiovascular and overall mortality risks of aromatase inhibitors, compared to tamoxifen treatment

Summary:

Among different side effects related to tamoxifen (TAM) and aromatase inhibitor (AI) therapies, increased risk of thromboembolic and cardiovascular events, respectively, emerged as competing causes of death. We performed an observational cohort study including women diagnosed with breast cancer and treated with TAM or AI to analyze the risk of thromboembolic and cardiovascular events, and the overall survival benefit in AI-treated patients, compared to TAM patients. Data were obtained from primary care records in a large population database (SIDIAP). Incidence rates of study outcomes are reported. Survival analyses included Kaplan–Meier estimation and Cox proportional hazards models. Propensity score adjustment was used to minimize confounding. A sensitivity analysis was conducted through Fine and Gray models to account for competing risk of death. Data were available for 9,537 women treated with TAM where of these, 3,082 were postmenopausal; and 18,455 treated with AI. Adjusted hazard ratios [95% confidence interval (CI)] for AI users, compared with postmenopausal-TAM group, were 0.93 [95%CI: 0.69 to 1.26] for thromboembolic events; 1.13 [95%CI: 0.79 to 1.63] for cardiovascular events, and 0.76 [95%CI: 0.70 to 0.82] for mortality; competing risk analysis detected a potential

risk of pulmonary embolism (2.15 [95%CI: 0.99 to 4.64]) in AI-treated patients.

In conclusion, AI users had >20% lower all-cause mortality compared to TAM users, without increasing cardiovascular and thromboembolic risk. This would locate AI therapy at the first line in clinical practice.

Reference:

Pineda-Moncusí M, Garcia-Giralt N, Diez-Perez A, Tusquets I, Servitja S, Albanell J, Prieto-Alhambra D, Nogués X. Thromboembolic, cardiovascular and overall mortality risks of aromatase inhibitors, compared with tamoxifen treatment: an outpatient-register-based retrospective cohort study. Ther Adv Med Oncol. 2020 Mar 25;12:1758835920909660. PubMed PMID: 32231712. doi: 10.1177/1758835920909660.

Therapeutic Advances in Medical Oncology

Original Research

Thromboembolic, cardiovascular and overall mortality risks of aromatase inhibitors, compared with tamoxifen treatment: an outpatient-register-based retrospective cohort study

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Abstract

Background: Tamoxifen (TAM) and aromatase inhibitor (AI) therapies have been associated with increased risk of thromboembolic and cardiovascular events, respectively, in addition to other side effects. This study analysed the risk of these events and the overall survival (OS) benefit in breast cancer patients treated with AI, compared with TAM-treated patients, in a large population-based cohort.

Methods: This observational cohort study included women diagnosed with breast cancer and treated with TAM or AI. Data were extracted from primary care records in a population database (SIDIAP, System for the Development of Research in Primary Care). Incidence rates of study outcomes are reported. Survival analyses included Kaplan–Meier estimation and Cox proportional hazards models. Sensitivity analysis was carried out, using Fine and Gray models to account for competing risk of death. Confounding was minimized using propensity score adjustment and inverse probability weighting (IPW) adjustment.

Results: Data from 3082 postmenopausal women treated with TAM, and 18,455 treated with AI, were available. Adjusted hazard ratios (HRs) [95% confidence interval (CI)] for AI users, compared with TAM group, were 0.93 (95%CI 0.69–1.26) for thromboembolic events (TEEs); 1.13 (95%CI 0.79–1.63) for cardiovascular events, and 0.76 (95%CI 0.70–0.82) for mortality. Additional analyses using competing risk analysis had similar results, while IPW adjustment showed a potential risk of pulmonary embolism (PE) [2.26 (95%CI 1.02–4.97)] in AI-treated patients.

Conclusions: AI users had >20% lower all-cause mortality compared with TAM users, without increasing risk to experience cardiovascular and TEEs. This would locate AI therapy on the first line in clinical practice. Thus, AI might be the most preferable option in adjuvant hormonal therapy choice.

Keywords: aromatase inhibitor, breast cancer, cardiovascular events, overall mortality, tamoxifen, thromboembolic events

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Introduction

Aromatase inhibitors (AIs) and tamoxifen (TAM) are known to be effective adjuvant endocrine therapies for patients with hormone-receptor-positive breast cancer. Generally, these patients have good prognosis, with an overall survival (OS) rate exceeding 80%.[1,2] However, these therapies have been associated with side effects that can affect quality of life and could impact on mortality, among them, cardiovascular events (CVEs)

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and thromboembolic events (TEEs) are emerging as competing causes of death.[3] A number of randomized controlled trials (RCT) have explored the cardiovascular effect, comparing AIs *versus* TAM, with heterogeneous results.[4,5] These studies have provide evidence of increased CVEs associated with AI therapies, compared with TAM, likely due to high depletion of estradiol levels and alteration of lipid metabolism related to AIs,[6] or to the cardioprotective role of tamoxifen *per se*.[7]

Although RCT and meta-analysis are gold standard experimental approaches for the study of efficacy and safety in 'ideal' conditions, they are sometimes not representative of clinical practice conditions or of the actual profile of the treated community,[8] and, thus, they cannot address definitively safety issues, particularly for side effects with low incidence. We therefore aimed to analyze the risk of CVE and TEE, and OS benefit during AI therapy, compared with TAM, in real-world conditions.

Thus, the present study used the SIDIAP (System for the Development of Research in Primary Care) database, which provided anonymized clinical information as coded by primary care practitioners in Catalonia, Spain, covering more than 7 million patients.[9] SIDIAP contains information on socio-demographics and extended clinical data. Moreover, SIDIAP is linked to pharmacy invoice data, which provides detailed information on drugs dispensed in community pharmacies under the universal health care system. Using this database, we performed a population-based study, including almost 28,000 women treated with AI or TAM for up to 10 years of follow up, to assess thromboembolic and cardiovascular events, and resulting OS in general clinical practice.

Methods

Data sources

SIDIAP (http://www.sidiap.org) is an anonymized clinical database of more than 7 million patient records collected from more than 370 primary care teams covering >80% of the total population of Catalonia. Among the available variables are socio-demographic data, lifestyle risk factors, prescriptions dispensed and comorbidities. Health professionals gather this information using ICD-10 codes and structured forms designed for the collection of clinical factors (alcohol use, smoking, body mass index, etc.). Migration out of the catchment area is also recorded, allowing for longitudinal follow up of patients.[10] Death is also registered in the SIDIAP database, as provided by the universal health insurance database for Catalonia (in Catalan, 'registre central de persones assegurades').

Study design and participants

Retrospective observational cohort study of women diagnosed with early breast cancer, defined as nonmetastatic breast cancer, stage I–III, and treated with monotherapy of TAM or AIs as registered in the SIDIAP database from January 2006 to December 2015. Therapeutic regimen in patients was identified by its anatomical therapeutic chemical classification in pharmacy dispensing records, coded as L02BG for AIs (L02BG03 for anastrozole, L02BG04 for letrozole, L02BG06 for exemestane), and L02BA01 for TAM.

Exclusion criteria were previous history of cancer (except nonmelanoma skin cancers) and patients who had received a switching therapy (TAM followed by AI or *vice versa*). No concomitant anticancer drugs other than TAM or AI were used.

Ethics statement

This study used only data collected routinely from the SIDIAP database. The Idiap Jordi Gol Research Ethics Committee and the SIDIAP Database Scientific Committee have approved the study protocol (P16/031). No human subjects or tissues were used in this study. Data provided by SIDIAP was anonymized and risk of identification was almost null according to Spanish law *LO 15/ 1999 13 December*. Thus, informed consent did not need to be obtained from participants.

Follow up

Participants were followed up from therapy initiation (first day of TAM or AI dispensing) until the earliest of three endpoints: treatment cessation (defined by a refill gap of 6 months or more with no dispensation of the index therapy), plus 1 month wash-out (for carry-over effects); evaluated outcomes date (as recorded in electronic medical records); or death, migration out of catchment area, or end of SIDIAP data availability (31 December 2015).

In the overall mortality assessment, patients were followed-up during all the study period (2006–2015).

Variables

Outcomes of the study. Analyzed outcomes were the first TEE [pulmonary embolism (PE) and deep vein thrombosis (DVT), including phlebitis and thrombophlebitis] and the first CVE [coronary artery disease (CAD), and cerebrovascular diseases (CVD), including stroke and intracerebral haemorrhage, among others] occurring during adjuvant therapy. In addition, PE, DVT, CAD and CVD were analysed separately as secondary outcomes. OS, expressed as mortality status during follow up, was also reported. ICD-10 codes used to identify the outcomes of the study are documented in Supplementary Table S1.

Confounders. A prespecified list of confounders was extracted from SIDIAP, informed by previous clinical knowledge and scientific literature. These confounding factors fell into five clusters:

(1) Sociodemographics: age (at treatment initiation), body mass index (BMI), and socioeconomic status (assessed by MEDEA, a validated deprivation index).[11]
(2) Menopausal status: defined as women >55 years old at diagnosis in the TAM group or all patients treated with AI. Menopausal status of patients <55 years old in TAM group is unknown.
(3) Lifestyle factors: smoking, alcohol use (defined according to The Catalan Health Care System: none/low, as a mean of 0 g of alcohol per week; moderate, not exceeding 170 g of alcohol per week; high/alcoholic, 170 g of alcohol or more per week).
(4) Past medical history: Charlson comorbidity index, and previous history of CVE and TEE.
(5) Concomitant use of antiplatelets or anticoagulants or statins at cohort entry (i.e. TAM/AI initiation).

Statistical analysis

Data from SIDIAP were managed using MySQL. Differences in baseline characteristics between TAM and AI participants were described and imbalances analysed using *t* test and Chi-square test.

Incidence rates of study outcomes (during treatment for TEE/CVE and at any time for OS) were estimated.[12]

For each outcome, survival analysis was done by Kaplan–Meier estimation and Cox proportional hazards model to estimate cumulative probability plots and HRs according to treatment/exposure, respectively.

Additionally, a subanalysis using Fine and Gray regression models were fitted to estimate subdistribution hazard ratios (SHR) for TEE and CVE (separately) according to treatment arm, accounting for a competing risk of death.[13]

HR and SHR are reported with 95% CI, and using TAM as a reference group (and AI as the 'exposed' group). Moreover, the assumption of proportionality was verified though proportional hazards assumption for a Cox regression model test.

Adjustment in survival analysis was conducted using the propensity score (PS). PS was estimated using logistic regression models, where treatment group was the outcome and the previously listed confounders were adjusted for. The final list of variables used in PS adjustments are listed in Table 1, including statins, anticoagulants and antiplatelet drugs. Missing data were imputed before the PS estimates, using multiple imputation by chained equations, obtaining 10 imputed datasets that were combined using Rubin's rules.[14] Previous TEE and CVE history were included in their respective analyses, and in OS evaluation. Additional analysis censoring patients with previous TEE and CVE was performed to account for potential baseline higher risk.

An additional analysis adjusting survival analysis by stabilized inverse probability weighting (IPW) and using a robust sandwich-type variance estimator was performed.[15]

In order to compare our results with an analysis not accounting for menopausal status, we repeated the same models using total TAM users, including those women younger than 55 years old.

All statistical analyses were conducted using R for Windows version 3.3.3 and the following R packages: foregin, Hmisc, compareGroups, survival and mice.

Table 1. Baseline characteristics of candidates in postmenopausal women.

Variable	AI *N* = 18,455	TAM *N* = 3082
Median age (years) [Q1;Q3]	67.0 [59.0;77.0]	69.0 [62.0;79.0]
Mean BMI (kg/m^2) ± (SD)	29.7 (5.36)	29.8 (5.09)
Missing, *n* (%)	13,555 (73.45)	2189 (71.03)
QMEDEA deprivation index, *n* (%):		
Rural population	3462 (18.8)	579 (18.8)
Urban area #1	3498 (19.0)	513 (16.6)
Urban area #2	2960 (16.0)	446 (14.5)
Urban area #3	2692 (14.6)	499 (16.2)
Urban area #4	2399 (13.0)	409 (13.3)
Urban area #5	2012 (10.9)	390 (12.7)
Missing	1432 (7.76)	246 (7.98)
Charlson comorbidity index, *n* (%):		
0	2315 (12.5)	399 (12.9)
1	704 (3.81)	115 (3.73)
2	9840 (53.3)	1671 (54.2)
3	3553 (19.3)	575 (18.7)
⩾4	2043 (11.1)	322 (10.4)
Smoking status, *n* (%):		
Never smokers	10,269 (55.64)	1579 (51.23)
Current smokers	1343 (7.28)	135 (4.38)
Ex-smokers (quit >1 year)	997 (5.4)	128 (4.15)
Missing, *n* (% of total)	5846 (31.68)	1240 (40.23)
Alcoholism, *n* (%):		
None/Low	2410 (13.06)	268 (8.7)
Moderate	390 (2.11)	44 (1.43)
High/Alcoholic	16 (0.09)	1 (0.03)
Missing	15,639 (84.74)	2769 (89.84)
Antiplatelet drug users, *n* (%)	1720 (9.32)	308 (9.99)
Anticoagulant drug users, *n* (%)	544 (2.95)	70 (2.27)
Statin drug users, *n* (%)	3518 (19.1)	511 (16.6)
Previous TEE history, *n* (%)	496 (2.69)	38 (1.23)
Previous CVE history, *n* (%)	693 (3.76)	122 (3.96)

Participants included in TAM group were older than 55 years old to ensure postmenopausal status.
AI, aromatase inhibitors; BMI, body mass index; CVE, cerebrovascular event; Q, quartile; QMEDEA, quintile MEDEA deprivation index; SDE, standard deviation; TAM, tamoxifen; TEE, thromboembolic event.

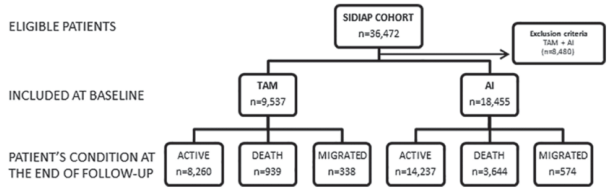

Figure 1. Flow chart of SIDIAP cohort study.
AI, aromatase inhibitor; SIDIAP, System for the Development of Research in Primary Care; TAM, tamoxifen; ⩽55y, patients equal or less than 55 years old.

Results

Of the 36,472 eligible participants, 21,537 (18,455 AI and 3082 TAM) monotherapy users were included in the analysis (see Figure 1), with a median (interquartile range) of treatment of 29 (10–53) months and a maximum of 119 months. Baseline characteristics of participants are presented in Table 1. Postmenopausal women treated with TAM were older, less likely to be current smokers, concomitant users of anticoagulants or statin therapy, and had lower prevalence of previous TEE than AI users; but had a similar BMI, Charlson comorbidity index, current alcohol drinker status, users of platelet inhibitors and prevalence of previous CVE.

Thromboembolic adverse events

A total of 49 patients in the TAM group experienced TEEs (1.59%), whereas these were 345 (1.87%) patients in the AI group. This is equivalent to incidence rates of 8.16/1000 person-years (95%CI 6.10–10.69) in TAM users, and 6.93/1000 person-years (95%CI 6.23–7.69) in AI patients (Figure 2a). No significant differences in thromboembolic risk were observed between both therapies [adjusted HR 0.93 (95%CI 0.69–1.26)] (Table 2). Survival analysis, adjusted for competing risk, showed similar findings (Table 2). Sensitivity analyses excluding patients with previous TEE did not change the estimates (data not shown).

Cardiovascular adverse events

A total of 33 (1.07%) TAM users had at least one CVE event during follow up, compared with 271 events (1.47%) in the AI user group. Incidence rates were therefore 5.50/1000 person-years (95%CI 3.85–7.63) in TAM, and 5.43/1000 person-years (95%CI 4.81–6.10) in AI users. Cumulative hazard plots of CVEs are shown in Figure 2b. No significant increase in cardiovascular risk was detected in AI-treated patients [adjusted HR 1.13 (95%CI 0.79–1.63)] (Table 2). Survival analysis, adjusted for competing risk, show similar findings (Table 2). Sensitivity analyses excluding patients with previous CVE did not change the estimates (data not shown).

Mortality

Overall mortality was 22.58% (696 participants) in the TAM group, and 19.75% (3644 subjects) in the AI group. Crude mortality rates were 40.68/1000 person-years (95%CI 37.74–43.78) in TAM, and 40.25/1000 (95%CI 38.95–41.57) in AI users. Cumulative hazard plots of mortality are shown in Figure 2c. Adjusted Cox models showed a better prognostic for AI users [HR of 0.76 (95%CI 0.70–0.82)] compared with TAM users (Table 2). Similar findings were observed after competing risk adjustment (Table 2).

Secondary outcomes

TEEs: PE and DVT. In our cohort, 100 PE events [7 in TAM group, incidence rate 1.17 (95%CI: 0.51–2.31); and 93 in AI group, incidence rate 1.87 (95%CI: 1.52–2.28)] and 294 DVTs [42 in TAM group, incidence rate 6.99 (95%CI: 5.10–9.36); and 252 in AI group, incidence rate 5.06 (95%CI: 4.47–5.72)] were reported. No differences in DVT risk were found between both groups. A nonsignificant increased risk of PE (Adjusted SHR of 2.15 [95%CI 0.99–4.64]) in AI group was observed (Table 3).

CVEs: CAD and CVD. Of 304 CVEs, 292 were CAD [32 in TAM, incidence rate 5.33 (95%CI: 3.71–7.44); and 260 in AI users, incidence rate 5.21 (95%CI: 4.60–5.87)] and 12 were CVD [1, incidence rate 0.17 (95%CI:0.01–0.82) in TAM; and 11, 0.22 (95%CI: 0.12–0.38) in AI users].

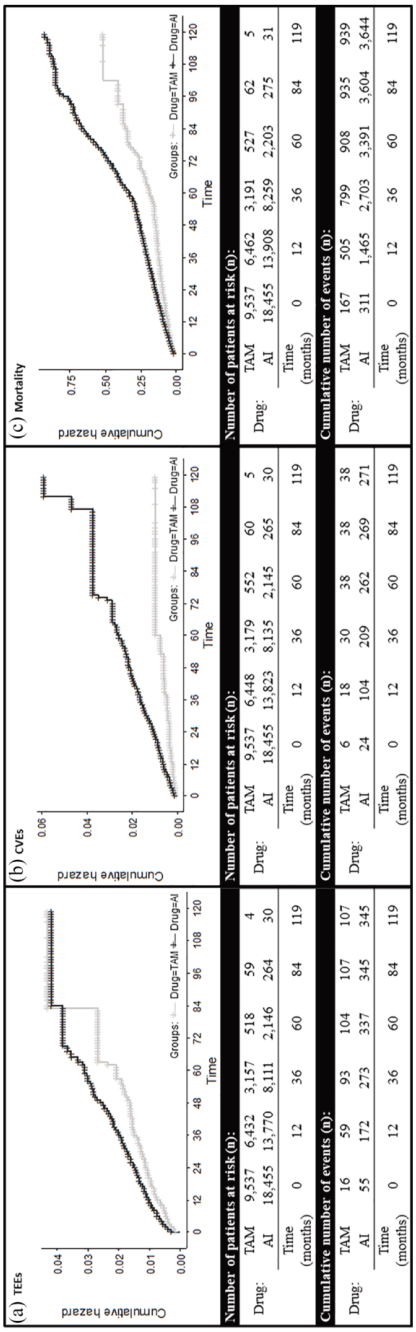

Figure 2. Cumulative hazard plot of (a) TEEs, (b) CVEs and (c) mortality in SIDIAP cohort according to drug therapy. Graphs show Kaplan–Meier curves representing the outcomes of the study in terms of cumulative hazards.
AI, aromatase inhibitor; CVE, cardiovascular event; SIDIAP, System for the Development of Research in Primary Care; TAM, tamoxifen; TEE, thromboembolic event.

No increased risk of CAD nor CVD was detected between both groups (Table 3).

A secondary analysis where patients with previous CVE and TEE were censored showed similar results (data not shown).

Inverse probability weighting analysis. Survival analyses adjusted for IPW are reported in Table 4. CVEs were similar to those obtained in Cox regression models, with no significant difference in risk between AI and TAM users. Likewise, lower mortality risk was detected in AI users. On the contrary, an increased risk of PE was detected in AI users [stabilized IPW HR 2.26 (95%CI 1.02–4.97)].

Survival analysis not accounting for menopausal status (including TAM women younger than 55 years old) showed an increased risk of CVE in AI patients [adjusted SHR 1.96 (95%CI 1.37–2.81)]. Nevertheless, the IPW analysis did not found differences between groups [HR 0.87 (95%CI 0.56–1.38)] (Supplementary Tables S2–5).

Discussion

In our cohort study, including 21,537 women with a breast cancer diagnosis treated in actual practice conditions, similar CVE and TEE risk was observed in both AI and TAM treatment groups. In addition, AI use seems to be a beneficial choice in terms of overall mortality reduction, with a >20% lower rate in AI users after adjusting for potential confounders.

A further analysis adjusting for IPW corroborated these findings but suggested a potential increase of PE associated with AI use.

In general, previous data on cardiovascular risk provided by RCT and meta-analyses, despite being heterogeneous, have identified a potential excess CVE risk associated with AI therapy, with slightly increasing odds of developing CVD, compared with patients receiving TAM therapy (OR 1.26, 95%CI = 1.10–1.43).[16] In this regard, Abdel-Qadir and colleagues published a similar population-based study using routinely collected data from Canada that observed an increased risk of myocardial infarction in AI users (HR 2.02; 95%CI = 1.16–3.53), but, exploring a lower-risk subgroup of patients aged <74 years, with stage I–II breast cancer and no prior ischaemic heart

Table 2. Thromboembolic, cardiovascular and mortality risk of AI treatment compared with TAM treatment in postmenopausal women.

Hazard risk estimates				
Outcome	**Number of events**		**Unadjusted HR (95%CI)**	**Adjusted HR (95%CI)**
TEEs	TAM AI	49 345	0.92 (0.68–1.24)	0.93 (0.69–1.26)
CVEs	TAM AI	33 271	1.02 (0.71–1.47)	1.13 (0.79–1.63)
Mortality	TAM AI	696 3644	0.65 (0.60–0.71)	0.76 (0.70–0.82)
Competing risk estimates				
Outcome	**Number of events**		**Unadjusted HR (95%CI)**	**Adjusted HR (95%CI)**
TEEs	TAM AI	49 345	0.99 (0.74–1.34)	1.05 (0.78–1.42)
CVEs	TAM AI	33 271	1.13 (0.79–1.62)	1.31 (0.91–1.88)

Adjusted results were obtained using continuous PS estimates.
AI, aromatase inhibitors; CI, confidence interval; CVEs, cardiovascular events; HR, hazard ratio; PS, propensity score; SHR, subdistribution hazard ratio; TAM, tamoxifen; TEEs, thromboembolic events.

disease, this excess risk was not detected (HR 1.53; 95%CI = 0.41–5.71, $P = 0.53$).[17]

In order to minimize imbalances when comparing treatment groups, we adjusted for a long list of potential confounders using propensity score equations, which is recommended in this type of study.[18] An additional analysis adjusting for IPW was performed to correct for potential attrition bias, confirming that AI-treated women did not experience an increased risk of CVE compared with postmenopausal women in the TAM group. Consistent with this, other population studies selecting older women also reported similar incidences in stroke and several heart diseases between treatment groups.[19,20] All together, these findings suggest that the increased risk detected in AI users compared with total TAM users is driven mainly by menopausal status.

In contrast with previous RCT findings,[3] an increasing risk of PE was observed after IPW adjustment of our data: patients treated with AIs had twice the risk of PE compared with TAM users. However, this was a *post hoc* analysis based on a limited number of events, and needs further confirmation in external cohorts. Further research is needed to explore this potential association between AI treatment and PE risk.

As a side note, and similar to the results of Abdel-Qadir and colleagues,[17] our supplemental survival analysis adjusted by computing risk of death including all TAM users (lower and older than 55 years old) suggested the AI group has nearly twice the risk of CVEs compared with the total TAM group, but later selection of postmenopausal women has shown similar hazard risks for all events in both treatment groups, proposing that menopause status is not a confounder but a potential interaction.

In addition to the lack of association between AI and CVE observed in our population, the significant OS in AI treated patients places these drugs in front to TAM in terms of cardiovascular safety and efficacy on recurrence incidence.[21] It is noteworthy that selective oestrogen receptor modulators (SERMs) have been associated with higher proportion of adverse drug reaction (ADR) reports related to QT prolongation, Torsade de Pointes, and ventricular arrhythmias compared with AIs, in the European database of suspected ADR reports. Nonetheless, the overall number of these events was very small.[22]

One limitation of the study is that data of previous exposure to chemotherapy or radiotherapy were not available. In any case, these treatments

Table 3. Risk of PE, DVT, CAD and CVD of AI treatment compared with TAM treatment in postmenopausal woman.

Hazard ratio estimates					
Outcome	**Subtype**	**Number of events**		**Unadjusted HR (95%CI)**	**Adjusted HR (95%CI)**
TEEs	PE	TAM AI	7 93	1.77 (0.82–3.82)	1.91 (0.88–4.13)
	DVT	TAM AI	42 252	0.92 (0.68–1.24)	0.81 (0.58–1.13)
CVEs	CAD	TAM AI	32 260	1.02 (0.71–1.47)	1.12 (0.77–1.62)
	CVD	TAM AI	1 11	1.02 (0.71–1.48)	1.49 (0.19–11.66)
Competing risk estimates					
Outcome	**Subtype**	**Number of events**		**Unadjusted SHR (95%CI)**	**Adjusted SHR (95%CI)**
TEEs	PE	TAM AI	7 93	1.91 (0.88–4.12)	2.15 (0.99–4.64)
	DVT	TAM AI	42 252	0.84 (0.61–1.17)	0.89 (0.64–1.24)
CVEs	CAD	TAM AI	32 260	1.12 (0.77–1.61)	1.29 (0.89–1.87)
	CVD	TAM AI	1 11	1.52 (0.20–11.76)	1.75 (0.22–13.71)

Adjusted results were obtained using continuous PS estimates.
AI, aromatase inhibitors; CAD, coronary artery disease; CI, confidence interval; CVD, cerebrovascular diseases, including stroke and intracerebral hemorrhage; CVEs, cardiovascular events; DVT, deep vein thrombosis, phlebitis and thrombophlebitis; HR, hazard ratio; PE, pulmonary embolism; PS, propensity score; SHR, subdistribution hazard ratio; TAM, tamoxifen; TEEs, thromboembolic events.

are given independently of endocrine therapy election.[23] Hence, potential toxic effects in heart would be allocated randomly among patients. In addition, we performed a sensitivity analysis using IPW to minimize the presence of a potential bias by indication due to other factors. Likewise, data of severity, grade and the clinical stage of breast cancer were not accessible. However, TAM and AI monotherapies are recommended and used mainly for hormone receptor-positive early breast cancer.[24] In our study, we excluded patients with sequential therapies (TAM/AI) and further studies analysing outcomes in these patients might provide additional safety data.

Additionally, the SIDIAP data were collected during routine clinical practice (not by an expert researcher), potentially limiting the validity of coded outcomes. However, high accuracy in coding for all of the study outcomes was previously validated in the SIDIAP database.[10]

The main strength of the study is the sample size, almost 22,000 participants, reflecting real population conditions. Likewise, the SIDIAP dataset includes all treatment centres and has the potential to include all patients in the source population, increasing the external validity of our findings.

In summary, no difference in CVD was observed between postmenopausal AI and TAM users. Furthermore, AI users had >20% lower all-cause mortality, yielding a positive risk–benefit for long-term use of these therapies.

Table 4. Risk of TEEs and CVEs in AI treatment compared with TAM treatment in postmenopausal women using stabilized IPW adjustment.

Outcome and subtypes	Number of events		Stabilized IPW HR (95%CI)
TEEs	TAM AI	49 345	0.96 (0.70–1.32)
PE	TAM AI	7 93	2.26 (1.02–4.97)
DVT	TAM AI	42 252	0.79 (0.55–1.11)
CVEs	TAM AI	33 271	1.05 (0. 71–1.54)
CAD	TAM AI	32 260	1.05 (0.71–1.54)
CVD	TAM AI	1 11	1.97 (0.25–15.54)
Mortality	TAM AI	696 3644	0.79 (0.72–0.86)

AI, aromatase inhibitors; CAD, coronary artery disease; CI, confidence interval; CVD, cerebrovascular diseases, including stroke and intracerebral hemorrhage; CVEs, cardiovascular events; DVT, deep vein thrombosis, phlebitis and thrombophlebitis; IPW HR, Inverse probability weighting hazard ratio; PE, pulmonary embolism; TAM, tamoxifen; TEEs, thromboembolic events.

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Supplemental material

Supplemental material for this article is available online.

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Supplemental material

Supplementary table 1. ICD-10 codes used to identify the outcomes of the study

Main outcome	Secondary outcome	ICD-10 code
Thromboembolic events (TEE)	Pulmonary embolism (PE)	I26, I26.0, I26.9
	Deep vein thrombosis (DVT)	G08, I67.6, I80, I80.0, I80.1, I80.2, I80.3, I80.8, I80.9, I81, I82, I82.0, I82.1, I82.2, I82.3, I82.8, I82.9, O22.2, O22.3, O22.5, O87.0, O87.1, O87.3
Cardiovascular events (CVE)	Coronary artery disease (CAD)	I20, I20.0, I20.1, I20.8, I20.9, I21, I21.0, I21.1, I21.2, I21.3, I21.4, I21.9, I22, I22.0, I22.1, I22.8, I22.9, I23, I23.0, I23.1, I23.2, I23.3, I23.4, I23.5, I23.6, I23.8, I24, I24.0, I24.1, I24.8, I24.9, I25, I25.0, I25.1, I25.2, I25.3, I25.4, I25.5, I25.6, I25.8, I25.9, Z95.1
	Cerebrovascular diseases (CVD)	I67.0, I67.1, I67.2, I67.3, I67.4, I67.5, I67.6, I67.7, G46, G46.0, G46.1, G46.2, G46.3, G46.4, G46.5, G46.6, G46.7, G46.8

Supplementary Table 2. Baseline characteristics of candidates using all TAM patients (including ≤55 years all).

Variable	AI N=18,455	Total TAM N=9,537
Median Age (years) [Q1;Q3]	67.0 [59.0;77.0]	49.0 [43.0;61.0]
Mean BMI (kg/m2) ± (SD)	29.7 (5.36)	28.3 (5.53)
Missing, *n* (%)	13,555 (73.45)	7,768 (81.45)
QMEDEA deprivation index, *n* (%):		
Rural population	3,462 (20.3)	1,699 (18.9)
Urban area #1	3,498 (20.5)	1,748 (19.5)
Urban area #2	2,960 (17.4)	1,559 (17.4)
Urban area #3	2,692 (15.8)	1,451 (16.2)
Urban area #4	2,399 (14.1)	1,334 (14.9)
Urban area #5	2,012 (11.8)	1,181 (13.2)
Missing, *n* (% of total)	1,432 (7.76)	565 (5.92)
Charlson co-morbidity index, *n* (%):		
0	2,315 (12.5)	1,062 (11.1)
1	704 (3.81)	171 (1.79)
2	9,840 (53.3)	6,797 (71.3)
3	3,553 (19.3)	1,073 (11.3)
>=4	2,043 (11.1)	434 (4.55)
Smoking status, *n* (%):		
Never smokers	10,269 (81.4)	3,572 (60.7)
Current smokers	1,343 (10.7)	1,527 (25.9)
Ex-smokers (quit >1 year)	997 (7.9)	788 (13.4)
Missing, *n* (% of total)	5,846 (31.68)	3,650 (38.27)
Alcoholism, *n* (%):		
None/Low	2,410 (85.6)	825 (78.3)
Moderate	390 (13.8)	222 (21.1)
High/Alcoholic	16 (0.06)	6 (0.6)
Missing, *n* (% of total)	15,639 (84.74)	8,484 (88.96)
Antiplatelet drug users	1,720 (9.32)	348 (3.65)
Anticoagulant drug users	544 (2.95)	75 (0.79)

Statin drug users	3,518 (19.1)	672 (7.05)
Previous TEE history	496 (2.69)	84 (0.88)
Previous CVE history	693 (3.76)	133 (1.39)

All patients' analysis, non-accounting for postmenopausal status. Abbreviations: AI, aromatase inhibitors; TAM, tamoxifen; BMI, body mass index; Q, quartile; QMEDEA, quintile MEDEA deprivation index; TEE, thromboembolic event; CVE, cerebrovascular event.

Supplementary Table 3. Thromboembolic, cardiovascular and mortality risk of AI treatment compared with TAM treatment (including all TAM users).

a. Hazard risk estimates

Outcome	Number of events		Unadjusted HR (95%CI)	Adjusted HR (95%CI)
TEEs	TAM	107	1.44 (1.16 to 1.79)	0.89 (0.71 to 1.14)
	AI	345		
CVEs	TAM	38	3.08 (2.19 to 4.33)	1.51 (1.06 to 2.15)
	AI	271		
Mortality	TAM	939	1.66 (1.54 to 1.78)	0.80 (0.74 to 0.87)
	AI	3,644		

b. Competing risk estimates

Outcome	Number of events		Unadjusted SHR (95%CI)	Adjusted SHR (95%CI)
TEEs	TAM	107	1.40 (1.13 to 1.75)	0.99 (0.78 to 1.26)
	AI	345		
CVEs	TAM	38	3.00 (2.14 to 4.22)	1.96 (1.37 to 2.81)
	AI	271		

In all patients' analysis, non-accounting for postmenopausal status, AI participants were 18,455 and TAM participants 9,537. Adjusted results were obtained using continuous Propensity Score estimates. Abbreviations: HR, hazard ratio; SHR, subdistribution hazard ratio; CI, confidence interval; TEEs, thromboembolic events; CVEs, cardiovascular events.

Supplementary Table 4. Risk of PE, DVT, CAD and CVD of AI treatment compared with TAM treatment (including all TAM users).

a. Hazard ratio estimates

Outcome	Subtype	Number of events		Unadjusted HR (95%CI)	Adjusted HR (95%CI)
TEEs	PE	TAM	13	3.24 (1.81 to 5.79)	1.80 (0.98 to 3.30)
		AI	93		
	DVT	TAM	94	1.44 (1.16 to 1.79)	0.79 (0.61 to 1.04)
		AI	252		

CVEs	CAD	TAM	37	3.08 (2.19 to 4.34)	1.49 (1.04 to 2.13)
		AI	260		
	CVD	TAM	1	3.08 (2.18 to 4.36)	2.44 (0.30 to 20.07)
		AI	11		

b. Competing risk estimates

Outcome	Subtype	Number of events		Unadjusted SHR (95%CI)	Adjusted SHR (95%CI)
TEEs	PE	TAM	13	3.17 (1.78 to 5.68)	2.13 (1.16 to 3.91)
		AI	93		
	DVT	TAM	94	1.16 (0.92 to 1.48)	0.88 (0.68 to 1.15)
		AI	252		
CVEs	CAD	TAM	37	2.96 (2.10 to 4.18)	1.93 (1.33 to 2.78)
		AI	260		
	CVD	TAM	1	4.63 (0.60 to 35.97)	2.93 (0.35 to 24.24)
		AI	11		

In all patients' analysis, non-accounting for postmenopausal status, AI participants were 18,455 and TAM participants 9,537. Adjusted results were obtained using continuous Propensity Score estimates. Abbreviations: HR, hazard ratio; SHR, subdistribution hazard ratio; CI, confidence interval; TEEs, thromboembolic events; PE, pulmonary embolism; DVT, deep vein thrombosis, phlebitis and thrombophlebitis; CVEs, cardiovascular events; CAD, coronary artery disease; CVD, cerebrovascular diseases, including stroke and intracerebral hemorrhage.

Supplementary Table 5. Risk of thromboembolic and cardiovascular events of AI treatment compared with TAM treatment (including all TAM users) using stabilized Inverse Probability Weighting adjustment.

Outcome and subtypes	Number of events		Stabilized IPW HR (95%CI)
TEEs	TAM	107	0.83 (0.61 to 1.14)
	AI	345	
PE	TAM	13	0.98 (0.44 to 2.20)
	AI	93	
DVT	TAM	94	0.79 (0.57 to 1.10)
	AI	252	
CVEs	TAM	38	0.87 (0. 56 to 1.38)
	AI	271	
CAD	TAM	37	0.85 (0.53 to 1.39)
	AI	260	
CVD	TAM	1	0.91 (0.11 to 7.62)
	AI	11	
Mortality	TAM	939	0.48 (0.44 to 0.53)
	AI	3,644	

In all patients' analysis, non-accounting for postmenopausal status, AI participants were 18,455 and TAM participants 9,537. Abbreviations: IPW HR, Inverse probability weighting hazard ratio; CI, confidence interval; TEEs, thromboembolic events; PE, pulmonary embolism; DVT, deep vein thrombosis, phlebitis and thrombophlebitis; CVEs, cardiovascular events; CAD, coronary

artery disease; CVD, cerebrovascular diseases, including stroke and intracerebral hemorrhage.

DISCUSSION

Woman receiving AI treatment undergo to several side effects that must be considered for their own well-being. Reference data obtained from RCT could not be completely representative and might not capture the real extend of AI side effects. In order to improve patient's quality of life and to reduce treatment discontinuation, this thesis has evaluated the impact of the most common side effects of AI in actual clinical practice through two approaches: using a prospective clinical cohort, B-ABLE; and using a primary care database, SIDIAP. Based on the incidence of ER+ BC and the wide use of AI, this has the potential to help numerous women.

There is a general awareness of a suboptimal adherence and persistence to AI therapy. Lack of adherence and early discontinuation of endocrine therapy have been related to increased mortality in women diagnosed with BC [107]. A systematic review reported discontinuation rates in clinical practice which ranged from 31 to 73% at the end of 5 years of treatment, while adherence range from 41 to 72% [108]. A meta-regression analysis including almost the same studies estimated 31.3% of AI patients ceases the treatment before reaching 5 years [109]. Entrance of generic AI (July 2010 for anastrozole and April 2011 for letrozole and exemestane) improved persistence after 36 months from diagnosis in 8%, compared to patients with high copays [110]. In our case, B-ABLE participants showed a good adherence (more that 90% of patients took >80% of the pills) and persistence (dropout rate of 14.5% among all included patients), being arthralgia as first discontinuation cause.

Arthralgia, or joint pain, is one of key factors that affect both early cessation and life quality. Treatment cessation by arthralgia can be labeled as AI intolerance since patients develop an intolerable toxicity that causes their treatment discontinuation. Having arthralgia during AI treatment is extremely common [70] and is the main cause of AI discontinuation [111]. Additionally, most of the AI discontinuations take place within the first year [64]. Thus, observational studies might underestimate arthralgia scores: participants intolerant to AI drop out the treatment, and hence, the study. Missing these participants also excludes high/extreme pain scores from the analysis, reducing the impact of arthralgia when analyzing longer periods of follow-up. Furthermore, and as it has been observed in this work, the risk is even higher for patients switching from TAM. Henry et al. proposed that identification of patients at high risk of early discontinuation could allow for interventions to improve tolerance before significant toxicities develop [64]. However, despite joint pain increment and AI intolerance are well-known facts, there are no consensus among arthralgia management. Supervision and education before treatment outset has been described as crucial [92], as well as some lifestyle modifications can reduce AI side effects [92,93]. Moreover, switching the AI type should be preferred than switching to TAM since Kadakia et al. reported that two thirds of AI intolerant patients elongated their treatment for at least six months after the first AI [95].

On the other hand, calcium and vitamin D have been identified as essential factors for bone health and maintenance. The most important role of calcium and vitamin D was attributed

to bone turnover: a decline of plasma calcium levels increases bone resorption to restore them. Therefore, adequate calcium intake is required to maintain this balance. To that end, vitamin D mediates calcium absorption by small intestines [112]. Moreover, vitamin D promotes bone resorption for maintaining calcium concentration in plasma. Vitamin D deficiency leads to decreased calcium absorption and increased osteoclasts formation [113]. Prior studies showed that calcium and vitamin D supplementation prevent fractures and bone loss in elderly patients [114,115]. Current guidelines recommend calcium and vitamin D assessment, including supplementation of both if required [100]. As a note, a meta-analysis point out that calcium and vitamin D supplementation was inadequate to prevent BMD loss in patients with antiestrogenic therapy [116], but this meta-analysis did not have a comparison group without supplements, and therefore cannot state a lack of effect in reducing BMD loss. Apart from the potential benefits of calcium and vitamin D supplementation in bone health, previous studies in B-ABLE have associated this supplementation with a relief of joint pain symptoms [117].

Furthermore, Vitamin D is also involved in the correct functioning of the immune, muscular and nervous systems; and it might play a role in controlling normal breast cell growth by blocking the growth of cancer cells [118]. In this thesis, we have observed that women diagnosed with breast cancer have lower vitamin D levels than healthy population, in special those who recently underwent chemotherapy. Deficiency of vitamin D has been associated with cancer, but it is still unknown whether is a cause

or consequence [119,120]. Moreover, previous studies in B-ABLE cohort showed better musculoskeletal outcomes in AI users with levels of vitamin D equal or higher to 40 ng/ml [117,121]. Positive effects of vitamin D supplementation on BMD levels can be easily related to its role on bone calcium homeostasis mentioned above. However, its association with arthralgia decrease is not so clear. Vitamin D role in immune system is to modulate innate and adaptive immunity [122], and suitable levels of vitamin D reduces oxidative stress and inflammation [123]. Indeed, low levels of vitamin D were associated with increased inflammatory biomarker profiles in people ≥50 years old [124]. Hence, one explanation to arthralgia decrease is the potential anti-inflammatory effect of Vitamin D. On the other hand, a recent study showed that peripheral effects of vitamin D reduced the inflammatory status in mice brain [125]. This could be related to the hypothesis of central nervous system alteration by AI use[72], explaining an improvement of joint pain outcomes through vitamin D supplementation.

For all these reasons, treatment adherence and musculoskeletal symptoms might be improved by calcium and vitamin D supplementation, and hence, physicians should take it into account when deal with patients. In this regard, B-ABLE cohort not only enhance patients' supervision, but also supplement them with calcium and vitamin D. These could lead to better outcomes than other studies, for instance, an improved adherence compared to RCTs (90% in B-ABLE compared to in RCTs 72%–78%) [126], or a lesser BMD loss further explained below.

Within this framework, participants from 5y-AI group (patients treated with AI for 5 years) without BP had a BMD reduction of −2.62% at LS, −3.42% at FN and −2.53% at TH, by the end of AI treatment. These values were much lower than values obtained in the ATAC trial (-6.08% at LS, -7.24% at TH), whose patients had no vitamin D nor calcium supplementation [76]. Up to date, the ATAC trial is the unique RCT reporting BMD values at 5 years of treatment, without prior TAM exposure. Conversely, there are more available data on sequential treatments (TAM followed by AI use). When AI were introduced in the market, many RCT included participants previously treated with TAM, or incorporated a new arm by switching part of their participants. In B-ABLE, pTAM-AI patients without BP showed a BMD reduction of −3.96% at LS, −3.33% at FN and −3.01% at TH at the end of treatment. Slightly higher results were observed in the IES trial (−4.17% at LS BMD and -3.11% at TH BMD) [127], while MA-17 trial reported an enhanced bone loss (−5.35% at LS BMD and -3.60% at TH BMD) [74].

Patients previously treated with TAM are a very interesting group. At the outset of AI treatment, prior TAM patients had similar or higher BMD values than women with AI monotherapy. However, they experimented a greater BMD loss during AI therapy, especially during the first months of treatment [128]. Moreover, our results suggest that BMD recovery in pTAM-AI group after AI cessation is slower that 5y-AI group. It has been proposed that TAM withdrawn induces a rebound effect in bone [129] and, in accordance with Cohen et al. suggestion, our findings showed that BP treatment can revert this effect: BMD

was maintained or increased in pTAM-AI patients, even though its increase was lower compared to 5y-AI group. In this line, rebound effect of TAM withdrawn could be as well associated with higher arthralgia and lower health-related quality of life observed within the first year of follow-up of pTAM-AI group, explaining why previous TAM users had an excess risk of abandoning AI treatment within the first year. However, and in contrast to BMD, VAS and ECOS-16 scores of pTAM-AI group returned to baseline values at one-year post-treatment, while values were maintained in 5y-AI group. These effects could be attributed by age differences between pTAM groups and AI monotherapy since differences in baseline values (mean years old: 60.3 in 3yTAM-2yAI, 58.1 in 2yTAM-3yAI, and 62.8 in 5yAI; $p<0.001$) would be enlarged at the end of treatment (62.3 in 3yTAM-2yAI, 61.1 in 2yTAM-3yAI, and 67.8 in 5yAI).

A cohort study like B-ABLE is very valuable for obtaining highly detailed patient medical history, especially for laboratory data (i.e. bone biomarker measurements, circulating vitamin D levels, among others) that may not be collected in other types of studies. However, outcomes at low incidence require larger sample size. In these cases, clinical databases like SIDIAP are more suitable. The additional value of SIDIAP database is the capacity to analyze the risk of mortality, incident fractures, and thromboembolic and cardiovascular events of AI treatment compared to TAM. Moreover, studied outcomes were previously validated in SIDIAP [130-133], enhancing the reliability and quality of the data source. On top of that, SIDIAP is a big database that represents most of Catalan population. Despite part of this

population might have an independent private health care, only a minority group does not have contact to the public primary care, and this rate is event less in issues as severe as cancer [134]. Thus, SIDIAP sample is highly representative among European population treated with AI or TAM, and hence, after correcting indication bias by different statistical approaches, the obtained estimates of treatment effects could be generalized to all women using AI.

Confounding by indication is an important type of confounding that occurs in clinical research, particularly in observational pharmacoepidemiologic studies. It is produced when a clinical indication for selecting certain treatment also affects the outcome, and hence randomization is not possible. In other words, two individuals are different since they are prescribed different medication [135]. An example in our study: AI users are always postmenopausal women, but this is not required in TAM users and, consequently TAM group has younger patients. Confounding can be prevented by different procedures in the study design (e.g. randomization, restriction, and matching) and reduced by different techniques in the statistical analysis (e.g. stratified analyses, regression modeling, and propensity scoring) [135]. Considering that, this thesis has used different approaches to ensure the comparability between TAM and AI patients.

For fracture risk estimation between TAM and AI therapies, sample size allowed a propensity score matching. Since menopause status was not available, participants were restricted to those >55-year-old when TAM and AI were compared. The

results showed a 40% increased risk of fracture in AI patients compared to TAM. Additionally, a stratification of participants according to their baseline fracture risk was performed: Comparing TAM and AI patients with low risk of fracture (participants without evidence of osteoporosis diagnosis nor BP exposure), we obtained similar results than the overall analysis (which included all patients), probably because low-risk patients represented the 78.68% of the participants of the total cohort. On the contrary, no significant differences were observed between AI and TAM patients at high risk of fracture (participants with evidence of osteoporosis diagnosis and/or BP exposure), likely due to the high risk of fracture is enough important per se independently to the administered endocrine therapy. However, we cannot rule out an insufficient statistical power because of a lack of sample size of TAM-highRF group (n=663). BP use in AI-highRF group reduced the fracture risk by 27%. However, BP treatment was not able to reduce the fracture rate to the levels of AI patients group with low fracture risk at baseline (incidence rates: 18.57 cases/1000 person-year in AI-highRF using BP vs 12.32 cases/1000 person-year in AI-lowRF).

For cardiovascular, thromboembolic and mortality risk estimations, TAM patients were restricted to those >55-year-old. Moreover, confounding was reduced by propensity score adjustment and inverse probability weighting adjustment. In this instance, age restriction was especially essential to avoid menopause status interaction since age and menopause have been described as two independent factors of cardiovascular disease [136]. Our findings showed an improved overall mortality,

a non-increased risk in cardiovascular events, and a potential increment of thromboembolic events in AI patients compared to TAM. Prior literature in cardiovascular risk of AI compared to TAM is inconsistent. Most of RCT and meta-analysis comparing AI and TAM treatments reported an excess risk of cardiovascular disease associated with AI use [87,89,137]. However, some studies suggest that it might be due to cardioprotective effects of tamoxifen [138,139], whereas it was found that cardiovascular risk did not increase when AI patients were compared to controls [140]. On the other hand, sub-analyses in a higher-risk subgroup (patients with prior ischemic heart disease) and lower-risk subgroup (aged <74 years, stage I-II BC and no prior ischemic heart disease) did not detect differences in cardiovascular risk between AI or TAM users [90]. Thus, differences detected in this study analyzing the overall cohort could be driven by differences in baseline cardiovascular risk of patients. In this line, cardiovascular risk in Spanish women is low [141], and hence SIDIAP population may also have a low baseline risk. Therefore, exclusion of menopause effect in SIDIAP patients would match baseline cardiovascular risk in our analysis.

In addition to that, death and discontinuation was considered a competing risk (an event that modifies the odds of the event of interest) for our outcomes in fracture, cardiovascular and thromboembolic risk analyses. This bias was managed by applying subdistribution hazards models (SHR) from Fine and Gray methodology [142]. It is required to mention that wrong application of SHR might overestimate the obtained estimates [143]. However, our results were similar (in fracture analysis HR

estimated was 40% risk and SHR estimated was 48% in AI users compared to TAM, whereas HR was 27% and SHR was 31% in AI-highRF patients compared to AI-lowRF; while no significant results were detected in cardiovascular and thromboembolic events using both HR and SHR).

As expected, B-ABLE and SIDIAP databases analysis showed a decrease in BMD and an increase of fracture risk during AI treatment. This bone loss was reverted, and fracture risk attenuated by BP use. Thus, assessment of bone status at the outset of AI treatment should be mandatory instead of recommended in order to distinguish patients at high risk of fracture. Up to date, the gold standard technique to assess bone health is still BMD measurements by DXA [37], which explains 70% of bone strength [102]. Complementary information from other procedures as TBS or assessment of bone remodeling markers could diminish BMD limitations. On the other hand, administration of BP should not imply a complete preservation of patient's bone health that excludes the need for supervision. Consequently, any patient at high risk of fracture will require an increased monitoring of their bone health during AI treatment. Greater efforts for establishing a suitable assessment period are needed. Improving bone health and arthralgia managing of AI patients would have a positive impact on their quality of life and life expectancy derived from the reduction of pain and osteoporotic fractures. Moreover, fracture prevention has the potential to lessen the state's economic burden for incident fragility fractures [144].

All in all, this thesis emphasizes the benefits of calcium and vitamin D supplementation during AI treatment, especially in patients who had recently underwent to chemotherapy. As well as the importance of having a good medical advice during the treatment, standing out an outset bone assessment and an enhanced supervision of patients at high-risk of fracture and/or prior TAM users. These interventions provide the potential to improve patient's adherence, life quality and life expectancy. Furthermore, B-ABLE and SIDIAP were good sources for monitoring existing public policies. The strategy of using both databases allowed us to overcome limitations linked to small or to large cohort studies. As a result, this thesis is an example of translational research, where its findings have the purpose of updating public health practices.

CONCLUSIONS

Conclusions from the evaluation of vitamin D levels of patients starting AI treatment in the B-ABLE cohort:

- Patients with ER+ BC cancer have reduced 25(OH)D levels compared to healthy population.
- Recent chemotherapy is a key factor contributing to 25(OH)D deficiency.
- Diminished 25(OH)D levels are partially recovered over the long term but remained much lower than healthy population.
- Vitamin D supplementation might improve prognosis and survival. Therefore, it is advisable, especially in patients receiving chemotherapy.

Conclusions from the assessment of bone health in ER+ BC patients one year after complete AI treatment in the B-ABLE cohort:

- AI-related bone loss stops at one year after AI completion in non-BP treated women. FN and TH BMD remains reduced, but LS BMD is totally recovered in most patients who received AI monotherapy and partially recovered in patients who were previously treated with TAM.
- BP treatment increases or maintains BMD values at the end of therapy and at one-year post-treatment.
- Monitoring bone health and supplement AI users with calcium and vitamin D is essential for the clinical management of patients.0

Conclusions from the assessment of life quality and treatment discontinuation of AI-treated patients in the B-ABLE cohort:

- AI therapy increases joint pain and reduces HRQoL, mainly during the first year of treatment.
- At 1-year post-treatment, joint pain and HRQoL return to baseline levels in patients previously treated with TAM, while levels on patients treated with AI monotherapy for 5 years remains greater than baseline.
- The proportion of early cessation of AI treatment caused by AI intolerance in the B-ABLE cohort is 3.96%.
- Patients previously treated with TAM experience greater pain when they switched to AI therapy and have an excess risk of discontinuation of 430% during the first 12 months.
- Strictly monitoring AI patients, especially previous TAM users, might reduce the incidence of AI treatment discontinuation.

Conclusions from the analysis of fracture incidence and risk during AI therapy and evaluation of the effectiveness of oral BP in reducing fracture risk, the SIDIAP study:

- During AI treatment, patients at low risk of fracture have and incidence rate of 13.55 cases/1000 person-year, while patients at high risk (diagnosed with osteoporosis and/or treated with BP) have and incidence rate of 21.35 cases/1000 person-year.
- In women older than 55 years old from actual clinical practice, AI treatment increments the risk of fracture by

>40% compared to TAM therapy. This corroborates previous RCT results.

- In patients at high-risk of fracture during AI treatment, BP users have a fracture risk reduction of 30% compared to non-BP users.
- Monitoring fracture risk and related risk factors in aromatase inhibitor patients is advisable.

Conclusions from the analysis of cardiovascular risk, thromboembolic risk, and overall survival benefit of AI compared to TAM treatment, the SIDIAP study:

- There is no increment in cardiovascular risk and thromboembolic risk between AI and TAM users.
- AI users have >20% lower all-cause mortality compared with TAM users.
- AI might be the most preferable option in adjuvant hormonal therapy choice.

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