EFFECTS OF CHEMOTHERAPY-INDUCED OVARIAN FAILURE ON BONE AND LIPID METABOLISM IN PREMENOPAUSAL BREAST CANCER PATIENTS

Impact of adjuvant clodronate and tamoxifen

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Academic Dissertation

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1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred in the text by their Roman numerals (I-IV):

I. Vehmanen L, Saarto T, Elomaa I, Mäkelä P, Välimäki M, Blomqvist C. Long-term impact of chemotherapy-induced ovarian failure on bone mineral density (BMD) in premenopausal breast cancer patients. The effect of adjuvant clodronate treatment. Eur J Cancer 37:2373-8, 2001

II. Vehmanen L, Saarto T, Risteli J, Risteli L, Blomqvist C, Elomaa I. Short-term intermittent intravenous clodronate in the prevention of bone loss related to chemotherapy-induced ovarian failure. Breast Cancer Res Treat 87:181-8, 2004

III. Vehmanen L, Elomaa I, Blomqvist C, Saarto T. Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status: Tamoxifen causes bone loss in patients who continue to menstruate but reduces bone loss in those with amenorrhea. (submitted)

IV. Vehmanen L, Saarto T, Blomqvist C, Taskinen MR, Elomaa I. Tamoxifen treatment reverses the adverse effects of chemotherapy-induced ovarian failure on serum lipids. Br J Cancer 91:476-81, 2004

2. ABBREVIATIONS

A	doxorubicin (Adriamycin®)
AC	doxorubicin –cyclophosphamide
AF1	activation domain 1
AF2	activation domain 2
AFOS	alkaline phosphatase
ATP	adenosine triphosphate
AVCF	doxorubicin -vincristine-cyclophospamide-5-fluorouracil
BMD	bone mineral density
CAF, FAC	cyclophosphamide, doxorubicin, 5-fluorouracil
CEF, FEC	cyclophosphamide, epirubicin, 5-fluorouracil
CHD	coronary heart disease
CI	confidence interval
CMF	cyclophosphamide, methotrexate, 5-fluorouracil
CMFp	cyclophosphamide, methotrexate, 5-fluorouracil, prednisone
CRP	C-reactive protein
CTX	cross-linked carboxytelopeptide of type I collagen
DCIS	ductal carcinoma in situ
DFS	disease-free survival
DNA	deoxyribonucleic acid
DXA	dual X-ray absorptiometry
ER	estrogen receptor
FSH	follicle stimulating hormone
G1	first growth phase of the replicative cycle
HDL	high-density lipoprotein
HER-2-neu	human epidermal growth factor receptor 2
HRT	hormone replacement therapy
ICTP	cross-linked carboxy-terminal telopeptide of type I collagen
i.v.	intravenous
LDL	low-density lipoprotein
LH	luteinizing hormone
Lp(a)	lipoprotein a
MPP	matrix metalloproteinase

NTX	cross-linked aminotelopeptide of type I collagen
OS	overall survival
PINP	aminoterminal propeptide of type I procollagen
PR	progesterone receptor
PST	primary/preoperative systemic therapy
PTH	parathyroid hormone
PTHrP	parathyroid hormone- related peptide
RR	response rate
SERM	selective estrogen receptor modulator
SD	standard deviation
T	docetaxel (Taxotere®)
TAC	docetaxel, doxorubicin, cyclophosphamide
TNM	primary tumor (T), regional lymph nodes (N), metastasis (M)
v.	versus

3. ABSTRACT

Adjuvant cytotoxic chemotherapy causes ovarian failure and amenorrhea in most premenopausal women with early breast cancer. This early menopause has profound effects on bone and lipid metabolism. The impact of clodronate and tamoxifen treatment on bone mineral density (BMD) and serum lipids in premenopausal breast cancer patients treated with adjuvant chemotherapy was studied here.

Two separate populations of premenopausal patients with early breast cancer were studied. The first population comprised 148 patients treated with adjuvant chemotherapy and randomized to oral clodronate for three years or controls. The second population included 159 patients treated with adjuvant chemotherapy. After the chemotherapy, adjuvant five-year tamoxifen was started in hormone receptor-positive patients. In addition, the first 48 patients were randomly allocated to receive intermittent intravenous clodronate treatment or no further therapy.

We examined the long-term effects of adjuvant peroral clodronate treatment as well as the effect of short-term intravenous adjuvant clodronate treatment in the prevention of bone loss related to chemotherapy-induced premature menopause. The impact of adjuvant tamoxifen treatment on BMD after adjuvant chemotherapy was also studied. In addition, we examined whether tamoxifen treatment could reverse the adverse effects of chemotherapy on serum lipid levels.

Adjuvant chemotherapy caused ovarian dysfunction and amenorrhea in the majority of the patients. Marked bone loss occured in women who developed chemotherapy-induced ovarian failure and early menopause, while those who continued to menstruate despite the chemotherapy preserved their baseline BMD levels. Three-year oral clodronate treatment significantly reduced bone loss associated with ovarian failure. Four-month intermittent intravenous clodronate treatment, on the other hand, did not prevent the rapid bone loss associated with chemotherapy-induced ovarian failure.

Tamoxifen treatment after adjuvant chemotherapy had opposite effects on BMD depending on menstrual status. Tamoxifen caused bone loss in patients who continued

to menstruate after adjuvant chemotherapy. Conversely, tamoxifen decreased bone loss in those women who developed chemotherapy-induced amenorrhea.

Changes in total and low-density lipoprotein (LDL) cholesterol during the chemotherapy correlated significantly with menstrual function. Only those patients who developed signs of ovarian failure had marked elevations in serum total and LDL cholesterol, while no significant changes occurred in those who preserved regular menstruation. Adjuvant tamoxifen therapy reversed the adverse effects of chemotherapy on total and LDL cholesterol and lowered their serum levels even below the baseline. The serum high-density lipoprotein (HDL) cholesterol levels, however, remained unchanged after chemotherapy followed by tamoxifen.

4. INTRODUCTION

Breast cancer is the most common malignancy in women of Western countries. In Finland, 3774 new cases were diagnosed in 2002. Although the incidence of breast cancer is increasing, the mortality figures are not. Today around 85 percent of Finnish breast cancer patients survive five years after diagnosis (1).

It is important to detect breast cancer as early as possible because the stage of the disease and the size of the tumor are the strongest prognostic factors for survival (2). Early detection programs through mass screening with mammography have been introduced in many countries. While some controversy exists about the survival benefits of mass mammography screening (3, 4), the effect of adjuvant therapies on breast cancer survival is well documented. Adjuvant therapy is systemic treatment given to kill any cancer cells that may have spread despite local therapy. Chemotherapy (or: cytotoxic therapy) and endocrine therapy (or: hormonal therapy) are used as adjuvant treatments to reduce breast cancer recurrence.

Postoperative radiotherapy has long been known to reduce the local relapse rates (5) and in recent trials utilizing modern radiotherapy techniques survival rates have also been shown to improve significantly (6, 7). Adjuvant polychemotherapy reduces mortality by 27% in women less than 50 years old and by 11% in those aged 50-69 years (8). Similarly, adjuvant endocrine therapy with the antiestrogen tamoxifen for five years reduces the risk of death by 28% (9).

Breast cancer is primarily a disease of older women; only approximately 25% of incident cases are women younger than 50 years (10). The reproductive health effects of breast cancer treatments specifically affect younger women as adjuvant chemotherapy often causes ovarian failure and amenorrhea leading to early menopause (11, 12). Menopause causes changes in serum lipids that are explained by the deficiency of estrogens: serum total and LDL cholesterol and triglyceride levels increase and HDL cholesterol levels decrease (13-17). Similarly, early chemotherapy-induced menopause leads to adverse and possibly atherogenic changes in serum lipids of breast cancer patients (18). Tamoxifen has an estrogen-like effect on serum lipids decreasing the levels of total and LDL cholesterol (19-22). Chemotherapy followed

by tamoxifen is an established adjuvant treatment in hormone receptor positive breast cancer. So far, no studies are available on the effects of adjuvant chemoendocrine therapy on serum lipid levels.

The chemotherapy-induced ovarian failure has adverse effects on bone metabolism as it causes rapid bone loss (11, 23-26). Bisphosphonates prevent bone loss in patients with established osteoporosis (27-32). In women with advanced breast cancer and clinically evident bone metastases, the use of bisphosphonates reduces the risk of skeletal complications (33). Clodronate, a bisphosphonate available both as an oral and intravenous remedy, has been shown to reduce the rapid bone loss related to chemotherapy-induced amenorrhea (26). The optimal duration and route (oral or intravenous) of adjuvant clodronate treatment is not known. Although tamoxifen prevents bone loss and increases BMD in postmenopausal women (34-38), it may induce bone loss in young, premenopausal patients (38). The effects of tamoxifen treatment on BMD after a period of adjuvant chemotherapy have not been studied before.

Today, more and more women survive breast cancer. While maximal long-term efficacy remains the mainstay of all adjuvant treatments, there is growing concern about the long-term safety and tolerability of these treatments as well. Chemotherapy-induced early menopause leads to adverse effects in serum lipid profile and causes accelerated bone loss. Consequently, many breast cancer survivors may have an increased risk of developing cardiovascular disease and osteoporosis. This thesis focuses on the long-term sequelae of adjuvant treatments on lipid and bone metabolism in premenopausal patients with early breast cancer.

5. ADJUVANT TREATMENT OF BREAST CANCER

In women with early breast cancer, the disease is, by definition, restricted to the breast and, in node-positive cases to the local lymph nodes. Although all detectable cancer tissue is removed surgically, undetected micrometastatic deposits of the disease may remain and subsequently develop into clinically evident metastatic disease. Adjuvant cytotoxic and endocrine therapies significantly reduce the risk of recurrence and improve survival in women with early-stage, primary breast cancer (39, 8, 9).

Today, adjuvant therapy is recommended for most breast cancer patients with the possible exception of women with a minimal risk for cancer recurrence (40). The most relevant factor for the estimation of risk of recurrence is the nodal status (41). The College of American Pathologists includes the TNM (primary tumor (T), regional lymph nodes (N), metastasis (M)) staging information, hormone receptor status of the tumor, histologic grade, histologic type and mitotic figure counts as factors proven to be of prognostic importance and useful in clinical patient management (42). Other, to date less extensively studied prognostic factors, include HER-2-neu (human epidermal growth factor receptor), proliferation markers and lymphatic and vascular channel invasion (42). Young age (< 35 years) is considered as an independent adverse prognostic factor (43).

Adjuvant treatments are recommended in the presence of unfavorable prognostic factors. The selection of a suitable adjuvant therapy is based primarily on the assessment of endocrine-responsiveness according to the presence of estrogen (ER) and progesterone receptors (PR) in the primary tumor (40). Patients with endocrine-nonresponsive disease (absence of detectable steroid hormone receptors) are treated with adjuvant chemotherapy alone as such patients do not benefit from endocrine therapy. Patients with endocrine-responsive tumors (presence of detectable steroid hormone receptors), on the other hand, are offered either endocrine therapy alone or a combination of chemotherapy and endocrine therapy.

5.1. Adjuvant chemotherapy of breast cancer

5.1.1 General aspects

The large meta-analysis conducted by the Early Breast Cancer Trialists`s Collaborative Group (EBCTCG) involved about 18 000 women in 47 trials of polychemotherapy versus no chemotherapy. According to this meta-analysis, adjuvant polychemotherapy produced substantial and highly significant reductions in the risk of both breast cancer recurrence and death. The risk reductions were age-specific. In women aged less than 50 years at randomization the proportional reduction in the risk of recurrence was 35% and in those aged 50-69 years 20 %. Only a few women aged 70 or over were studied. For mortality, the reductions were also significant both in women aged less than 50 years (27%) and in those aged 50-69 (11%). The proportional reductions in risk of death were similar for women with node-negative and node-positive disease. Applying the proportional mortality reduction observed in women aged under 50 years at randomisation would typically change a 10-year survival of 71% for those with node-negative disease to 78% (an absolute benefit of 7%), and of 42% for those with node-positive disease to 53% (an absolute benefit of 11%). For women aged 50-69 years, adjuvant polychemotherapy would translate into smaller absolute benefits, changing a 10-year survival of 67% for those with node-negative disease to 69% (an absolute benefit of 2%) and of 46% for those with node-positive disease to 49% (an absolute gain of 3%) (8).

The adjuvant chemotherapy should be started no later than a few months after the breast cancer surgery. If both systemic chemotherapy and tamoxifen are given, the sequential administration of tamoxifen after chemotherapy is superior to concurrent use of these modalities. A recent trial compared CAF (cyclophosphamide, doxorubicin and 5-fluorouracil) for six cycles followed by tamoxifen for five years to CAF administered concurrently with tamoxifen. The disease-free survival (DFS) was significantly better after sequential treatment as compared to concurrent treatment. These results are consistent with the hypothesis that tamoxifen may antagonize some chemotherapeutic agents and support the practice standard of starting adjuvant tamoxifen when chemotherapy is completed (44).

The optimal duration of adjuvant chemotherapy has been studied in a few trials. Three cycles of FEC (5-fluorouracil, epirubicin, cyclophosphamide) has been shown to be inferior to six cycles (45). Four courses of AC (doxorubicin, cyclophosphamide) have been shown to be equivalent to six cycles of classical CMF (46). Continuing adjuvant CMF beyond the standard treatment of six cycles did not improve survival (47). Four to six cycles of adjuvant chemotherapy is considered adequate according to international consensus guidelines (40, 48).

The question of dose intensity remains controversial in the adjuvant chemotherapy of breast cancer. Lower than standard doses have been shown to result in an inferior outcome (45, 49). On the other hand, intensifying and increasing the total dose of cyclophosphamide in AC adjuvant treatment did not demonstrate any survival benefit (50). Adjuvant high-dose therapy with autologous bone marrow support does not seem to offer survival benefit as compared to conventional treatment (51-52). However, increasing dose density (administration of drugs with a shortened intertreatment interval) may improve survival (53).

Some primary breast cancers are diagnosed as locally advanced (e.g. inflammatory cancer or involving the skin, chest wall or clavicular nodes). Primary or preoperative systemic therapy (PST) refers to the first postdiagnosis systemic treatment and is considered the standard of care for initially nonoperable, locally advanced breast cancer. For operable but large breast cancer, PST provides an additional opportunity for breast-conserving surgery. PST offers the same survival benefits, as does conventional postoperative adjuvant therapy (54).

5.1.2 Different adjuvant chemotherapy regimens

As adjuvant therapy, combination chemotherapy regimens (polychemotherapy) are superior to single-agent chemotherapies (39). The classical CMF (cyclophosphamide, methotrexate, 5-fluorouracil) treatment was introduced by Bonadonna in the mid 1970`s and became a standard adjuvant chemotherapy for breast cancer (55). The Bonadonna CMF treatment consisted of cyclophosphamide 100 mg/m² orally from day 1 to 14, methotrexate 40 mg/m² intravenously on days 1 and 8 and 5-fluorouracil 600 mg/m² intravenously on days 1 and 8. The long-term results of adjuvant CMF

therapy confirmed the preliminary observations of the effectiveness of the treatment in women with node-positive breast cancer. After nearly 30 years of follow-up, the patients given adjuvant CMF chemotherapy still had significantly better rates of relapse-free survival and overall survival (OS) than the controls (56).

Anthracyclines like doxorubicin and epirubicin are among the most active drugs in metastatic breast cancer with response rates (RRs) of approximately 30% to 50% (57-58). Anthracycline-combinations yield significantly more objective responses in patients with metastatic breast cancer than the classical CMF (59). A few randomized studies have demonstrated the superiority of an anthracycline-containing combination over CMF, also in the adjuvant setting. Levine et al compared an intensive CEF (cyclophosphamide, epirubicin, 5-fluorouracil) adjuvant chemotherapy regimen to classical CMF in 710 node-positive breast cancer patients. The five-year DFS rates for the CEF and CMF groups were 63% and 53%. The five-year OS rates were 77% and 70%, respectively (60). In the study by Misset et al, 249 node-positive breast cancer patients were randomized to receive 12 monthly cycles of either AVCF (doxorubicin, vincristine, cyclophosphamide and fluorouracil) or CMF. With a median follow-up time of 16 years, the survival rates were significantly higher in the AVCF versus (v.) CMF arm: for DFS 53% v. 36% and for OS 56% v. 41%, respectively (61). Similarly, early results (median follow-up 32 months) of the NEAT and SCTBG BR9601 trials (n=2391) showed that adding epirubicin to either classical or intravenous CMF resulted in significant benefits for both relapse-free and overall survival as compared to CMF alone (62). Finally, the large meta-analysis by EBCTCG confirmed that anthracycline-containing combinations are more effective than the CMF regimens and provide an extra absolute benefit of 3% at five years in terms of OS (8).

The taxanes paclitaxel and docetaxel have significant antitumor activity in patients with metastatic breast cancer (57, 58, 63-65). Randomized trials of single-agent paclitaxel have reported RRs of 21% to 54% (58, 64). RRs of 30% to 48% have been reported in randomized trials of single-agent docetaxel treatment in metastatic breast cancer (57, 63, 65). The efficacy of the taxanes has been proven also in patients with disease progression after an anthracycline-based chemotherapy regimen (63). The potential benefits of adding taxanes to anthracycline-containing regimens in the adjuvant setting have been studied in a few major trials. The results of the largest

paclitaxel (CALGB 9433 and NSABP B-28) and docetaxel (BCIRG 001 and PACS 01) adjuvant trials are discussed below (Table 1). All these four trials included pre- and postmenopausal patients with node-positive early breast cancer and the results are reported after an approximate follow-up of five years.

Two trials have examined four courses of paclitaxel after four cycles of AC in the adjuvant setting, but these results are difficult to interpret because of the confounding by duration, receptors, and the concurrent administration of tamoxifen. In the CALGB 9344 study, patients were randomly assigned to receive a combination of cyclophosphamide (C) 600 mg/m², with one of three doses of doxorubicin (A), 60, 75, or 90 mg/m², for four cycles followed by either no further therapy or four cycles of paclitaxel at 175 mg/m². Tamoxifen was given to most patients with hormone receptor–positive tumors. In this study, there was no evidence of a doxorubicin dose effect. The addition of four cycles of paclitaxel after the completion of a standard course of AC significantly improved both DFS and OS of the patients (66). In the NSABP B-28 trial, patients were randomized to be treated either with adjuvant AC (doxorubicin 60 mg/m², cyclophosphamide 600 mg/m²) for four cycles followed by four cycles of paclitaxel 225 mg/m² or with AC for four cycles alone. Tamoxifen was started with chemotherapy for five years for patients of 50 years of age or older and to those younger with hormone receptor-positive tumors. The addition of paclitaxel significantly improved DFS as compared to AC alone, but OS did not differ between the treatment groups (67).

Two major trials have examined the benefits of adding docetaxel to anthracycline-containing regimens. A BCIRG 001 trial evaluated six cycles of docetaxel, doxorubicin and cyclophosphamide (TAC) versus six cycles of 5-fluorouracil, doxorubicin and cyclophosphamide (FAC). Patients received tamoxifen if appropriate. Both DFS and OS were significantly better in the TAC group as compared to FAC (68, 69). Quite recently, results of the PACS 01 trial comparing a FEC 100 regimen (5-fluorouracil 500 mg/m², epirubicin 100 mg/m² and cyclophosphamide 500 mg/m²) for six cycles to three cycles of FEC 100 followed by three cycles of 100 mg/m² of docetaxel were reported. Combining docetaxel with FEC significantly improved both DFS and OS. However, the docetaxel-FEC combination did not seem to offer any extra advantage for patients younger than 50 years (70).

In summary, adding taxanes to anthracycline-containing regimens seems to improve survival in patients with node-positive early breast cancer. The improvements observed by the sequential addition of paclitaxel to AC in the CALGB 9344 and NSABP B-28 trials could be related to a true ability of paclitaxel to kill anthracycline-resistant cancer cells or simply to the longer duration (eight v. four cycles) of the treatment. Adding docetaxel to AC (TAC regimen) and combining CEF and docetaxel sequentially has resulted in survival benefits (69, 70). More than 20 000 additional women have been included in randomized clinical trials investigating the role of taxanes that have yet to report results and the results of earlier trials are maturing. The findings of the major adjuvant trials comparing anthracycline-based regimens and taxanes are summarized in Table 1.

The HER-2-neu gene, which encodes the human epidermal growth factor protein HER-2-neu, is amplified or HER-2-neu protein overexpressed in 25 to 30 % of breast cancers, increasing the aggressiveness of the tumor (71). Trastuzumab (Herceptin®) is a recombinant humanised monoclonal antibody that specifically targets the HER-2-neu protein. Trastuzumab when used in combination with chemotherapy (cyclophosphamide plus anthracyline or paclitaxel) is more effective than chemotherapy alone for the treatment of metastatic breast cancer overexpressing HER-2-neu. However, it seems to be associated with congestive heart failure particularly in combination with anthracycline based chemotherapy (72). Many trials that study the potential role of trastuzumab in the adjuvant therapy of breast cancer are underway and the first results are awaited during the next few years.

Table 1. Major adjuvant trials comparing anthracycline-regimens and taxanes

Trial	n	Treatment	Patients	Follow-up	DFS	OS
CALGB 9344	3121	ACx4 v.	Pre/post	5 years	65%	77%
		ACx4 + Px4	N+		70%*	80%*
NSABP B-28	3060	ACx4 v.	Pre/post	5 years	72%	85%
		ACx4 + Px4	N+		76%*	85%
BCIRG 001	1491	FACx6 v.	Pre/post	4.5 years	68%	81%
		TACx6	N+		75%*	87%*
PACS 01	1999	FECx6 v.	Pre/post	5 years	73%	87%
		FECx3 + Tx3	N+		78%*	91%*

A=doxorubicin, C=cyclophosphamide, DFS=disease-free survival, E=epirubicin, F=5-fluorouracil, n=number of patients, N+=node-positive, OS=overall survival, P=paclitaxel, pre=premenopausal, post=postmenopausal, T=docetaxel, *=significant difference

5.2. Adjuvant endocrine therapy of breast cancer

5.2.1 General aspects

Many breast cancers depend on estrogens for their continued growth. The binding of estradiol to ER in the nucleus activates interaction of the receptor-hormone complex with specific regions of deoxyribonucleic acid (DNA), which ultimately triggers cellular growth. Depriving the tumor of this stimulus is an established method of treating the disease (73). Regression of advanced breast cancer because of endocrine therapy was first described over 100 years ago (74).

More than 70% of all breast carcinomas are ER positive while PR positivity is detected in about 60% of cases (75). ER and PR content in the primary tumor are powerful markers predicting endocrine responsiveness (9). The exact threshold of ER and PR (with immunohistochemical staining), which should be used to distinguish between endocrine-responsive and endocrine-nonresponsive tumor, is unknown. Even a low number of cells stained positive (as low as 1% of tumor cells) identify a cohort of tumors having some responsiveness to endocrine therapies (76). According to an adjuvant consensus meeting, 10% positive staining of cells for either receptor might be considered as a reasonable threshold for definite endocrine responsiveness (48).

The rationale for the use of adjuvant endocrine therapy is its steady efficacy for patients with receptor-positive breast cancer in a multitude of randomized trials, as summarized by the Early Breast Cancer Trialists' Collaborative Group (9). The antiestrogen tamoxifen is still the gold standard for adjuvant hormonal treatment of breast cancer for both pre- and postmenopausal women. Aromatase inhibitors were initially used in the treatment of advanced breast cancer in postmenopausal women for whom tamoxifen failed. Since then, aromatase inhibitors have shown superior efficacy over tamoxifen as first-line treatment for postmenopausal women with advanced breast cancer (77-79). Nowadays the efficacy and tolerability of aromatase inhibitors has also been demonstrated in an adjuvant setting (80-86). Ovarian ablation significantly improves long-term survival for young premenopausal women, at least in the absence of chemotherapy (87).

5.2.2 Different endocrine therapy regimens

5.2.2.1 Selective estrogen receptor modulators

Tamoxifen

Over the past few decades, the desire to develop compounds to counteract the actions of estrogen for controlling breast cancer led to the identification of antiestrogens. In addition to the well-demonstrated antagonist effects of these compounds in estrogen-stimulated breast tissue, it was observed that antiestrogens displayed substantial agonist activities in other organs. The phenomenon of variable pharmacological attributes of agonist/antagonist, depending on the target tissue, suggested the name "selective estrogen receptor modulator" or SERM (88).

Tamoxifen is the prototype of a group of nonsteroidal SERMs possessing a triphenylbutene core and basic side chain (89). It is an antiestrogen, which blocks ER at tumor level by competitively inhibiting estradiol binding. This causes the cell to be held at the G1 phase (the first growth phase of the replicative cycle) (90, 91). Though the primary action of tamoxifen is competitive antagonism of estrogen at the cellular receptor, it also exhibits estrogen agonist properties. The main effect of tamoxifen on breast tissue is antiestrogenic, whereas many of its other effects, such as those on the uterus, lipid metabolism, the vasculature, blood clotting mechanisms, and bone resorption, are considered to be estrogen-agonistic (34, 89, 92, 93).

The estrogen agonist/antagonist properties of SERMs can be partly explained through the two activation domains AF1 and AF2 of ER that mediate the transcriptional control of the receptor. Tamoxifen blocks the effect of estrogen by inhibiting AF2 but it does not inhibit AF1. Therefore, tamoxifen has primarily antagonist activity in breast tissue where AF2 is dominant but more agonistic activity in other tissues such as uterus where AF1 is dominant. Estrogen agonist or antagonist effects are thus dependent on the organ-specific type and amount of ER available for ligand binding as well as the type of target gene promoter (90). Also the menopausal status modulates the effect of SERMs. Tamoxifen seems more estrogen antagonist than agonist in premenopausal women while the estrogen agonist properties prevail in postmenopausal women (90).

In metastatic breast cancer, tamoxifen induces RRs up to 35% in patients with ER- or PR-positive disease when used as a first line treatment. Another 20% of these patients have stable disease lasting for approximately six months (94). A large meta-analysis by EBCTCG studied the effects of adjuvant tamoxifen treatment in 18 000 women with estrogen-receptor (ER) positive primary tumors and nearly 12 000 more with untested tumors (of which an estimated 8 000 would have been ER-positive). Adjuvant tamoxifen for one year, two years and five years produced proportional recurrence reductions of 21%, 29% and 47 %, respectively, during 10 years of follow-up. The corresponding proportional mortality reductions were 12%, 17% and 26%, respectively (9). However, more than five years of adjuvant tamoxifen did not lead to any additional benefit in a Scottish study (95) and in the NSABBP B-14 study 10 years of tamoxifen was in fact associated with more recurrences than five years of tamoxifen (96). Other trials are still ongoing to address the question of tamoxifen treatment beyond five years.

According to the EBCTCG meta-analysis, the proportional mortality reductions were similar for women with node-positive and node-negative disease, but the absolute mortality reductions were greater in node-positive women. With five years of adjuvant tamoxifen treatment, the absolute improvements in 10-year survival were 11% for node-positive and 6% for node-negative breast cancer patients. The proportional reductions in breast cancer recurrence and mortality appeared to be largely unaffected by other patient characteristics such as age or other treatment modalities given (9).

In addition to its efficacy in metastatic and early breast cancer, tamoxifen has also been shown to prevent invasive breast cancer (97). Five years of adjuvant tamoxifen produced a proportional reduction in contralateral breast cancer of 47% in breast cancer patients (9). In a NSABBP B-24 trial 1 804 women with ductal carcinoma in situ (DCIS) were randomly assigned to either tamoxifen or placebo for five years after lumpectomy and radiation therapy. Women in the tamoxifen group had significantly fewer breast-cancer events at five years than did those on placebo (8% v. 13%) (98).

Other SERMs

Other SERMs in clinical use today include toremifene, raloxifene and fulvestrant. Raloxifene and fulvestrant are not used in the adjuvant setting in early breast cancer.

Preclinical studies suggest that toremifene has antiestrogenic and estrogenic effects similar to those of tamoxifen. However, toremifene may have a lower estrogenic-to-antiestrogenic ratio than tamoxifen (99). Toremifene has shown comparable efficacy to tamoxifen both in the treatment of metastatic breast cancer (100, 101) and as adjuvant therapy (102, 103) in postmenopausal women. In the adjuvant trial conducted by the Finnish Breast Cancer Group (FBCG) toremifene 40 mg/day was compared with tamoxifen 20 mg/day, both given for three years to postmenopausal women with early breast cancer. The DFS or OS did not differ between the treatment groups, but a trend towards an improved survival for patients on toremifene was seen in a subgroup of ER-positive patients (102). Another adjuvant trial (IBCSG 12-93 and 14-93) compared toremifene 60 mg/day with tamoxifen 20 mg/day in peri- and postmenopausal patients; most patients had also received adjuvant chemotherapy. The DFS and OS were similar in the two treatment groups, also in the ER-positive cohort (103) (Table 2).

Raloxifene is a selective estrogen receptor modulator that binds to ER to competitively block estrogen-induced DNA transcription in the breast and endometrium (104) and exhibits beneficial estrogenic effects on the bone and lipid metabolism (105). In animal studies, raloxifene inhibits estrogen-stimulated growth of mammary cancers (106). Raloxifene is used mainly in the prevention or treatment of postmenopausal osteoporosis (107) but it also reduces the incidence of ER-positive breast cancer (108).

Fulvestrant is the first of a new type of ER antagonist that downregulates the ER and is devoid of the partial agonist properties of tamoxifen when tested in laboratory models (108). Randomized trials have showed fulvestrant (250 mg, once monthly via intramuscular injection) to be at least as effective as the aromatase inhibitor anastrozole for the treatment of postmenopausal women with hormone receptor–positive advanced breast cancer progressing on prior endocrine therapy (110, 111). In

a trial comparing fulvestrant versus tamoxifen as first line treatment in advanced breast cancer, fulvestrant demonstrated similar efficacy as tamoxifen in those patients with hormone-receptor positive tumors (112).

5.2.2.2 Aromatase inhibitors

The aromatase enzyme is critically responsible for the conversion of androgen precursors into estrogen. The rationale for using aromatase inhibitors is to decrease the levels of both circulating estrogen and intratumoral estrogen by inhibiting the conversion of androstenedione into estrogen. In premenopausal women, the primary site of aromatase activity and hence of estrogen synthesis, is the ovary. Aromatase inhibitors are ineffective in suppressing such premenopausal ovarian aromatase activity (113) and may even provoke an ovarian hyperstimulation syndrome (114). In postmenopausal women, on the other hand, they are effective in suppressing extraovarian sites of aromatase enzyme activity (i.e. adipose tissue, muscle, liver, breast tumor). This peripheral aromatization in postmenopausal women is almost completely inhibited by administration of the third-generation inhibitors (115, 116). There are two classes of third-generation oral aromatase inhibitors: reversible nonsteroidal inhibitors, such as anastrozole and letrozole (117) and irreversible steroidal inactivators, exemplified by exemestane (118).

The third generation aromatase inhibitors have shown superior efficacy over tamoxifen as first-line treatment of postmenopausal women with advanced hormone-sensitive breast cancer (77-79). Exemestane can also benefit some patients after failure of nonsteroidal aromatase inhibitors such as anastrotzole or letrozole (119). There are insufficient data to recommend one aromatase inhibitor over another. A randomized unblinded trial compared the efficacy of anastrozole and letrozole in metastatic breast cancer and showed no significant difference between the two agents in time to progression (120).

Few phase III randomized, adjuvant trials have assessed the third-generation aromatase inhibitors in comparison with tamoxifen or to a placebo following some years of tamoxifen therapy in postmenopausal patients with early breast cancer (Table 2). The ATAC trial evaluated anastrozole alone or in combination with tamoxifen

compared with tamoxifen alone, as a five-year adjuvant treatment (80, 81, 121). The BIG 1-98 trial randomized the patients to receive either tamoxifen for five years, letrozole for five years, tamoxifen for two years followed by letrozole for three years or letrozole for two years followed by tamoxifen for three years (86). In both of these trials, DFS was superior in the aromatase inhibitor arm as compared to tamoxifen, but so far, no significant difference in OS has emerged between the treatment groups (121, 86). Also switching patients on adjuvant tamoxifen to aromatase inhibitor (anastrozole or exemestane) seems to decrease the risk of relapse (84, 85, 122-124). Furthermore, aromatase inhibitor therapy after the standard five-year tamoxifen treatment seems beneficial. Letrozole, when started after the completion of five-year tamoxifen therapy, improved DFS when compared with placebo and even an OS advantage was seen in the subset of node-positive women (83, 84).

Until recently, tamoxifen has been considered as a first choice for adjuvant treatment of postmenopausal hormone-receptor positive breast cancer and aromatase inhibitors have been recommended only for patients in whom tamoxifen is contraindicated or not tolerated (48). The updated ASCO Technology assessment on the use of aromatase inhibitors, however, states that optimal adjuvant hormonal therapy for a postmenopausal woman with receptor-positive breast cancer includes an aromatase inhibitor either as initial therapy or after treatment with tamoxifen (125). At present, it is not known whether tamoxifen pre-primes the cells to make them sensitive to aromatase inhibitors and thus, it is not known whether tamoxifen followed by an aromatase inhibitor is better than aromatase inhibitor as initial treatment. The findings of the major adjuvant trials comparing tamoxifen and other endocrine therapies in postmenopausal patients are summarized in Table 2.

In addition, preliminary data suggests that there might be special subgroups of patients who derive most benefit from aromatase inhibitors. A subgroup analysis of the ATAC trial showed that the women with ER-positive but PR-negative breast cancer might derive greater benefit from initial therapy with an aromatase inhibitor (126). Two neoadjuvant studies have reported better RRs for aromatase inhibitors as compared with tamoxifen in patients with breast cancer overexpressing HER-2-neu (127, 128).

In conclusion, adjuvant therapy with aromatase inhibitors seems to improve DFS as compared to tamoxifen in all major adjuvant trials but an OS advantage has so far emerged only in one trial of continued letrozole after five-year tamoxifen (node-positive subset). If the benefits of aromatase inhibitors can be confirmed in the long term, they will challenge the place of tamoxifen as the gold standard for adjuvant therapy in hormone-sensitive postmenopausal breast cancer. For the premenopausal patients, tamoxifen remains the recommended endocrine therapy.

Table 2. Major adjuvant trials comparing tamoxifen and other endocrine therapy regimens in postmenopausal breast cancer

Trial	n	Treatment	Patients	Follow-up	DFS
FBCG	1480	TAM 3 years v.	N+ 100%	3.4 years	66%
	(899)	TOR 3 years	ER+ 62%		70%
IBCSG 12/14-93	1035	TAM 5 years v.	N+ 100%	5.5 years	69%
		TOR 5 years	ER+ 75%		72%
ATAC	9366	TAM 5 years v.	N+ 40%	4 years	85%
		ANA 5 years	ER/PR+ 84%	(5 years)	87%*
BIG 1-98	8023	TAM 5 years v.	N+ 41%	3 years	81%
		LET 5 years v.	ER/PR+ 100%		84%*
IES	4742	TAM 5 years v.	N+ 44%	2.5 years	87%
		TAM+EXE 5 years	ER+ 81%		92%*
ABCSG 8+	3224	TAM 5 years v.	N+ 26%	2.3 years	93%
ARNO 95		TAM+ANA 5 years	ER/PR+ 81%		96%*

ANA=anastrozole, ER+=ER-positive, EXE=exemestane, DFS=disease-free survival, LET=letrozole, n=number of patients, N+=node-positive, PR+=PR-positive, TAM=tamoxifen, TOR=toremifene, *=significant difference

5.2.2.3 Ovarian ablation

Ablation of the ovaries has been used in the treatment of breast cancer for more than 100 years and its value as an adjuvant treatment for premenopausal women has been clearly demonstrated by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis (87). Ovarian ablation consists of removal of the main source of estrogen synthesis in premenopausal women. Traditionally ovarian ablation has been achieved by surgery or irradiation. The failure rate of pelvic irradiation in achieving amenorrhea may be rather high (129). If successful, it produces irreversible ovarian suppression like surgical oophorectomy with the possibility of serious long-

term effects. More recently, luteinizing hormone releasing hormone (LHRH) agonists, that suppress estradiol concentrations to postmenopausal levels, have become more popular at least in clinical trials. The benefit of the LHRH agonists is that the ovarian suppression achieved is potentially reversible (130).

Young age is considered as an independent adverse prognostic factor in early breast cancer (43). Young premenopausal breast cancer patients with ER-positive tumors treated with adjuvant CMF chemotherapy have a particularly unfavorable prognosis if they do not achieve amenorrhea (131). It has been postulated that proportion of the benefit of adjuvant breast cancer chemotherapy in premenopausal receptor-positive women is mediated through a castration effect, as chemotherapy-induced ovarian failure is associated with better DFS (132, 133).

According to the EBCTCG meta-analysis (n=3456), ablation of functioning ovaries in women aged less than 50 years with early breast cancer significantly improves long-term survival, at least in the absence of chemotherapy (87). In women aged less than 50 years when randomized, most of whom would have been premenopausal at diagnosis, the 15-year OS was highly significantly improved in those undergoing ovarian ablation (52% v. 46%), as was also recurrence-free survival (45% v. 39%). The proportional reduction in the risk of recurrence was 19%. The benefit was significant both for those with node-positive and for those with node-negative disease. In women aged 50 years or over when randomized, most of whom would have been perimenopausal or postmenopausal, no significant improvement in survival was noted (87, 134).

The benefits of ovarian ablation might well be lessened by the presence of polychemotherapy, as such chemotherapy induces ovarian dysfunction in many premenopausal women (87, 135). In the EBCTCG meta-analysis, the risk reduction produced by ovarian ablation appeared to be nonsignificant in the presence of chemotherapy (i.e., in trials of ablation plus chemotherapy versus the same chemotherapy alone) and, in these trials, the benefits appeared to be greater in women with ER-positive primary tumours. These comparisons were, however, based on numbers too small to be statistically significant (87, 134).

Adjuvant treatment with ovarian ablation seems to have comparable efficacy to CMF chemotherapy. In the few randomized trials comparing ovarian ablation and CMF chemotherapy, no difference in DFS has been noted between these treatment modalities (136, 137). However, patients with ER-negative tumors seem to fare better with CMF (137, 138) and those with ER-positive equally (138) or better (137) with ovarian ablation. Also the efficacy of combined endocrine treatment with ovarian ablation and tamoxifen has been compared to CMF chemotherapy in hormone-receptor positive early breast cancer. In one study, DFS was significantly better in the ovarian ablation plus tamoxifen group as compared to CMF alone (139), while in another study, DFS did not differ between the combined endocrine treatment and chemotherapy groups (140).

The question of whether adding ovarian ablation to adjuvant chemotherapy improves outcome, has been assessed in a few trials. Adding ovarian ablation to adjuvant CMFp (cyclophosphamide, methotrexate, 5-fluorouracil, prednisone) chemotherapy did not improve DFS as compared to this chemotherapy alone, even for patients with ER-positive tumors (141). Similarly, CMF chemotherapy with goserelin yielded similar DFS as compared to either modality alone in patients with ER-positive early breast cancer (142). Adding ovarian ablation (goserelin) to an anthracycline-containing chemotherapy had no significant effect on survival when compared to chemotherapy alone. However, a trend towards DFS benefit with goserelin was seen in a subgroup of women under 40 years of age and who were not amenorrheic after chemotherapy (143).

Also, the potential benefits of adding combined endocrine treatment with both ovarian ablation and tamoxifen to adjuvant chemotherapy have been studied. Adding goserelin and tamoxifen to an CMF/anthracycline-containing chemotherapy did not affect OS as compared to chemotherapy alone, though the risk of relapse was reduced in the combined modality group (144). Moreover, ovarian ablation in addition to tamoxifen and CMF chemotherapy offered no survival benefit over tamoxifen and CMF chemotherapy (145).

The studies described above demonstrate the efficacy of ovarian ablation as adjuvant therapy for premenopausal women. In those women with endocrine-responsive

disease, ovarian ablation with (139) or without tamoxifen (138) seems to be at least as effective as CMF chemotherapy alone. On the other hand, adding ovarian ablation to chemotherapy has not demonstrated any survival benefit over chemotherapy alone (141, 142) with the possible exception of women under 40 years of age and who do not achieve amenorrhea after chemotherapy (143).

Anthracycline-containing chemotherapy plus tamoxifen or tamoxifen alone are nowadays the most commonly used adjuvant therapies in hormone-receptor positive breast cancer. The fact that the studies described above lack an anthracycline-containing chemotherapy plus tamoxifen arm for comparison has lead to uncertainty about the position of ovarian ablation in clinical practice. Long-term side effects, mainly in young women, are still a significant issue when ovarian ablation is offered. As LHRH agonists produce a reliable but reversible suppression of ovarian estrogen production they represent a beneficial therapeutic option for premenopausal patients with hormone-sensitive disease.

5.2.2.4 Side effects of different endocrine therapy regimens

While tamoxifen has long been the gold standard of adjuvant endocrine therapy with well-documented side effects, significant differences in the safety profiles between various SERMs and aromatase inhibitors have now become evident during the follow-up of the studies described above.

Both tamoxifen and raloxifene have increased the rates of venous thromboembolic events in prevention and adjuvant trials (97). According to a large meta-analysis, (n=52 929 patients), tamoxifen is associated with a significantly increased risk of stroke and pulmonary embolus (146). The venous complications may be less frequent among toremifene-treated patients (102). The incidence of thromboembolic events is significantly lower in patients treated with adjuvant aromatase inhibitors than with tamoxifen (86, 121, 124). Arthralgia is more common with aromatase inhibitor than tamoxifen use (121, 124).

The use of tamoxifen in postmenopausal women is associated with a 2–3 fold increased risk of endometrial cancer (146). No such excess of endometrial cancers has

been seen with the use of toremifene though far less patients have been treated with toremifene than with tamoxifen (147). Raloxifene has not been associated with an increased rate of endometrial carcinoma compared with placebo (108, 148). Anastrozole, an aromatase inhibitor with the longest adjuvant tolerability data, does not seem to increase the risk of endometrial cancer (121). In general, gynecologic symptoms such as vaginal bleeding (86, 121) and discharge (121) are more common with tamoxifen than with aromatase inhibitor use.

The effects of endocrine therapies on bone and lipid metabolism are discussed in more detail in chapters 6 and 8.

6. LONG-TERM EFFECTS OF ADJUVANT TREATMENTS ON BONE METABOLISM

6.1 Bone structure and metabolism

Bone in adults consists of an outer part called cortical or compact bone and an inner part named cancellous or trabecular bone. Cortical bone is hard and dense and is found in flat bones, the shafts of long bones and as a thin covering over all other bones. Trabecular bone tissue is located inside the ends of long bones and in short bones such as vertebral bodies. The hollow centre of long bones contains yellow bone marrow, which consists predominantly of fat cells (149, 150).

Bone is composed of mineral, organic matrix and bone cells. The organic matrix consists predominantly of collagen fibers and the mineral consisting of calcium and phosphate is deposited on these collagen fibers (149, 150). Bone contains four cell types, osteoblasts, osteocytes, osteoclasts and bone lining cells (151). Bone is constantly being turned over in a process called remodeling employing predominantly two cell types, osteoblasts and osteoclasts. During remodeling, new bone is being formed by the osteoblasts and old bone is being dissolved by the osteoclasts. Osteoblasts secrete both the type I collagen and the non-collagenous proteins of organic matrix and they regulate the mineralization of this matrix. Osteoclasts resorb bone by attaching themselves to the matrix and secreting enzymes that digest the matrix and dissolve the bone mineral (149).

Normally the amount of bone formed equals the amount destroyed. In osteoporosis, this “coupling” between bone formation and resorption is disturbed. As a result, more bone is being resorbed than formed resulting in negative balance (149). Osteoporosis is a disorder of bone turnover where the total amount of bone tissue decreases, but its composition remains normal (152). This is in contrast to osteomalacia, where the amount of bone tissue is normal but its mineralization is defective. Osteoporosis is first seen in cancellous bone where the bone turnover is far more active than in the cortical bone. The main contributory factors in the development of osteoporosis are a too little peak bone mass formed during adolescence and the continuous loss of bone in later decades.

Postmenopausal bone loss is a consequence of defective bone remodeling stemming from estrogen deficiency. The withdrawal of sex steroids leads to bone loss because bone formation, albeit enhanced, is unable to keep pace with an even more abundant stimulation of osteoclastic bone resorption (153), a phenomenon known as uncoupling. The mechanism driving this uncoupling is central to the pathogenesis of postmenopausal osteoporosis but remains poorly understood.

Calcium homeostasis in the body is set by an interaction between the blood calcium and its target organs: bone, intestine and the kidneys. As calcium is the principal mineral of the bone, disturbances in the blood calcium levels will ultimately affect bone metabolism (149). Physiological regulation of blood calcium is maintained by the parathyroid hormone (PTH). Secretion of PTH by the parathyroid glands is inversely related to ionized serum calcium. PTH stimulates bone resorption and increases renal calcium reabsorption. It also increases the hydroxylation of 25-hydroxy-vitamin D to 1,25-dihydroxy-vitamin D (calcitriol) in the kidney. Calcitriol, in turn, increases absorption of calcium from the gut but also independently stimulates bone resorption (154). Calcium deficiency due to dietary factors or decreased calcium absorption by the intestine as well as vitamin D deficiency may thus induce bone loss and contribute to the development of osteoporosis (149).

6.2 Methods of examining bone metabolism

6.2.1 Bone mineral density (BMD)

Single (SXA) and dual X-ray absorptiometry (DXA) are used to assess mineral content of the entire skeleton and that of specific sites. Bone mineral content is the amount of mineral in the specific site scanned and, when divided by the area measured, can be used to derive a value for BMD (155). Other techniques used to assess bone density are those utilizing ultrasound (broad-band ultrasound attenuation and ultrasound velocity at the heel) and computed tomography. Today, DXA is regarded as the gold standard for diagnosis of low bone mass and osteoporosis (156). The recommended sites for diagnosis are the proximal femur and the lumbar spine. (157). The presence of osteomalacia, osteoarthritis or even aortic calcification is a potential source of error in the DXA measurements (158).

BMD measurements are usually given as standard deviations (SDs) from the mean. In relation to the bone mass of 30-year-old subjects of the same sex, this SD value is expressed as a T score, and in relation to an age-matched population, as a Z score (155). A World Health Organization (WHO) expert panel proposed that women with a T score of -2.5 SD below the young adult mean value be considered osteoporotic and those with T scores between -1 and -2.5 SD be considered osteopenic (159).

6.2.2 Biochemical markers of bone turnover

Biochemical markers of bone turnover are substances in blood and urine that are indicative of events occurring during the bone remodeling cycle. They are divided into markers of bone formation and markers of bone resorption and reflect the relative activity of osteoblasts and osteoclasts, respectively (160). The most common markers of bone formation are osteocalcin, bone alkaline phosphatase (AFOS) and aminoterminal propeptide of type I collagen (PINP). Markers of bone resorption include pyridinoline, deoxypyridinoline, hydroxyproline, cross-linked aminotelopeptide of type I collagen (NTX), cross-linked carboxytelopeptide of type I collagen (CTX) and cross-linked carboxy-terminal telopeptide of type I collagen (ICTP). Markers of bone turnover demonstrate significant day-to-day and circadian variability (161, 162).

Under most circumstances, the levels of resorption and formation markers change in the same direction (i.e., increase or decrease). An increase of bone turnover occurs during the menopause and is reflected by elevation of bone markers already during the perimenopause (163, 164). During hormone replacement treatment these markers decrease to the premenopausal level (165, 166). Similarly, treatment with most antiresorptive agents, such as bisphosphonates and SERMs, results in rapid and large reductions in markers of bone resorption coupled with more modest reductions in markers of bone formation (167).

High levels of bone turnover markers predict postmenopausal bone loss (168) and seem to be an independent risk factor for fracture (169, 170). Thus, much interest has been focused on the potential of predicting treatment efficacy with bone turnover markers. The early bone marker changes during hormone replacement therapy (171)

and bisphosphonate treatment (172, 173) seem to predict long-term effects on bone mass, even though variability of the marker changes decreases their predictive value in individualized therapy. Among women treated with antiresorptive agents such as raloxifene or bisphosphonates, greater short-term reductions in bone turnover are associated with fewer subsequent fracture events (174, 175).

One potential use of biochemical markers of bone turnover is the prediction of antifracture efficacy on the basis of pre-treatment levels of biochemical markers. The findings in this area are conflicting, though. The nonvertebral fracture risk reduction with alendronate treatment has been shown to be greater among those women with high levels of bone formation markers before therapy as compared to those with low pre-treatment levels (176). On the contrary, the efficacy of risedronate to reduce vertebral fractures in women with postmenopausal osteoporosis seems to be largely independent of pretreatment bone resorption rates (177).

In conclusion, clinical questions that might be answered by bone turnover markers include diagnosing osteoporosis, identifying "fast bone losers" and patients at high risk of fracture, selecting the best treatment for osteoporosis, and providing an early indication of the response to treatment. Additional information is needed to define specific situations and cut points to allow marker results to be used with confidence in making decisions about individual patients (178).

6.2.2.1 PINP and ICTP as markers of bone turnover

PINP is a marker of bone formation intended to reflect the synthesis of type I collagen (179). Type I collagen is the major structural organic component of bone tissue and is secreted into the extracellular matrix as an extended precursor called procollagen with amino and carboxy-terminal propeptide domains (termed PINP and PICP, respectively). Before incorporation into the bone matrix, both propeptides are cleaved by specific proteinases and are released into the circulation, where they can be measured (180).

Serum concentrations of PINP increase after surgical (181) or chemotherapy-induced menopause (182). PINP levels increase in postmenopausal osteoporosis (183). During

hormone replacement therapy (184) and treatment with the bisphosphonate clodronate (182) the PINP levels decrease reflecting the reduced bone turnover. Moreover, a decrease in PINP observed during raloxifene therapy in postmenopausal women with osteoporosis seems to predict a reduction in the vertebral fracture risk (185).

ICTP is a degradation product of mature type I collagen. Its serum concentration reflects type I collagen breakdown (186). ICTP has been reported to increase in cases of elevated bone matrix degradation, as in patients with osteolytic metastases (187). The results of ICTP as a resorption marker in postmenopausal osteoporosis or as an indicator of efficacy of antiresorptive treatment have been conflicting (182,188, 189).

6.3 Chemotherapy and bone metabolism

Women with breast cancer are at increased risk for osteoporosis as compared with women in general (190). In a majority of premenopausal breast cancer patients, adjuvant chemotherapy causes an early menopause and a rapid bone loss that may increase the risk of osteoporosis later in life (11, 12, 25, 26). Although the incidence of vertebral and hip fracture is unknown in breast cancer patients who develop ovarian failure, early menopause is a risk factor for osteoporosis in other settings.

The incidence of adjuvant chemotherapy-induced amenorrhea varies from 26% to 89% depending on the drug combination used (191). Generally, the higher the cumulative dose of cyclophosphamide, the higher the observed incidence of menopause (192). Two thirds of premenopausal women experience amenorrhea with the adjuvant regimen CMF (11), while rates of amenorrhea associated with anthracycline therapy show significant variation among studies (11, 60, 68). Women most prone to develop ovarian failure and early menopause are those in their 40s, while younger women have better preservation of menstruation after adjuvant chemotherapy (11, 193, 194).

The changes in BMD are strongly associated with menstrual function after chemotherapy. Women who develop chemotherapy-induced ovarian failure undergo accelerated and highly significant bone mineral loss while those who continue to menstruate have only minimal changes in BMD (26, 195). In a study by Saarto et al,

the change in BMD at 12 months in patients with chemotherapy-induced amenorrhoea was -6.8% in the lumbar spine and -1.9% in the femoral neck, respectively (26). In a study by Shapiro et al, the first annual BMD decrease in patients with chemotherapy-induced amenorrhoea was 7.7% in the lumbar spine and 4.6% in the femoral neck, respectively (195). A similar rapid bone loss has also been demonstrated after surgical ovarian ablation (oophorectomy) (24). Significant increases in bone formation markers serum osteocalcin and bonespecific AFOS were observed in the women who developed chemotherapy-induced ovarian failure at six and 12 months. Among those who retained menstrual function, significant increases in these markers were observed at six months; however, between six and 12 months the markers declined (195).

In addition to the risk for early menopause induced by chemotherapy, some chemotherapeutic agents used in the treatment of breast cancer may have a direct adverse effect on BMD. Methotrexate increases bone resorption in vivo as assessed by both increases in urinary hydroxyproline levels and histomorphometry (196). Severe osteoporosis with fractures has been reported after high-dose and long-term methotrexate treatment (197). Doxorubicin has been observed to cause a decrease in the trabecular bone volume (198).

Breast cancer itself may predispose to osteoporosis. Patients with cancer but without bone metastases show increased bone resorption as indicated by biochemical markers of bone turnover (198). In a study by Kanis et al, a high incidence of vertebral fracture was noted in women with breast cancer but without evident bone metastases (199).

6.4 Endocrine therapy and bone metabolism

Data on the effects of tamoxifen on bone turnover markers are limited. Decreases in the urinary excretion of the bone resorption (pyridinoline, deoxypyridinoline, hydroxyproline and NTX) and formation markers (osteocalcin and PINP) during tamoxifen treatment have been demonstrated in a few studies (200, 201). These findings probably reflect decreased bone remodelling similar to that observed with oral estrogen.

The effects of tamoxifen on BMD have been studied in both postmenopausal breast cancer patients and healthy postmenopausal women with concordant results. The data accumulated to date confirm that in postmenopausal women tamoxifen significantly decreases the loss of BMD in the lumbar spine and to a somewhat lesser degree at the femoral neck. Tamoxifen at least prevents bone loss in the lumbar spine or even increases the lumbar spine BMD (34-38, 200-205). In most trials, tamoxifen preserved or even increased BMD also in the femoral neck (35, 37, 38, 200, 202- 204) or had no effect (37). The positive effect of tamoxifen on BMD has been shown to last over the treatment period of five years (201, 205). However, a rapid decrease of 4.8% in the lumbar spine BMD was noted when BMD was measured one year after the cessation of five-year adjuvant tamoxifen (205).

There is some data on the effects of tamoxifen on the risk of fracture. A large placebo-controlled study using tamoxifen in the prevention of breast cancer in more than 13 000 patients at high risk showed a nonsignificant reduction of about 20% in the overall incidence of spine, Colles and hip fracture (206). In an adjuvant study on 1 716 postmenopausal women with breast cancer the femoral fracture rate was not decreased among tamoxifen users (207). In a randomized study on 140 postmenopausal women with early breast cancer, no significant difference in the fracture rate between the tamoxifen and placebo groups was noted (201).

While tamoxifen prevents bone loss in postmenopausal women, the effects may be opposite for premenopausal women. Powles et al studied the effects of tamoxifen on BMD in 179 pre- and postmenopausal healthy women who participated in a placebo-controlled tamoxifen chemoprevention trial. In premenopausal women, both lumbar spine and femoral neck BMD decreased progressively in tamoxifen users. In accordance of prior studies, in postmenopausal patients tamoxifen increased the lumbar spine and femoral neck BMD compared with a nonsignificant loss for women on placebo (38). Thus, in premenopausal women, tamoxifen seems to act as an antiestrogen on bone tissue, probably competing with the endogenous estradiol and resulting in bone loss.

The effects of another SERM toremifene on bone have been less extensively studied. Similarly to tamoxifen, the urinary excretion of bone resorption markers decreases

during toremifene treatment reflecting reduced bone turnover (200). Adjuvant toremifene at a dose level of 60 mg/day prevented bone loss in postmenopausal breast cancer patients (202) while toremifene 40 mg/day failed to do so (203).

Raloxifene is the first SERM approved for the prevention of osteoporosis and reduction of new vertebral fractures in postmenopausal women with osteoporosis. Raloxifene decreases bone loss in healthy postmenopausal women (208), women with osteopenia (209) and women with osteoporosis (107). The increase in BMD and decrease in biochemical markers of bone turnover noted in raloxifene treated patients compare well with those noted during estrogen treatment (210). The largest osteoporosis intervention trial so far randomized 7 705 postmenopausal women with osteoporosis to receive either raloxifene 60 mg or 120 mg or placebo. After three years of follow-up, raloxifene increased the BMD in the lumbar spine and femoral neck as compared to the placebo group. Moreover, raloxifene decreased the cumulative risk of new vertebral fractures but the risk of nonvertebral fractures was similar in the raloxifene and placebo groups (107). The update at four years of follow-up did not significantly change these results (211).

While the SERMs provide protection against bone loss in postmenopausal women, the aromatase inhibitors may reduce bone mineral density by decreasing estrogen levels. Estrogen deficiency in turn may lead to osteoporosis (212). Recent studies have shown that both anastrozole and letrozole increase bone resorption (213-215). In the ATAC trial comparing adjuvant anastrozole and tamoxifen, tamoxifen had a slightly positive effect on BMD while anastrozole had a negative effect on BMD. Also fractures were significantly more common with anastrozole than with tamoxifen during the follow-up of 68 months (11.0% v. 7.7%, respectively). The greatest increase in fractures on anastrozole treatment seemed to be in the spine while no increase in hip fractures was seen (121, 216). A four-group trial evaluating adjuvant tamoxifen, letrozole or a sequential combination of these (BIG 1-98) also reported significantly more fractures with patients on letrozole as compared to tamoxifen (5.8% v. 4.1%, respectively) (86). In a MA-17 adjuvant study on letrozole after five years of tamoxifen, newly diagnosed osteoporosis was significantly more common among women on letrozole as compared to those on placebo. No significant difference in fracture rates between the groups emerged during the short follow-up of

2.5 years (83, 84). In the IES adjuvant study on exemestane, a trend towards a higher incidence of reported fractures in the exemestane group as compared to the tamoxifen group was reported (85, 124).

Ovarian ablation with LHRH analogs causes a sudden decline in estrogen levels and this chemical castration in turn leads to a rapid decrease in BMD (217). Both bone resorption and formation markers increase during the treatment of premenopausal women with LHRH agonist (217). As suggested in the preclinical setting (212), tamoxifen seems to partially counteract the demineralising effects of LHRH analogs. A Swedish study randomized premenopausal women with early breast cancer to receive goserelin alone, goserelin plus tamoxifen or tamoxifen alone for two years. The loss in total body BMD was greatest in women receiving goserelin alone, while tamoxifen plus goserelin and tamoxifen alone resulted in smaller declines in BMD. Though, one year after cessation of treatment, the goserelin alone group showed a partial recovery from the bone loss (219).

The effects of adjuvant treatments on BMD and fracture risk are summarized in Table 3.

Table 3. Effects of adjuvant treatments on bone mineral density (BMD) and fracture risk in postmenopausal breast cancer patients

	Chemotherapy	Tamoxifen	Toremifene	Anastrozole	Letrozole	Exemestane
BMD	↓↓*	↑↑	↑	↓↓	↓↓	↓
Fracture risk	NE*	↓	NE	↑↑	↑↑	↑

↓ decrease (trend), ↓↓ decrease (significant), ↑ increase (trend), ↑↑ increase (significant), NE not evaluated, * in chemotherapy-induced amenorrhea

6.5 Osteoporosis

Osteoporosis is defined as a disease characterized by low bone mineral mass, microarchitectural deterioration of bone tissue leading to increased bone fragility and consequent increase in fracture risk with minimal or no trauma (220). Based on the

World Health Organization criteria, about a third of postmenopausal white women have osteoporosis (221). Age is the most important risk factor for osteoporosis. Also the risk of osteoporotic fractures increases dramatically with aging. It has been estimated that a 50-year old white woman has a 40% risk of suffering a hip, Colles or vertebral fracture during her remaining lifetime (222).

Strong risk factors of low bone density and osteoporosis for women include advanced age, previous fragility fracture, maternal family history of hip fracture, premature menopause, low body-mass index, corticosteroid therapy and some disorders associated with osteoporosis (hypogonadism, hyperparathyroidism, malabsorption syndromes, chronic renal failure, Cushing`s syndrome etc.) (223). Other risk factors include smoking, high caffeine intake, physically inactive lifestyle and a low dietary intake of calcium and vitamin D. On the other hand, estrogen use, high body-mass index and late age at menopause seem to protect from osteoporosis (224). Regular weight-bearing exercise can conserve or improve BMD in peri- and postmenopausal women (225, 226).

6.5.1 Treatment of osteoporosis – general

Several therapeutic options with well-documented improvements in BMD and reductions in fracture risk are available to women for the prevention and treatment of postmenopausal osteoporosis. The bisphosphonates clodronate, etidronate, risedronate and alendronate, raloxifene, calcitonin and (hydroxylated) vitamin D all reduce vertebral fractures, while only alendronate and risedronate significantly reduce nonvertebral fractures (227).

The majority of evidence for the efficacy of hormone replacement therapy (HRT) on BMD and fracture risk is based on case-control and cohort studies instead of randomized trials. Observational studies have indicated a significant decrease in the risk of hip fracture in HRT users (228, 229). The largest randomized, placebo-controlled trial Women`s Health Initiative (WHI) including 16 608 postmenopausal women, demonstrated a significant increase in BMD and decrease in the risk of both vertebral and nonvertebral fracture in women receiving estrogen plus medroxyprogesterone as compared to those on placebo. However, an increased risk of

cardiovascular disease and breast cancer was observed in the HRT users relative to the placebo arm (230). Among the 10 739 postmenopausal women randomized to either estrogen or placebo, estrogen alone also decreased the risk of hip fracture, but, in contrast to the estrogen plus progestin treatment, also insignificantly decreased the risk of breast cancer (231).

Risedronate and alendronate are third- and second generation bisphosphonates, respectively, approved for prevention and treatment of postmenopausal osteoporosis. Alendronate increases the BMD in postmenopausal osteoporosis and decreases the risk of both vertebral and nonvertebral fracture (232, 233). Similarly, risedronate has been shown to increase the BMD in postmenopausal osteoporosis and to decrease the risk of both vertebral and nonvertebral fracture (234, 235). They can be administered orally either daily or more conveniently once weekly as these dosing options are equally effective (236, 237).

The first generation bisphosphonate, clodronate, has also demonstrated beneficial effects on BMD in patients with osteopenia or osteoporosis. Both continuous and cyclical regimens given orally or intravenously have resulted in significant increases in BMD, especially in the lumbar spine (29, 31, 32, 238-240). Clodronate has been shown to reduce the risk of vertebral fracture (32, 240). Another first generation bisphosphonate, etidronate, significantly increases BMD at the spine and hips and slightly reduces the rate of spinal fractures (241).

As already discussed above, the SERM raloxifene increases the BMD, decreases the markers of bone turnover and decreases the risk of vertebral fractures as compared to placebo (107, 208).

Calcitonin is a polypeptide hormone derived from salmon. Calcitonin increases the BMD in spine and reduces the risk of new vertebral fractures. However, calcitonin has not demonstrated an ability to lower the frequency of nonvertebral fractures (242). Calcitonin is available as an injection or a nasal spray.

Teriparatide is a recombinant human parathyroid hormone analogue available as an injection. While other therapeutic agents for osteoporosis reduce bone resorption,

intermittently administered teriparatide stimulates new bone formation by preferentially stimulating osteoblasts. Consequently, the effects on bone turnover differ dramatically from those observed with antiresorptive therapy. With teriparatide, bone formation markers rise quickly and remain elevated for the duration of treatment (243). After a brief delay, PTH therapy also increases markers of bone resorption. Teriparatide has been shown to increase bone mineral density and to significantly reduce the risk of vertebral and nonvertebral fracture (244).

Supplementation with (calcium and) vitamin D reduces risk of vertebral and probably also nonvertebral fractures (245, 246). Although the evidence from clinical trials demonstrates that vitamin D could prevent fractures, there is a wide variability in the magnitude of this effect. Therefore, calcium and vitamin D supplements should not be considered an adequate intervention for fractures in high-risk women but rather as a standard supplement to other osteoporosis drug therapy. Vitamin D supplementation appears to even reduce the risk of falls among ambulatory or institutionalized older individuals possibly by increasing musculoskeletal function (247).

6.5.2 Treatment of osteoporosis in women with a history of breast cancer

Osteoporosis is first seen in cancellous bone (e.g. the vertebral bodies) where bone turnover is fast. Reflecting this, a high incidence of vertebral fracture has been noted in women with breast cancer (199). Breast cancer patients found to have osteoporosis based on BMD results (T-score –2.5 or lower) should have pharmacological therapy initiated with an agent demonstrated to have efficacy. There is currently insufficient evidence to recommend a particular agent in this category (248). Some remarks, however, can be made based on indirect evidence.

All postmenopausal women, also those with a history of breast cancer, should be encouraged to maintain adequate calcium and vitamin D intake and sustain regular physical activity in order to help preserve BMD. Appropriate calcium intake ranges between 1000 and 1500 mg/day depending upon age, and appropriate vitamin D intake ranges between 400 and 800 IU/day (246). Regular weight-bearing exercise can conserve or improve BMD in postmenopausal women (225).

Although raloxifene is approved for osteoporosis prevention and therapy, its use after five years of adjuvant tamoxifen therapy may not be advisable. Raloxifene is a SERM, and it has been suggested that the usage of another SERM tamoxifen beyond five years might worsen the prognosis of breast cancer. In the NSABBP B-14 study 10 years of tamoxifen was associated with more recurrences than five years of tamoxifen (96). Moreover, raloxifene has been shown to stimulate tamoxifen-dependent cancer cells in laboratory studies (249).

Teriparatide has been associated with osteosarcoma development in animal studies (317). Though no cases of osteosarcoma have been observed in humans treated with teriparatide for osteoporosis, it is not recommended for use in women diagnosed with breast cancer (248, 250).

According to the recent findings in the WHI trial, HRT with estrogen and progestin is associated with an increased risk of breast cancer (230). Estrogen therapy has long been contraindicated in women with a history of breast cancer because of the probable increases in the risk of recurrence and second primary breast cancers (251). Recently, a Scandinavian open randomized trial addressing the question whether HRT is safe for women with breast cancer history was terminated because of safety concerns. New breast cancer events were more common in the HRT group relative to placebo group (252). HRT should not be used in the treatment or prevention of osteoporosis in breast cancer survivors.

Of the treatment options available for osteoporosis prevention and therapy, the bisphosphonates may seem most appropriate for women with a history of breast cancer. According to recent reviews they seem to be the most efficient drugs in the treatment of osteoporosis (227).

7. BISPHOSPHONATE TREATMENT IN BREAST CANCER

7.1 Bone metastasis – general

The skeleton is the most common organ to be affected by metastatic breast cancer: around 70 percent of patients dying with breast cancer have bone metastases (253, 254). Breast cancer patients with bone metastases alone tend to have a protracted disease course when compared to those with visceral and in particular liver metastases. The median survival in patients with liver metastases averages one year (355) while the median survival in patients with disease confined to the skeleton survive at least twice that long (253). For patients with breast carcinoma, good prognostic factors for survival after the development of bone metastases are low histologic grade, positive estrogen receptor status, bone disease at initial presentation, a long disease free interval, and increasing age. Up to 20 percent of breast cancer patients with metastatic bone disease survive five years after the diagnosis of bone metastases (254).

Paget first proposed the idea of "seed and soil" hypothesis in the pathophysiology of bone metastases more than one hundred years ago (256). Micrometastatic deposits of cancer ("seed") access the bone via the bloodstream. The bone microenvironment contains several growth factors that encourage the growth of cancer cells. As the metastatic cells alter the regulatory mechanisms of bone, it becomes a more favorable "soil" for the development of metastases. The tumor cells release different growth factors and cytokines (e.g. parathyroid hormone-related peptide (PTHrP), interleukins IL-1 and IL-6, TGFa, TGFb and prostaglandins) that accelerate the osteoclast-mediated bone resorption and lead to local erosion of the bone (257). In breast cancer, the main mediator causing osteolysis is probably PTHrP (258).

Bone resorption in metastatic bone disease leads to pathological fractures, pain and hypercalcemia. Bone pain is the most common complication of metastatic bone disease, resulting from structural damage, periosteal irritation, and nerve entrapment. The increased bone destruction brings about a release of calcium from the skeleton to the bloodstream. Other mechanisms behind the hypercalcemia of malignancy include

PTHrP- mediated increased reabsorption of calcium in the kidney and dehydration (259).

7.2 Mechanism of action of the bisphosphonates

Bisphosphonates are synthetic compounds characterized by a P-C-P (phosphate-carbon-phosphate) structure, in contrast with their naturally occurring analogue, pyrophosphate, with a P-O-P (phosphate-oxygen-phosphate) sequence. Unlike natural pyrophosphate, bisphosphonates are resistant to breakdown by enzymatic hydrolysis (260). The negative charges on the two phosphate groups of the bisphosphonate nucleus give these compounds a high affinity for mineralized bone. Modification of one of the two side chains coupled to the central carbon atom determines the antiresorptive potency of the bisphosphonate. According to the structure of this side chain, the bisphosphonates can be divided into those resembling natural bisphosphonate or non-aminobisphosphonates (clodronate and etidronate) and to those with a nitrogen-containing side chain, aminobisphosphonates (pamidronate, alendronate, zoledronic acid, risedronate, ibandronate). Over the years, first, second and third generation bisphosphonates have been developed with increasing potency from generation to generation. They are used in medicine mainly to inhibit bone resorption in diseases like osteoporosis, Paget's disease and metastatic bone disease. The more potent aminobisphosphonates have demonstrated higher efficacy than the non-aminobisphosphonates in preclinical studies, but the possible clinical differences between these compounds have not been extensively studied (261).

Bisphosphonates inhibit bone resorption by a variety of mechanisms. The principal biologic effect appears to be the inhibition of osteoclast activity. When osteoclasts ingest bisphosphonate-containing bone, their cytoskeleton becomes disrupted and apoptosis of the osteoclasts is activated (260). The non-aminobisphosphonates act as analogues of ATP (adenosine triphosphate) and inhibit ATP dependent intracellular enzymes leading to apoptosis and death of the osteoclasts (262). The aminobisphosphonates, on the other hand, inhibit enzymes of the mevalonate pathway by disrupting the signalling functions of key regulatory proteins and lead to osteoclast apoptosis (263-265). Bisphosphonates also inhibit the development of osteoclasts from monocyte-precursors (266). The net effect of both non-aminobisphosphonates

and aminobisphosphonates is inhibition of osteoclast function, which leads to a decrease in bone resorption (150). It has also been suggested that bisphosphonates prolong the survival of osteoblasts (267).

The bisphosphonates inhibit normal and ectopic calcification by disturbing the formation of calcium phosphate crystals (262). Reflecting this, the first generation bisphosphonate etidronate has been shown to cause osteomalacia in the early studies (268). More recently, bisphosphonates have been shown to inhibit matrix metalloproteinases (MMPs) that are involved in bone resorption and tumor invasion. They have also been found to inhibit the adhesion of tumor cells to the bone and to induce tumor cell apoptosis in vitro (269-271). Bisphosphonates have demonstrated direct antitumor activity against neoplastic cells especially in the bone, where bisphosphonates reduce skeletal tumor burden and increase tumor cell apoptosis in skeletal metastases (270, 272, 273). The effect of bisphosphonates on non-skeletal metastases is still controversial. Even an adverse effect on the development of non-skeletal metastases by bisphosphonate therapy has been suggested in some preclinical studies (274, 275).

The clinical pharmacology of bisphosphonates is characterized by low intestinal absorption, but highly selective localization and retention in bone. The bioavailability of an oral dose of a bisphosphonate is only 1% - 10%. Absorption is further diminished if oral bisphosphonates are given at mealtimes and especially with dairy products or iron supplements. Bone takes up most of the absorbed bisphosphonate, the remainder being rapidly excreted in the urine. The skeletal retention of bisphosphonates is very lasting, possibly life-long. However, the bisphosphonate deposited in the skeleton probably has little pharmacological activity once the administration is ceased (276).

Bisphosphonate therapy is generally well tolerated. The probability of hypocalcemia is greater with the use of intravenous aminobisphosphonates and therefore supplementation with calcium (and vitamin D) is recommended (277). When bisphosphonates are administered intravenously, too rapid an infusion may lead to renal complications (277). Thus, infusion times less than two hours with pamidronate or less than 15 minutes with zoledronic acid should be avoided (248). Ibandronate is

given via a one- to two-hour infusion (278). In a study comparing intravenous pamidronate and zoledronic acid, six to 8 percent of breast cancer patients showed some deterioration of renal function during the first 12 months of bisphosphonate therapy (279). Though the renal complications during bisphosphonate therapy are often mild and apparent only as transient increases in serum creatinine, there are limited data on the long-term renal safety of bisphosphonates. Few case reports have been published about the adverse renal consequences of prolonged, high-dose bisphosphonate administration (280, 281). Consequently, the serum electrolyte and creatinine levels should be monitored during bisphosphonate therapy (248).

Gastrointestinal discomfort, nausea, dyspepsia, vomiting, diarrhea and even esophageal ulceration may accompany oral administration of bisphosphonates. In a large adjuvant clodronate study, especially diarrhea was significantly more common in clodronate users than in those on placebo (15% v. 7%, respectively) (282). Similarly, in a pooled analysis of two oral ibandronate trials in breast cancer patients with bone metastases, patients receiving ibandronate were twice as likely to experience adverse gastrointestinal events than those receiving placebo (283).

The intravenous administration of the aminobisphophonates can induce transient flu-like symptoms with fever, myalgia and arthralgia. These side effects occur primarily after the first infusion (284). Recently, an association between long-term aminobisphosphonate therapy and osteonecrosis of the jaws has been reported (285). Few ocular disturbances including scleritis have been described during pamidronate use (286).

7.3 Bisphosphonate treatment in metastatic breast cancer

At least 14 randomized placebo-controlled prospective studies have been published on the effects of bisphosphonates in patients with metastatic breast cancer. These include five studies on clodronate (287-291), five studies on pamidronate (292-296), three studies on ibandronate (278, 283, 297) and one on zoledronic acid (298). In addition, there is one study comparing the efficacy of zoledronic acid and pamidronate in patients with breast cancer (299). These studies show that bisphosphonates, in addition to hormone therapy or chemotherapy, reduce the risk of developing a skeletal

event (pathological fracture, hypercalcemia, bone pain or need for palliative radiotherapy) and the skeletal event rate. As summarized in a recent Cochrane review, bisphosphonates also increase the time to skeletal event but have no effect on survival in women with advanced breast cancer and bone metastases (33).

Oral bisphosphonates, including clodronate and ibandronate, may be self-administered at home and thus do not acquire frequent hospital admissions. However, patient compliance may be compromised by the gastrointestinal toxicity of these oral agents (282, 283). Although oral bisphosphonates seem somewhat less effective than the intravenous remedies at least for the treatment of hypercalcemia (277), at present the data is insufficient to recommend one bisphosphonate over another in the treatment of skeletal metastases (248). No studies directly compare the effectiveness of oral and intravenous bisphosphonates in the prevention of skeletal complications. The one study comparing two intravenous bisphosphonates showed a lower risk of skeletal events with zoledronic acid than with pamidronate (299).

In addition to the trials in patients with breast cancer and bone metastases mentioned above, there are three placebo-controlled trials (one on oral pamidronate, two on oral clodronate) examining the role of bisphosphonates in patients with advanced breast cancer but no bone metastases (302-304). No effect on the development of bone metastases was found in the pamidronate and the smaller clodronate study (302, 304). In the larger clodronate study, however, the total number of skeletal metastases was significantly lower with clodronate treatment than with placebo, but the number of patients developing bone metastases did not differ between the treatment groups (303).

7.4 Adjuvant bisphosphonate treatment in breast cancer

7.4.1 Effect on survival

There are three randomized prospective controlled trials available on adjuvant bisphosphonate treatment in early stage breast cancer. These trials on oral clodronate provide conflicting data on the potential role of adjuvant bisphosphonates among patients with no evidence of distant metastases after definitive local surgery.

The first trial conducted by Diel et al randomly assigned 302 women with node-positive and node-negative primary breast cancer and a positive bone marrow aspirate for tumor cells to receive either clodronate 1600 mg/day for two years or no bisphosphonate. The type of adjuvant systemic therapy was selected in accordance with specific guidelines (hormonal therapy, chemotherapy, or both, or no systemic therapy). In the initial report, with a median follow-up of 36 months, the incidence of overall metastasis (13% v. 29%), bone metastasis (8% v. 17%), and visceral metastasis (8% v. 19%) was significantly lower in the clodronate group. At five years of follow-up, the incidence of bone metastases was still significantly lower in the clodronate group, but the extra-skeletal effect was no longer significant (305, 306). Quite recently, the investigators provided an update of their initial report after an extended follow-up of 103 months. Although the incidence of osseous and visceral metastases was now similar in both groups, the OS was still significantly better in the clodronate group (307).

Saarto et al reported the results of a trial of 299 women with node-positive breast cancer who were randomly assigned to receive clodronate 1600 mg/day or placebo for three years. All patients received adjuvant therapy; premenopausal women received CMF chemotherapy and postmenopausal women were treated with antiestrogens. After a follow-up of five years, there was no significant difference in the frequency of bone metastases between the clodronate and control arms. In contrast to the Diel study, however, the incidence of nonosseous metastases was significantly higher in the clodronate arm (43% v. 25%). Both DFS (56% v. 71%) and OS were significantly worse in the clodronate arm than in the controls (70% v 83%). Although more patients in the clodronate group had hormone-receptor negative tumors at baseline, the adverse effect of clodronate on DFS (but not on OS) remained significant in multivariate analyses after adjustment for prognostic factors like hormone receptor status, number of lymph nodes and tumor size (308). Quite recently, Saarto et al have updated their results after ten years of follow-up. The results remained stable: the incidence of skeletal metastases did not differ between the treatment groups, while the incidence of extraskeletal metastases was significantly higher in the clodronate group. Similarly, ten-year DFS remained significantly lower in the clodronate than in the control group (45% v. 58%), but this did not significantly compromise OS. The adverse effect of

clodronate was especially seen in estrogen-receptor negative patients, where the 10-year DFS was 25% in the clodronate group and 58% in the control group (309).

In the double-blind trial by Powles et al, 1 069 women with node-negative or node-positive breast cancer were randomly assigned to receive either clodronate 1600 mg/day or placebo for a duration of two years. The categories of no adjuvant therapy, chemotherapy, tamoxifen, or both chemotherapy and tamoxifen were balanced between the arms. During the two years of clodronate use, bone metastases were significantly less frequent in the treatment group as compared to the placebo group (2.3% v. 5.2%). At five years of follow-up, the incidence of bone metastases was no longer significantly different between the clodronate and control arms (12% v. 15%). No difference in the frequency of nonosseous metastases was observed (21% v. 24%, respectively). OS was significantly improved in the clodronate arm (82% v. 76%) (310).

Thus, two of the adjuvant clodronate trials yielded favorable results and one demonstrated an adverse impact. The three trials differ with regard to the patient characteristics and the study protocol. In the Diel study, all patients had bone marrow micrometastases. In the study by Saarto only node-positive patients were included as compared to only 37% and 50% of node-positive patients in the Powles and Diel study, respectively. The duration of clodronate treatment was three years in the Saarto study and two years in the other two trials (305, 308, 310).

In all three trials clodronate prevented or delayed the appearance of bone metastases; even in the study by Saarto et al, bone as the first site of relapse was less frequent in the clodronate than in the control group. The results of the influence of clodronate on nonosseous metastases, however, are controversial. Given that the three trials are inconsistent, it remains uncertain whether bisphosphonates are beneficial as adjuvant treatment. The largest adjuvant bisphosphonate study so far is ongoing (NSABP trial B34), with 2 400 early stage breast cancer patients randomly assigned to adjuvant clodronate or placebo, and will hopefully answer some questions raised by the former studies. Additionally, the North American Intergroup will conduct an adjuvant bisphosphonate trial (S0307) comparing oral clodronate to risedronate and zoledronic

acid. At present, adjuvant bisphosphonates cannot be recommended for women about to undergo systemic adjuvant therapy outside clinical trials (248).

7.4.2 Effect on bone mineral density

Adjuvant therapy for breast cancer can be associated with decreased BMD that may lead to osteoporosis and skeletal morbidity. Current evidence from clinical trials indicates that bisphosphonates are effective in maintaining bone density in women receiving adjuvant therapy for breast cancer.

Women who develop chemotherapy-induced ovarian failure undergo accelerated and highly significant bone mineral loss while those who continue to menstruate have only minimal changes in BMD (26, 195). Risedronate and clodronate have been evaluated in breast cancer patients with chemotherapy-induced early menopause. Intermittent oral risedronate for two years prevented effectively the bone loss observed in the placebo group (311). Another oral bisphosphonate, clodronate, significantly reduced bone loss in premenopausal patients who developed amenorrhea after adjuvant CMF chemotherapy (26). Similarly, oral clodronate for two years reduced the loss of BMD in patients who received adjuvant chemotherapy and/or tamoxifen (312).

Ovarian ablation with LHRH analogs causes chemical castration, which in turn leads to a rapid decrease in BMD in premenopausal breast cancer patients (219). Intravenous bisphosphonate zoledronate maintained bone density in premenopausal breast cancer patients made functionally postmenopausal with LHRH agonist goserelin use (313).

In postmenopausal patients with early breast cancer, aromatase inhibitors reduce BMD and increase the risk of osteoporosis (84-86, 216). Preliminary data suggests that the bone loss associated with adjuvant letrozole may be preventable with zoledronic acid given every six months (314).

8. LONG-TERM EFFECTS OF ADJUVANT TREATMENTS ON LIPID METABOLISM

8.1 Lipid metabolism and atherosclerosis

High levels of total and low-density lipoprotein (LDL) cholesterol are well-recognized risk factors for atherosclerotic disease, in particular coronary heart disease (CHD) (315). Small, dense and oxidized LDL particles are considered particularly atherogenic (316). Lipoprotein(a) (Lp(a)) consists of an LDL particle covalently attached to apolipoprotein(a). High plasma levels of Lp(a) have been positively associated with atherosclerosis, myocardial infarction and stroke (317). The atherogenity of Lp(a) is at least partly associated with an elevated concentration of LDL cholesterol (318). Plasma levels of high-density lipoprotein (HDL) cholesterol, on the other hand, are consistently inversely associated with CHD risk in observational studies (319, 320). The cardioprotective effects of HDL cholesterol have been attributed to its role in reverse cholesterol transport, its effects on endothelial cells, and its antioxidant activity (321, 322). The role of triglycerides in the development of CHD is less evident. A meta-analysis of 17 prospective population-based studies found hypertriglyceridemia to be an independent risk factor for cardiovascular disease (323). Nevertheless, it is possible that, rather than being actual atherogenic agents in themselves, elevated triglycerides merely serve as a marker for increases in triglyceride-rich remnant lipoproteins and that it is these latter particles that are involved in the development of atherosclerosis (316, 324).

Atherosclerosis is a slowly progressing, pathological process involving the intima and media of large- and medium-sized arteries. The response-to-injury hypothesis behind the pathogenesis of atherosclerosis currently emphasizes endothelial dysfunction because of injury to the endothelial wall. Different forms of injury increase the adhesiveness and permeability of the endothelium and induce the endothelium to have procoagulant instead of anticoagulant properties (325). In addition to endothelial dysfunction, monocyte adhesion, lipid accumulation and oxidation, smooth muscle proliferation and extracellular matrix deposition contribute to the formation of atherosclerotic plaques (326). Concurrently with lipid accumulation, signs of inflammation occur in the artery wall (327). The atherosclerotic plaques do not usually interfere with arterial flow in the early stage of the disease. When thrombosis

is superimposed on non-stenotic lesions, however, blood flow is impaired and may lead to ischaemia of target organs heart, brain or extremities.

Endothelial injury may result from free radicals caused by elevated LDL cholesterol levels, smoking, hypertension, diabetes or hyperhomocystinemia (325). An infectious etiology has also been suggested as microorganisms like Chlamydia pneumoniae have been identified in atheromatous coronary artery lesions (328). Further, elevated levels of the inflammatory marker C-reactive protein (CRP) can indicate low-grade chronic inflammation and thus help to identify patients at risk for atherosclerotic complications (327).

While the role of other factors in the development of atherosclerosis and CHD are being extensively studied, LDL cholesterol remains the primary target of therapy for the prevention and treatment of coronary heart disease (316). Reduction of total and LDL cholesterol is associated with numerous sequelae which attenuate atherogenesis, including improved endothelial function, reduced oxidative stress and reduced inflammation (329). Clinical trials of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have confirmed that LDL cholesterol reduction significantly reduces cardiovascular morbidity and mortality in primary and secondary cardiovascular disease prevention (330, 331). A recent meta-analysis on the effectiveness of statin therapy reviewed 25 studies enrolling 69 511 individuals with CHD. According to this meta-analysis, statin therapy reduced CHD mortality by 23% and all-cause mortality by 16% as compared to placebo. The risk of nonfatal infarction was reduced by 25% with statins (332).

8.2 Estrogen and lipid metabolism

Estrogen decreases the serum levels of LDL cholesterol by inducing the expression of hepatic LDL receptors and thus enhancing the clearance of LDL cholesterol from plasma. Estrogens are thought to increase HDL cholesterol by reducing hepatic triglyceride lipase activity that catabolizes HDL (333). Estrogen has also been shown to lower Lp(a) levels (334). Menopause in turn causes changes in serum lipids that are explained by the deficiency of estrogens: serum total and LDL cholesterol and triglyceride levels increase and HDL cholesterol levels decrease (13-17). These

changes in serum lipid profile are considered atherogenic and contribute to the increased risk of CHD in postmenopausal women. Consequently, the incidence of CHD in women increases after the menopause (335).

Estrogen replacement therapy reduces plasma levels of LDL cholesterol and increases levels of HDL cholesterol, changes known to be associated with a reduced risk of cardiovascular disease (336). Estrogen has also been shown to inhibit oxidation of low-density lipoprotein, improve endothelial vascular function and reverse postmenopausal increases in fibrinogen and plasminogen-activator inhibitor type 1 - changes that should also reduce the risk of cardiovascular disease (337). However, estrogen also has adverse physiological effects, including increasing the plasma levels of triglycerides, small, dense LDL particles, CRP and some thrombotic markers (338, 339).

Observational studies have suggested that postmenopausal hormone replacement therapy (HRT) reduces the risk of CHD (340). Recent randomized trials, however, have either provided no evidence of cardiac protection or even some evidence of harm when HRT has been given to women with CHD (336, 341). Moreover, the first randomized trial to directly study the effects of estrogen plus progestin therapy on CHD incidence in previously healthy postmenopausal women, was stopped early because it was found that the health risks associated with HRT exceeded the benefits. In this Women's Health Initiative (WHI) trial, HRT with estrogen plus progestin increased the risk of CHD among generally healthy postmenopausal women by 24% as compared to placebo (338). Though, as the criticism against the WHI trial states, many of the “healthy” women included actually had either CHD or several risk factors for CHD at randomization. HRT with estrogen only did not affect the risk of CHD (342).

8.3 Adjuvant chemotherapy and serum lipids

The effect of adjuvant chemotherapy on serum lipids has not been extensively studied. A few small studies on breast cancer patients at various stages of the disease have been published with conflicting findings (18, 343, 344).

As explained earlier, adjuvant chemotherapy causes ovarian failure in a majority of premenopausal women with early breast cancer. The incidence of adjuvant chemotherapy induced amenorrhea varies depending on the drug combination used (191). The changes in serum lipids after adjuvant chemotherapy seem to correlate to changes in ovarian function: patients who preserve menstruation have stable lipid levels while those with chemotherapy-induced menopause develop possibly atherogenic changes in serum lipids. Total cholesterol, LDL and HDL cholesterol levels have been shown to increase in patients with chemotherapy-induced ovarian dysfunction (18), but no long-term data is available on this subject.

8.4 Adjuvant endocrine therapy and serum lipids

Tamoxifen has favorable and possibly antiatherogenic effects on lipid metabolism. Tamoxifen reduces the plasma concentrations of total and LDL cholesterol by downregulation of cholesterol synthesis (345). The effect of tamoxifen treatment on HDL cholesterol has been small (19-22, 346). In contrast to the favorable effects of tamoxifen on total and LDL cholesterol, tamoxifen has been shown to increase the serum triglyceride concentrations (19, 21, 347) and contribute to the development of fatty liver (hepatic steatosis) (348). Further, tamoxifen has demonstrated effects on inflammatory markers that are consistent with reduced cardiovascular risk. Reductions in CRP and fibrinogen have been noted with tamoxifen use (349).

In line with the findings of the recent trials on postmenopausal hormone replacement therapy and CHD risk, the favorable lipid effects of tamoxifen do not necessarily translate to reductions in cardiac risk. Few randomized trials suggested that adjuvant tamoxifen might have a cardioprotective effect in postmenopausal women (350-352). However, a large (n= 13 388) placebo-controlled randomized chemoprevention study failed to show any effects of tamoxifen on cardiovascular risk (206).

The effects of toremifene on plasma lipids seem even more favorable than those of tamoxifen. Like tamoxifen, also toremifene lowers total and LDL cholesterol by downregulating cholesterol synthesis and reduces Lp(a) levels (345, 354, 355). In contrast to tamoxifen, however, HDL cholesterol levels have been shown to increase and triglyceride levels either steady or even decrease during toremifene use (21, 354).

Randomized trials of raloxifene's use in postmenopausal women have consistently demonstrated a reduction in serum LDL cholesterol levels, while no apparent effect on HDL cholesterol or triglyceride levels has been noted (211, 356). Secondary analysis of data from the MORE trial showed a significant reduction in the risk of cardiovascular endpoints in a subset of women with increased risk for cardiovascular disease (357). A randomized, placebo-controlled trial of raloxifene for the prevention of coronary heart disease events is under way.

The aromatase inhibitors anastrozole, letrozole and exemestane have shown somewhat different effects on serum lipid profile. In the metastatic setting, anastrozole did not show any major effects on lipids (358). In a small adjuvant study on Japanese women anastrozole did not induce any significant changes in total and LDL cholesterol but led to a increase in HDL cholesterol and decrease in triglyceride levels (359). The largest adjuvant aromatase inhibitor study ATAC reported that the patients receiving anastrozole had higher cholesterol levels than those receiving tamoxifen (81). In few studies, letrozole had no effect on serum lipid profiles (83, 360), while in one small study levels of both total and LDL cholesterol increased during letrozole administration (361). In contrast, exemestane has shown no adverse effects on cholesterol levels and seems even to decrease the serum triglycerides in patients with metastatic breast cancer (362).

The effects of two SERMs (tamoxifen and toremifene) and two aromatase inhibitors (anastrozole and exemestane) on serum lipids were compared in an adjuvant study on 180 postmenopausal women with early breast cancer. In line with previous studies, both tamoxifen and toremifene lowered significantly the levels of total and LDL cholesterol, but the effects on HDL cholesterol and triglycerides differed. Tamoxifen lowered and toremifene raised the levels of HDL cholesterol, while triglycerides increased during tamoxifen administration and decreased during toremifene treatment. The aromatase inhibitors anastrozole and exemestane induced modest decreases in total and LDL cholesterol. Anastrozole had no effect on serum HDL cholesterol or triglyceride levels, while exemestane somewhat lowered the levels of both (363).

In conclusion, while all SERMs reduce the total and LDL cholesterol concentrations, the lipid profile seems most favorable with toremifene as it also reduces triglyceride

levels and raises those of HDL cholesterol. The effects of aromatase inhibitors on serum lipids seem modest. Still, all the major adjuvant studies have suggested a somewhat increased, albeit not significant, risk of cardiovascular events during treatment with an aromatase inhibitor as compared to tamoxifen (86, 124, 121).

The effects of adjuvant treatments on serum lipids are summarized in Table 4.

Table 4. Effects of adjuvant treatments on serum lipids

	Chemotherapy	Tamoxifen	Toremifene	Anastrozole	Letrozole	Exemestane
Total cholesterol	↑↑*	↓↓	↓↓	−/↑	−/↑	↓
LDL cholesterol	↑↑*	↓↓	↓↓	−/↑	−/↑	↓
HDL cholesterol	↑↑*	−/↓	↑↑	−/↑	−	↓
Triglycerides	−*	↑↑	↓	−/↓	−	↓

↓ decrease (trend), ↓↓ decrease (significant), ↑ increase (trend), ↑↑ increase (significant), − no change, * in chemotherapy-induced amenorrhea

9. AIMS OF THE PRESENT STUDY

This study in premenopausal patients with early breast cancer aimed to investigate:

1. The long-term effects of chemotherapy-induced premature menopause and adjuvant peroral clodronate treatment on bone mineral density (I).

2. The effect of short-term intravenous adjuvant clodronate treatment in the prevention of bone loss related to chemotherapy-induced premature menopause (II).

3. The impact of adjuvant tamoxifen treatment on bone mineral density after adjuvant chemotherapy (III).

4. The effect of adjuvant tamoxifen treatment on serum lipids after adjuvant chemotherapy (IV).

10. PATIENTS AND METHODS (I-IV)

10.1 Patients

Two separate populations of premenopausal patients with early breast cancer were studied. The earlier population (named MACLO) comprised 148 node-positive patients treated with adjuvant chemotherapy and randomized to oral clodronate for three years or controls (I). The later population (named MACLOT) included 159 patients with either node-positive disease or node-negative disease with other risk factors. After adjuvant chemotherapy, five-year tamoxifen was started to hormone receptor-positive patients (II-IV). In addition, the first 48 patients were randomly allocated to receive intermittent intravenous clodronate treatment or no further therapy. The patients were treated at the Helsinki University Hospital, Department of Oncology. Informed consent was obtained from all participants. The Local Ethical Committee approved the studies.

The patients included were ≤50 years of age. Premenopausal status at entry was defined as women with regular menstruation or last menstrual cycle within three months and serum follicle stimulating hormone (FSH) levels <30 IU/l (I) or as ongoing menstruation during the last six months (II-IV). The MACLO population included three hysterctomized women with premenopausal FSH levels (I), but hysterectomized subjects were excluded from the MACLOT population (II-IV). Patients with a Karnofsky performance index below 70%, other malignancies, peptic ulcer, serum creatinine above 150 umol/l, pregnancy, osteoporosis, medication affecting bone metabolism or untreated hypo- or hyperthyroidism were excluded.

The patients had undergone surgery with axillary evacuation and total mastectomy or breast-conserving resection. Postoperative radiotherapy was given to those treated with breast conserving surgery and to those diagnosed with axillary lymph node metastases. The patients in the MACLO population were treated with six cycles of CMF chemotherapy (cyclophosphamide 600 mg/m², methotrexate 40 mg/m² and 5-fluorouracil 600 mg/m²) intravenously on day one at three weeks intervals. In addition, the patients were randomized to receive or not to receive oral clodronate (Bonefos®, Leiras) 1600 mg daily for three years (I). Patients of the MACLOT

population received either CMF or CEF (cyclophosphamide 600 mg/m², epirubicin 60 mg/m²and 5-fluorouracil 600 mg/m²) chemotherapy and adjuvant five-year tamoxifen was started to those with hormone-receptor positive tumors after chemotherapy. In addition, 48 patients from the MACLOT population were randomly allocated to receive 1500 mg clodronate intravenously before each chemotherapy infusion or no further therapy.

Patients who developed metastatic disease during follow-up were excluded from the analyses. Those lost for follow-up or having medication other than prescribed according to study protocol affecting bone (I-III) or lipid metabolism (IV) were also excluded. Patients having received intravenous clodronate (II) were excluded from the study examining the effect of tamoxifen on BMD (III) but were included in the study on the effects of tamoxifen on lipid metabolism (IV).

10.2 Methods

10.2.1 Clinical investigation and menopausal status

At entry, all patients underwent clinical examination, liver ultrasound, chest x-ray and bone scintigraphy to rule out hematogenic metastases. Basic laboratory tests before randomization included a complete blood count and sedimentation rate, liver enzymes (transaminase, AFOS, 5-nucleotidase), serum creatinine, calcium and electrolytes. During the follow-up visits, the patients were clinically investigated and interviewed regarding menstrual status, medications and other diseases at one, two, three and five years (I) or at six months, one and three years thereafter (II-IV). Bone scintigraphy (I) and measurements of serum FSH, luteinizing hormone (LH) and estradiol were repeated before treatment and at one, two, three and five years (I) or before treatment and at three, six and 9 months and at one and three years (II-IV).

The menstrual status of the patients was defined during each follow-up visit as part of the interview. Amenorrhea was defined as absent menstruation for at least six months.

10.2.2 Dual energy X-ray absorptiometry (DXA)

BMD (grams per square centimeter) was measured by DXA using a Hologic QDR-densitometer (Hologic, Inc., Waltham, MA). BMD was measured in the lumbar vertebrae (L1-4) and the femoral neck, femoral trochanter and Ward´s triangle, intertrochanteric and total femoral area in the right femoral area, both before initiation of chemotherapy, and at one, two, three and five years (I) or at six months, one and three years thereafter (II-III).

10.2.3 Radioimmunoassays for bone markers PINP and ICTP

For the 48 patients randomized to intermittent intravenous clodronate parallel to chemotherapy or to controls, the biochemical measurements (radioimmunoassays) of the bone turnover markers PINP and ICTP were performed at the beginning of chemotherapy and at three, six, 9 and 12 months thereafter (II). The serum samples were stored at –20º C. The reference interval of PINP for adult women is 19-84 ug/l and that of ICTP is 1.7-4.6 ug/l. The methods for the PINP and ICTP assays have been described elsewhere (179, 364).

10.2.4 Assays of serum lipid levels

Fasting serum levels of total, LDL and HDL cholesterol and triglycerides were measured before chemotherapy and at three, six, 9 and 12 months thereafter (IV). The serum cholesterol level was determined with an enzymatic colorimetric CHOD-PAP method and the triglyceride level with an enzymatic colorimetric GPO-PAP method (Roche Diagnostics). The concentration of HDL cholesterol was measured by an enzymatic HDL-C plus 2nd generation method (Roche Diagnostics). The equipment used to measure serum cholesterol, HDL-cholesterol and triglyceride levels was a Hitachi 917 or Modular analysator (Hitachi Ltd, Tokio, Japan). LDL cholesterol was calculated according to Friedewald equation (LDL-cholesterol = cholesterol - HDL-cholesterol - Trigly / 2.2) (365).

10.2.5 Statistical methods

All statistical analyses were performed with the program SPSS for Macintosh. BMD values are presented as percentages of the baseline value (I-III). The repeated measurements ANOVA model was used to test the effect of the treatments (chemotherapy, clodronate and tamoxifen) and menstrual status on BMD. Other comparisons were performed using the Mann-Whitney test or Wilcoxon matched pair test. Ninety-five percent confidence intervals were calculated for the main outcome measures.

Changes in the collagen metabolites were analyzed as the logarithmic transform of the PINP and ICTP levels divided by the baseline level. The logarithmic transformations were used because the collagen metabolites were non-normally distributed. The correlations between bone marker levels and the BMD were assessed using Spearman's rank-order correlation coefficient (r) (II).

The Wilcoxon matched pair test was used to compare lipid and hormonal changes within the treatment (tamoxifen/control) and menstrual groups. The repeated measurements ANOVA model was employed to test the effect tamoxifen treatment and menstrual status on serum lipid levels. The correlations between the changes in serum lipids and weight were assessed by Spearman´s rank-order correlation coefficient. Other comparisons were performed using the Mann-Whitney test (IV).

To address the statistical problem of multiple comparisons, the significance level was set at 0.01 in all studies. The information on patients and methods is summarized in Table 5.

Table 5. Patients and methods in Studies I (MACLO) and II-IV (MACLOT)

	MACLO (n=148)		MACLOT (n=159)	
	Study I	**Study II**	**Study III**	**Study IV**
n	73	45	111	146
Follow-up	5 years	1 year	3 years	1 year
Menstrual status: *				
Menstruating	26%	31%	29%	46%
Amenorrheic	74%	69%	71%	53%
Investigation	p.o. CLO → BMD	i.v. CLO →BMD	TAM →BMD	TAM →lipids
Methods	DXA	DXA, bone markers	DXA	serum lipids

CLO = clodronate, i.v. = intravenous , n= number of patients, p.o. = peroral, TAM = tamoxifen, * = at follow-up. All patients were treated with adjuvant chemotherapy.

11. RESULTS

11.1 Chemotherapy, clodronate and tamoxifen treatment: Effects on bone mineral density (I, II, III)

11.1.1 Effect of chemotherapy on bone mineral density (I, II, III)

Adjuvant chemotherapy caused ovarian dysfunction and amenorrhea in the majority of the patients. In the MACLOT population, 69% and 71% of the patients had developed amenorrhea during one and three years of follow-up, respectively (II, III). In the MACLO population with the longest follow-up, 74% of the patients were permanently amenorrheic five years after chemotherapy (I). The risk of amenorrhea was age-dependent: the mean age of the patients at the start of the chemotherapy was 46 and 47 years for those who developed amenorrhea and 37 and 39 years for those who continued to menstruate (III and I, respectively).

Changes in BMD correlated significantly with menstrual function after adjuvant chemotherapy. At three years of follow-up, patients with chemotherapy-induced amenorrhea had lost -9.5% (95% confidence interval of change (95%CI) -12.4% to -6.5%) of their baseline lumbar spine BMD, while those who continued to menstruate had a modest gain of +0.6% (95%CI –1.8% to +3.0%) For the femoral neck, the corresponding BMD losses were -4.9% (95%CI –8.6% to –1.2%) and –1.4% (95%CI –4.0% to +1.2%), respectively (III). At five years of follow-up, a similar significant correlation between BMD changes and menstrual function was observed. Five years after adjuvant chemotherapy, amenorrheic patients had lost –10.4% (95%CI –12.0% to –8.9%) of their baseline lumbar spine BMD values while those with ongoing menstruation had only minimal loss of –1.3% (95%CI –3.5% to +0.9%) (p=0.0001). The corresponding changes at the femoral neck were –5.8% (95%CI –7.5% to –4.0%) and –0.3% (95%CI –3.3 to + 2.7%) (p=0.001). The rate of bone loss in amenorrheic patients decreased from the first few years of rapid bone loss to the fifth year of follow-up (I).

Thus, a marked bone loss occurred in women who developed chemotherapy-induced ovarian failure and early menopause, while those who continued to menstruate despite

the chemotherapy preserved their baseline BMD levels. The bone loss was most marked during the first few years after chemotherapy.

11.1.2 Effect of clodronate on bone mineral density (I, II)

11.1.2.1 Effect of peroral long-term clodronate on bone mineral density (I)

In the MACLO population (n=148), the patients were randomized to oral clodronate for three years or to controls in addition to adjuvant CMF chemotherapy. 73 disease-free patients were included in the five-year follow-up study on peroral clodronate and BMD. After three years of clodronate treatment, the bone loss in the lumbar spine was significantly less than in the controls, –3.0% and –7.4%, respectively (p=0.003), while no significant differences between the treatment groups were found in the femoral neck BMD. At five years, two years after termination of the clodronate treatment, the bone loss in the lumbar spine was still significantly less in clodronate-treated patients compared to controls, -5.8% and –9.7%, respectively (p=0.008). Following discontinuation of the treatment, both the patients previously treated with clodronate and the control patients lost bone mass at similar rates. Thus, no acceleration of bone loss after treatment termination was observed (Figure 1).

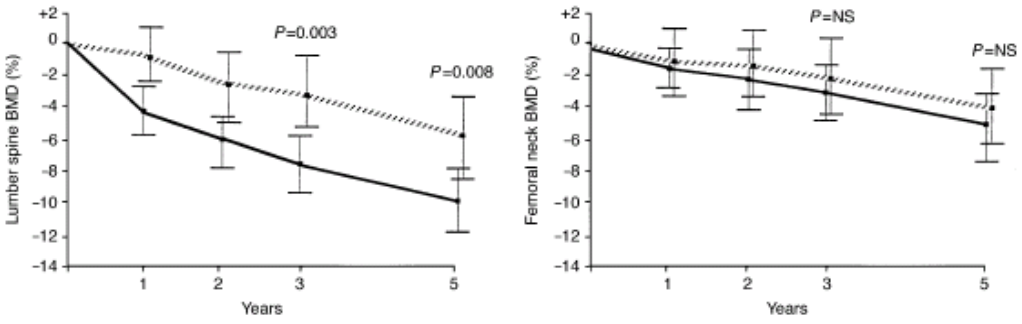

Figure 1. Percentual changes and 95%CIs in lumbar spine and femoral neck BMD in clodronate (dotted line) and control (bold line) groups

At five years, the patients were further divided into those who preserved menstruation and into those who became amenorrheic during follow-up. The beneficial effect of clodronate on BMD was evident in both menstruating and amenorrheic women. The small bone loss of the menstruating women was totally prevented by clodronate. However, the rapid bone loss seen among patients who experienced chemotherapy-

induced ovarian failure was reduced 29-40% but not prevented by clodronate (Figure 2).

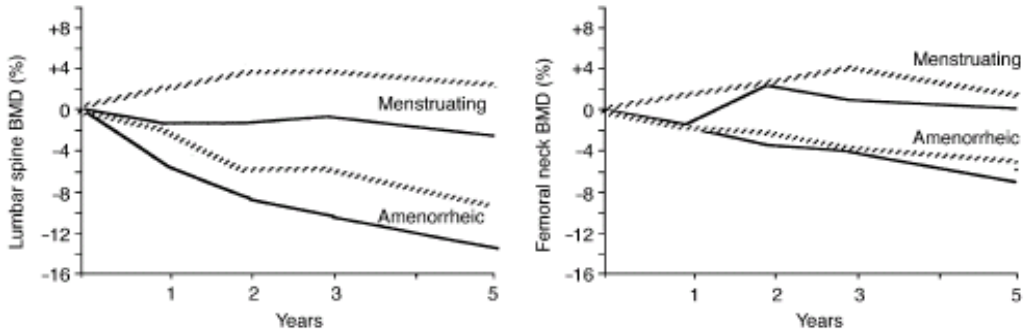

Figure 2. Percentual changes and 95%CIs in lumbar spine and femoral neck BMD according to menstrual status in clodronate (dotted line) and control (bold line) groups

11.1.2.2 Effect of intravenous short-term clodronate on bone mineral density (II)

In the MACLOT population (n=159), the first 48 patients were randomized to intravenous clodronate parallel to adjuvant CMF or CEF chemotherapy. 45 disease-free patients were included in the study on short-term intravenous clodronate on BMD. Chemotherapy caused amenorrhea in 69% of the patients during one year of follow-up. The intermittent, short-term intravenous clodronate did not prevent the bone loss associated with chemotherapy-induced ovarian failure in this small study. At six months, the change in lumbar spine BMD was -0.5% in the clodronate group and -1.4% in the control group (p=0.22), and, in the femoral neck -0.4% and -1.9%, respectively (p=0.37). The bone loss in the lumbar spine at 12 months was -3.9% in the clodronate group and -3.6% in the control group (p=0.62), and, in the femoral neck -1.4% and -2.9% (p=0.43), respectively (Figure 3). While the effect of clodronate treatment on BMD change at 12 months was not significant, a highly significant effect of menopausal status (amenorrhea vs. irregular or regular menstruation) was found in the lumbar spine (p=0.008). In the femoral neck, the effect of menopausal status on BMD was not statistically significant (p=0.31).

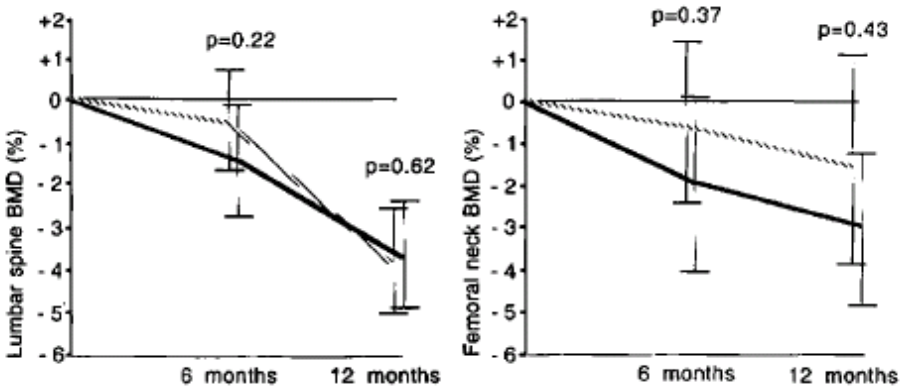

Figure 3. Percentual changes and 95%CIs in lumbar spine and femoral neck BMD in the clodronate (dotted line) and control (bold line) groups

In conclusion, oral clodronate treatment for three years significantly reduced bone loss in the lumbar spine. A four-monthly intermittent intravenous clodronate treatment, on the other hand, did not prevent the bone loss associated with chemotherapy-induced ovarian failure.

11.1.2.3 Effect of short-term intravenous clodronate on bone markers PINP and ICTP (II)

Although intravenous intermittent four-monthly clodronate could not prevent the rapid bone loss associated with chemotherapy-induced early menopause, the serum levels of bone turnover marker PINP decreased significantly during clodronate treatment. The median PINP levels at three and six months were significantly lower in the clodronate group than in the control group: at three months 17.5 ug/l (range 11.6 - 59.10 ug/l) and 29.3 ug/l (range 19.8 - 54.0 ug/l), and at six months 22.6 ug/l (range 15.7 - 55.8 ug/l) and 44.0 ug/l (range 12.5 - 91.9 ug/l). Thereafter, at 9 and 12 months, no significant difference between the treatment groups was found. The PINP levels decreased in the patients treated with clodronate while the PINP levels rose in the control group at six months. The mean change in PINP values from randomization to six months was -28.7% and +21.8% (p=0.0003), and from randomization to 12 months +44.9% and +37.7% (p=0.34) in clodronate and control groups, respectively (Figure 4).

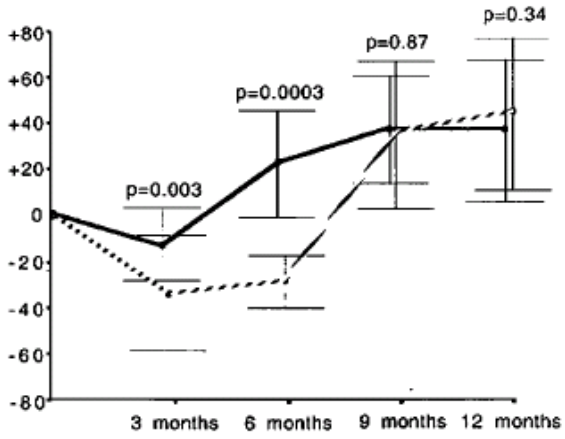

Figure 4. Percentual changes and 95%CIs in PINP values in clodronate (dotted line) and control (bold line) groups

The serum levels of the other bone turnover marker ICTP did not differ between the clodronate and control groups.

In conclusion, serum levels of the bone marker PINP decreased significantly during clodronate treatment reflecting reduced bone turnover.

11.1.3 Effect of tamoxifen on bone mineral density (III)

In the MACLOT population (n=159), adjuvant five-year tamoxifen after CMF or CEF chemotherapy was started to those patients with hormone-receptor-positive tumors (tamoxifen group) while patients with hormone-receptor-negative tumors received no further treatment (control group). 111 disease-free patients were included in the study of tamoxifen and BMD, 88 in the tamoxifen group and 23 in the control group. Again, chemotherapy caused amenorrhea in the majority (71%) of the patients.

Tamoxifen treatment caused a significant bone loss in lumbar spine BMD in premenopausal patients who continued to menstruate three years after adjuvant chemotherapy. At three years of follow-up, the mean bone loss at lumbar spine was -4.6% in the tamoxifen group while a modest gain of +0.6% was noted in the control group (Figure 5). At the femoral neck, tamoxifen-treated women lost –1.8% and women in the control group –1.4% of their baseline BMD values during the three-year observation period.

Tamoxifen treatment reduced bone loss in patients with chemotherapy-induced amenorrhea. At three years of follow-up, women on tamoxifen had lost –6.8% of their baseline lumbar spine BMD while those without tamoxifen had lost –9.5% (Figure 5). At the femoral neck, tamoxifen-treated women lost –3.6% and those without tamoxifen –4.9% of their baseline values.

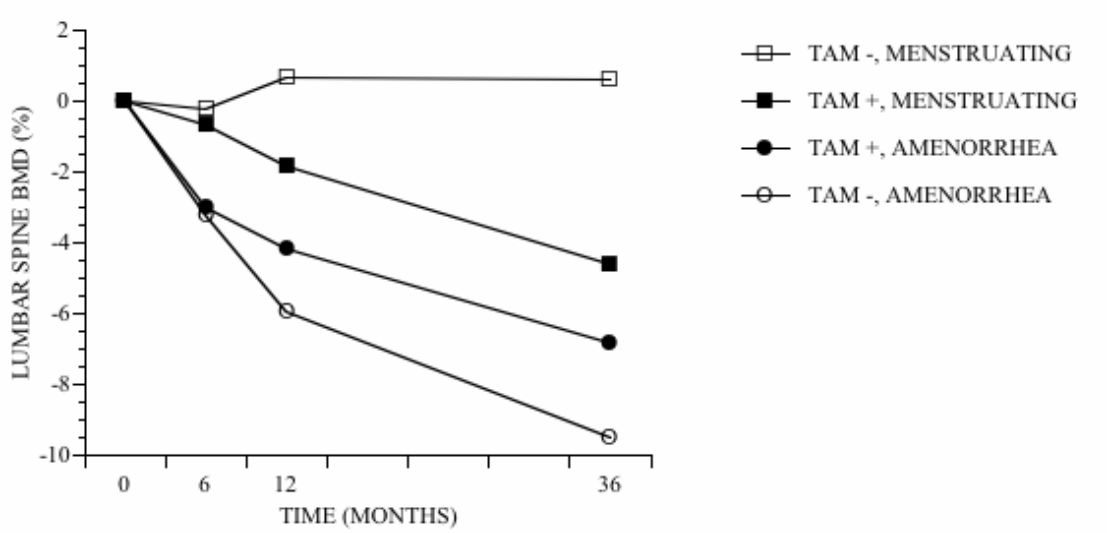

Figure 5. Percentual changes in lumbar spine BMD according to tamoxifen use and menstrual status

The interaction between tamoxifen therapy and menstrual status on lumbar spine BMD changes seen during the three-year follow-up was highly significant ($p<0.0001$). A similar trend towards an interaction was noted between tamoxifen therapy and menstrual status on BMD changes at the femoral neck ($p=0.075$).

In conclusion, tamoxifen had opposite effects on BMD depending on menstrual status. Tamoxifen caused bone loss in patients who continued to menstruate after adjuvant chemotherapy. Contrarily, tamoxifen decreased bone loss in those women who developed chemotherapy-induced amenorrhea.

11.2 Chemotherapy and tamoxifen treatment: Effects on serum lipids (IV)

11.2.1 Effect of chemotherapy on serum lipids (IV)

In the MACLOT population (n=159), all patients received adjuvant CMF or CEF chemotherapy. 146 disease-free patients were included in the study on chemotherapy,

tamoxifen and serum lipids. During one year of follow-up, 53% of the patients had developed amenorrhea, 32% had irregular menstruation and only 14% menstruated regularly. The mean age of the patients at the start of the chemotherapy was 47 years for those who developed amenorrhea, 41 years for those with irregular menstruation and 36 years for those who still menstruated regularly, respectively. The gonadotropin FSH and LH changes during the chemotherapy period correlated with the changes observed in the menstrual cycle.

Changes in total and LDL cholesterol during chemotherapy (0-6 months) correlated significantly with menstrual function. In patients who developed amenorrhea, the total cholesterol increased by +9.5% and the LDL cholesterol by +16.6% ($p<0.00001$ and $p<0.00001$, respectively). The LDL/HDL ratio increased by +21.7% ($p<0.00001$) and the total cholesterol/HDL ratio by +13.3% ($p<0.00001$). The total cholesterol increased by +7.3% and LDL cholesterol by +11.8% in patients with irregular menstruation ($p=0.003$ and $p=0.017$, respectively). The LDL/HDL ratio increased by +14.7% ($p=0.02$) and the cholesterol/HDL ratio +9.4% ($p=0.005$). In patients who still menstruated regularly, the total cholesterol increased only +2.4% and the LDL cholesterol +3.0% ($p=0.52$ and $p=0.57$, respectively). Accordingly, LDL/HDL cholesterol and cholesterol/HDL cholesterol ratios remained unchanged.

The differences in the changes of serum total and LDL cholesterol were insignificant between patients with amenorrhea and irregular menstruation ($p=0.61$ and $p=0.22$, respectively) but the differences in the changes of serum total and LDL cholesterol between patients with regular menses and irregular or absent menstruation (amenorrhea) were more marked ($p= 0.04$ and $p=0.008$). Similarly, the differences in the changes of LDL/HDL ratios and total cholesterol/HDL ratios were insignificant between patients with amenorrhea and irregular menstruation ($p=0.50$ and $p=0.84$), but the differences in the changes of LDL/HDL ratios were significant ($p=0.006$) and of total cholesterol/HDL ratios nearly significant ($p=0.02$) between patients with regular menses and irregular or absent menstruation (amenorrhea). Serum triglyceride levels increased and HDL cholesterol levels slightly decreased regardless of menstrual function and the differences between the groups were statistically insignificant.

In conclusion, changes in total and LDL cholesterol during the chemotherapy correlated significantly with menstrual function. Only those patients who developed signs of ovarian failure had marked elevations in serum total and LDL cholesterol, while no significant changes occurred in those who menstruated regularly.

11.2.2 Effect of tamoxifen on serum lipids (IV)

Six months after the beginning of adjuvant chemotherapy, tamoxifen was started and continued for five years to those 112 patients with hormone-receptor-positive tumors (tamoxifen group) while the 34 patients with hormone-receptor-negative tumors received no further treatment (control group). The serum lipid levels were monitored in both groups during chemotherapy (0-6 months) and during the first six months thereafter (6-12 months).

The total and LDL cholesterol and triglyceride levels increased during chemotherapy in all patients. However, during the first six months of tamoxifen treatment the total and LDL cholesterol decreased even below the pre-chemotherapy levels. In the control group, no significant decrease in the cholesterol levels was seen during the same six months of follow-up. The serum triglyceride remained at an increased level in both tamoxifen and control groups.

Chemotherapy caused an increase of +6.5% and +12.2% in total cholesterol (tamoxifen and control groups, respectively), which was reversed during the following six months in patients on tamoxifen but not in control patients. The total cholesterol decreased –9.7% from the post-chemotherapy levels during the first six months of tamoxifen treatment but only –1.6% in the control group ($p=0.001$). Similarly, chemotherapy increased the LDL cholesterol levels (+11.5% and +18.0% in tamoxifen and control groups, respectively). During the first six months of tamoxifen the LDL cholesterol levels decreased by -16.7% while a decrease of only –1.5% was noted in control patients ($p<0.0001$) (Figure 6). The changes in HDL cholesterol levels were minimal during chemotherapy and tamoxifen treatment had no significant effect on HDL cholesterol. The LDL/HDL cholesterol ratio increased in all patients during chemotherapy, but thereafter, a significant decrease was seen only in patients treated with tamoxifen (Table 6).

Notably already after three months of tamoxifen therapy both total and LDL cholesterol levels had decreased even below the baseline levels measured before the chemotherapy: total cholesterol had decreased -4.6% and LDL cholesterol -8.3% below the baseline levels ($p<0.0001$ and $p<0.0001$, respectively).

Table 6. The effect of tamoxifen (6-12 months) after chemotherapy (0-6 months) on lipid levels.

	Tamoxifen group		Control group	
	0-6 months	**6-12 months**	**0-6months**	**6-12 months**
Total cholesterol	+6.5%	-9.7%*	+12.2%	-1.6%*
LDL	+11.5%	-16.7%**	+18.0%	-1.5%**
HDL	-2.0%	+0.6%	+1.2%	+0.4%
Triglyceride	+22.3%	+8.8%	+32.8%	+0.1%
LDL/HDL	+16.8%	-15.0%*	+18.9%	+0.5%*
Total cholesterol/HDL	+10.5%	-8.4%	+12.0%	-0.6%

Significance of the difference between tamoxifen and control groups: * $p<0.01$, ** $p<0.001$

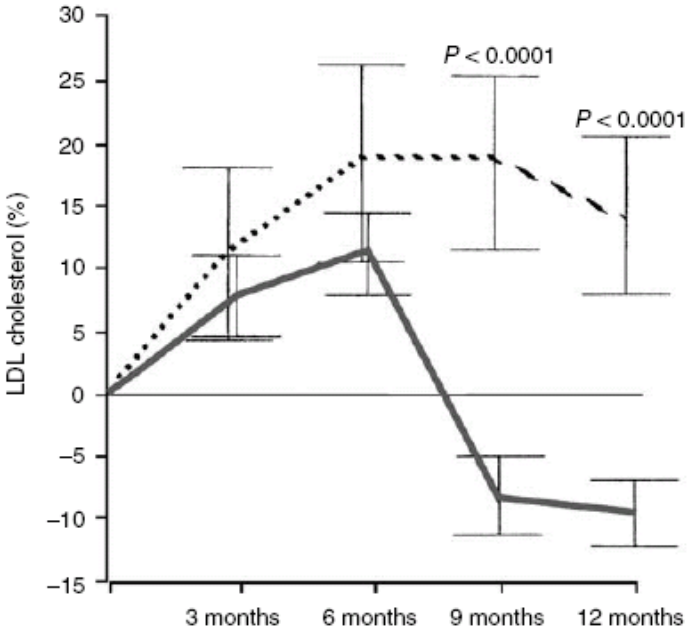

Figure 6. Percentual changes (and 95%CIs) from pre-chemotherapy levels in serum LDL cholesterol in tamoxifen (bold line) and control (dotted line) groups

In conclusion, adjuvant tamoxifen therapy reversed the adverse effects of chemotherapy on total and LDL cholesterol and lowered their serum levels even

below the baseline. The serum HDL cholesterol levels, however, remained unchanged after chemotherapy followed by tamoxifen.

12. DISCUSSION

12.1 Adjuvant chemotherapy and bone mineral density

During our follow-up period of five years, 74% of the premenopausal patients treated with adjuvant chemotherapy developed ovarian failure and amenorrhea. The risk of amenorrhea was age-dependent: the mean age of the patients at the start of the chemotherapy was 47 years for those who developed amenorrhea and 39 years for those who continued to menstruate (I). This is in line with prior findings that women most prone to develop ovarian failure and early menopause after chemotherapy are those 40 years of age or older (11, 193, 194).

In the current study, marked bone loss occurred only in women who developed chemotherapy-induced ovarian failure and early menopause, while those who continued to menstruate preserved their BMD levels. Five years after adjuvant chemotherapy, the bone loss among amenorrheic patients was as high as –10.4% in the lumbar spine while menstruating patients had only minor BMD changes (I). The association between menstrual function and the BMD changes is in accordance with previous studies (26, 195).

The rate of bone loss was greatest during the first few years after chemotherapy-induced menopause and decreased thereafter. While the rate of bone loss observed after natural menopause is slower (366), the rapid bone-losing phase after chemotherapy resembles that seen after surgical oophorectomy and probably reflects the severe estrogen deficiency of iathrogenic menopause. In two earlier studies on the subject, the lumbar spine bone loss has averaged -7% during the first year after chemotherapy-induced ovarian failure (26, 195). In the current studies, the first annual bone loss at lumbar spine was slightly less (-4% to –6%).

Chemotherapy-induced ovarian failure caused rapid and highly significant bone loss especially in the spine. The spine consists mainly of cancellous bone where bone turnover is fast and estrogen deficiency causes rapid bone loss. In the current study, bone loss at the femoral neck was less than that seen in the lumbar spine. This

probably reflects the slower bone turnover of cortical bone tissue found at the femoral neck (367).

The results of the current and earlier studies imply that women who develop chemotherapy-induced ovarian failure undergo significant bone mineral loss most pronounced in the lumbar spine. Thus, long-term breast cancer survivors may be at a higher than average risk for osteoporosis. Women with breast cancer and chemotherapy-induced early menopause should probably have their BMD monitored. Measures such as adequate calcium and D vitamin intake, regular weight-bearing exercice and avoidance of cigarette smoking should be encouraged. If osteoporosis develops, bisphosphonates may seem the most appropriate treatment for women with a history of breast cancer.

12.2 Effect of clodronate on bone loss induced by adjuvant chemotherapy

Oral clodronate for three years significantly reduced bone loss in the lumbar spine. As shown for the first time, still two years after termination of the clodronate treatment, the bone loss in the lumbar spine was significantly less in clodronate-treated patients compared to controls: the bone loss observed in the lumbar spine was –5.8% for patients on clodronate and -9.7% for controls, respectively (I). The bone loss was not accelerated after termination of the clodronate treatment. As most of the patients in both clodronate and control groups experienced early chemotherapy-induced menopause, clodronate could not prevent the rapid bone loss although diminished it. The effect of clodronate at the femoral neck was less marked than in the lumbar spine. This is probably related to the fact that bone turnover is faster in the spine than at the femoral neck (367).

While long-term oral clodronate offered significant protection against bone loss, intermittent, intravenous short-term clodronate did not seem to prevent clinically significantly the bone loss related to chemotherapy-induced ovarian failure. However, a significant reduction of a biochemical bone turnover marker (PINP) was seen during the therapy (II). This suggests that even though the short-term intermittent intravenous clodronate treatment did reduce the bone turnover rate, the duration of the treatment (around four months) was insufficient to lead to long lasting clinically significant

reduction of bone loss. The number of patients included in the study on intravenous clodronate, however, is small and the study may well be underpowered to detect a significant difference between the clodronate and control groups even if there is one.

In our study on intermittent intravenous clodronate, there was a small difference in BMD favouring clodronate during the short treatment period but this was not statistically significant. This may be due to low statistical power or insufficient duration of treatment. It has been suggested that bisphosphonates need to be given for at least six months before an effect on skeletal morbidity is seen (150). Bisphosphonates are ingested by osteoclasts, which subsequently die, removing the drug from the site of active bone resorption. To cover the rapid bone loss seen after chemotherapy-induced ovarian failure, the bone should probably be loaded with bisphosphonates under longer periods than a few months.

The optimal dose or route of clodronate administration for the treatment of osteoporosis is not yet established. Oral doses of 400-800 mg/day given cyclically or continuously have been successfully used in several large studies (29, 239, 240, 368). When comparing the dosing of peroral and intravenous routes, it should be noted that the bioavailability of clodronate is about 2% of the oral dose while around 20-30% of the intravenously administered dose remains in the bone (369). According to this, the intravenous dosage of 1500 mg every three weeks used in our study should compare with an oral dosing of 800 mg/day.

A spectrum of different dosing regimens has been used in studies available on intermittent intravenous clodronate. In women with early postmenopausal bone loss, cyclical 200 mg clodronate given intravenously every month for two years was found to prevent bone loss (31). A long-term cyclical clodronate therapy with 200 mg infusion every three weeks for six years increased BMD significantly and reduced the incidence of vertebral fractures in women with postmenopausal osteoporosis (32). In a small study with patients on long-term parenteral nutrition and osteopenia, 1500 mg clodronate given intravenously every three months for one year significantly inhibited bone resorption as assessed by changes in biochemical markers of bone turnover. However, like in our study cyclic intravenous clodronate therapy failed to increase spinal BMD (370).

Continuous peroral clodronate 400 mg/day has been compared with intermittent intravenous clodronate administered either as an 18-hour infusion of 1800 mg or by separate infusions of 300 mg over six consecutive days every 6 months in postmenopausal women with osteopenia. After two years of treatment, continuous peroral clodronate was found to be significantly more effective than the intermittent intravenous remedies (368).

Current evidence indicates that bisphosphonates are effective in maintaining bone density in women receiving adjuvant therapy for breast cancer (26, 195, 311, 312). Both risedronate and clodronate have been shown to reduce bone loss in patients with chemotherapy-induced early menopause. Intermittent oral risedronate for two years prevented effectively the bone loss observed in the placebo group (311) and peroral clodronate for two years reduced the loss of BMD in patients who received adjuvant chemotherapy and/or tamoxifen (312). A significant bone loss was noted in premenopausal breast cancer patients treated with goserelin plus tamoxifen or goserelin plus anastrozole. When the same combined endocrine treatment was given with intravenous zoledronate every six months, no treatment-induced bone loss was seen (313).

In conclusion, 1600 mg/day of peroral clodronate for three years significantly reduced bone loss in chemotherapy-induced early menopause. On the other hand, 1500 mg of clodronate given intravenously every three weeks for four months did not significantly reduce bone loss in breast cancer patients treated with adjuvant chemotherapy. This may be due to insufficient duration of the clodronate treatment. Bisphosphonate treatment, if given to prevent chemotherapy-induced bone loss, should probably cover the one to two years of most rapid bone turnover. The efficacy of different bisphosphonates has not been compared in this respect. So far, adjuvant bisphosphonate treatment is not standard practice outside clinical trials.

12.3 Effect of intravenous clodronate on bone markers PINP and ICTP

Serum levels of the bone formation marker PINP decreased significantly during clodronate treatment reflecting reduced bone turnover (II). PINP is a marker of bone formation intended to reflect the synthesis of type I collagen (179). While menopause

(181, 182) and osteoporosis (183) increase PINP levels, effective treatment of bone loss and osteoporosis decreases its serum concentrations (182, 185). PINP may be used in clinical practice among other bone turnover markers to help to identify bone loss and to monitor response to antiresorptive treatment such as bisphosphonate therapy.

The serum ICTP levels did not change during clodronate treatment (II). ICTP is a degradation product of mature type I collagen and its serum concentration reflects type I collagen breakdown (186). ICTP has been reported to increase in such pathological conditions as bone metastases and rheumatoid arthritis (187). However, ICTP is rather insensitive to detect changes in bone turnover induced by osteoporosis or antiresorptive treatment (182, 188, 189). As shown recently, ICTP probably reflects the MMP-mediated bone resorption as seen with osteolytic bone metastases, but not the cathepsin K-mediated osteoclastic bone resorption of osteoporosis (371). ICTP does not seem useful in monitoring response to bisphosphonate treatment.

12.4 Effect of tamoxifen after adjuvant chemotherapy on bone mineral density

In the current study tamoxifen treatment after adjuvant chemotherapy had opposite effects on BMD depending on menstrual status. Tamoxifen treatment caused significant bone loss in patients who continued to menstruate after chemotherapy. At three years of follow-up, menstruating patients on tamoxifen had lost –4.6% of their baseline BMD values while a modest gain of +0.6% was noted in the control group. In contrast, tamoxifen reduced bone loss in patients who developed chemotherapy-induced early menopause. In amenorrheic patients the lumbar spine BMD values decreased –6.84% in tamoxifen-users and –9.46% in the controls, respectively (III).

The effects of tamoxifen on BMD are well established in postmenopausal patients. In this group of patients, tamoxifen significantly decreases the loss of BMD in the lumbar spine and to a somewhat lesser degree at the femoral neck (34-38, 200-205). While tamoxifen prevents bone loss in postmenopausal women, the opposite has been suggested for premenopausal women. In a placebo-controlled tamoxifen chemoprevention study, both lumbar spine and femoral neck BMD decreased progressively in tamoxifen users who remained premenopausal throughout the

observation period (38). Similar findings were observed in the ZIPP trial comparing different endocrine approaches in early breast cancer, where a significant decline in total-body bone density was seen in premenopausal patients on tamoxifen (219). In our study, no bone loss occurred in women who continued to menstruate after chemotherapy and who were not given tamoxifen, whereas a significant bone loss was observed in menstruating patients given chemoendocrine therapy with tamoxifen. These findings are in accordance with results of the tamoxifen prevention trial and the ZIPP trial, which also reported bone loss in premenopausal women receiving tamoxifen (38, 219).

Why do the effects of tamoxifen on BMD differ in pre- and postmenopausal patients? Tamoxifen is a SERM with estrogen antagonist or agonist effects dependent on the surrounding physiologic conditions and target tissue. Menopausal status modulates the effect of SERMs. Tamoxifen seems more estrogen antagonist than agonist in premenopausal women while the estrogen agonist properties prevail in postmenopausal women (90). In the presence of premenopausal levels of estrogen, tamoxifen seems to act as an estrogen-antagonist and cause bone loss. This has been suggested also in preclinical studies, where tamoxifen reduced bone mass in rats with intact ovaries (372, 373).

In patients with chemotherapy-induced amenorrhea tamoxifen reduced bone loss but it did not totally prevent the bone loss. As stated before, bone loss in patients who develop amenorrhea after chemotherapy is extremely rapid and comparable to that seen after surgical oophorectomy (24, 26, 195). Even though tamoxifen is effective in preventing bone loss in the later postmenopause, it cannot counteract the sudden and rapid bone turnover seen during chemotherapy-induced perimenopausal transition period.

To conclude, those patients with early breast cancer who continue to menstruate despite chemotherapy and are given adjuvant tamoxifen, seem to be at an increased risk of bone loss as compared to those still menstruating but not on tamoxifen. However, the bone loss is even more marked in patients who develop chemotherapy-induced menopause. In these women, tamoxifen offers some protection against bone loss. Probably most long-term survivors of breast cancer who have received adjuvant

therapy are at increased risk of osteoporosis and bone health intervention should be considered as part of their follow-up.

12.5 Adjuvant chemotherapy and serum lipids

We noted that changes in total and LDL cholesterol during the chemotherapy correlated significantly with menstrual function. Only those patients who developed either amenorrhea or irregular menstruation had marked elevations in serum total and LDL cholesterol, while no significant changes occured in those who continued to menstruate. Serum triglyceride levels increased during chemotherapy regardless of menstrual function while no significant changes in HDL cholesterol were noted (IV).

A few small studies have looked at the effect of chemotherapy on lipid levels with somewhat inconsistent findings (18, 343, 344). In an earlier study on the subject, however, the serum levels of total and LDL cholesterol but also of HDL cholesterol have been shown to increase in patients with chemotherapy-induced ovarian dysfunction (18).

In a premenopausal woman, circulating estrogens decrease the serum levels of LDL cholesterol (and thereby also total cholesterol) by enhancing the clearance of LDL cholesterol from plasma and increase HDL cholesterol levels by reducing hepatic lipase activity that degrades HDL (333). Natural menopause in turn causes changes in serum lipids that are explained by the deficiency of estrogens: serum LDL and total cholesterol levels increase and HDL cholesterol levels decrease. The triglyceride levels also tend to rise during menopause (13-17).

Most patients in the current study developed either amenorrhea or irregular menstruation during the first year post-chemotherapy reflecting estrogen deficiency (IV). Thus, the increase in LDL and total cholesterol and triglycerides noted resembles the changes seen during natural menopause. Although changes in HDL cholesterol were negligible in the current study, the other changes seen in serum lipid profile are considered atherogenic. Consequently, those breast cancer patients who experience early menopause with premature adverse lipid effects may be at an increased risk of coronary heart disease (CHD).

12.6 Effect of tamoxifen after adjuvant chemotherapy on serum lipids

The total and LDL cholesterol and triglyceride levels increased during chemotherapy. However, during the first six months of tamoxifen treatment the total and LDL cholesterol decreased even below the pre-chemotherapy levels. In the control group, no significant decrease in the cholesterol levels was seen during the same six months of follow-up. The serum triglyceride remained at an increased level both in patients treated with tamoxifen and the controls. Tamoxifen treatment had no significant effects on HDL cholesterol.

High levels of total and LDL cholesterol are well-recognized risk factors for atherosclerotic disease, in particular CHD (315). Also, hypertriglyceridemia may be an independent risk factor for cardiovascular disease (323). Plasma levels of HDL cholesterol, on the other hand, are inversely associated with CHD risk in observational studies (319). Tamoxifen has favorable and possibly antiatherogenic effects on lipid metabolism as it reduces the plasma concentrations of total and LDL cholesterol in postmenopausal patients (345). While the levels of total and LDL cholesterol have uniformly decreased in tamoxifen-treated women, the effect of tamoxifen treatment on HDL cholesterol has been minimal. However, tamoxifen may increase the serum triglyceride concentrations (347).

In the present study, adjuvant tamoxifen therapy reversed the adverse effects of chemotherapy on total and LDL cholesterol. Tamoxifen therapy had no effect on HDL cholesterol. Triglyceride levels increased during adjuvant chemotherapy and remained high both in patients on tamoxifen and those with no further therapy. The possible clinical implications of these findings still need to be studied as many factors other than serum cholesterol levels affect the risk of cardiovascular disease. The effects of menopause, estrogen, chemotherapy and tamoxifen (including findings of the present study) on serum lipids are summarized in Table 7.

Table 7. Effects of estrogen, menopause, chemotherapy and tamoxifen on serum lipids

	Estrogen	Menopause	Tamoxifen	Chemotherapy and menopause	Tamoxifen after chemotherapy
Total cholesterol	↓	↑	↓	↑	↓
LDL cholesterol	↓	↑	↓	↑	↓
HDL cholesterol	↑	↓	−	↑/−	−
Triglycerides	↑	↑	↑/−	↑/−	↑

↓ decrease, ↑ increase, − no change

13. CONCLUSIONS

1. Marked bone loss occurred in premenopausal women who developed chemotherapy-induced ovarian failure and early menopause, while those who continued to menstruate preserved their BMD levels. Oral clodronate for three years significantly reduced bone loss associated with ovarian failure. The positive effect of clodronate on BMD was still evident two years after termination of the treatment.

2. Intermittent intravenous clodronate treatment for four months did not prevent the rapid bone loss associated with chemotherapy-induced ovarian failure. However, serum levels of the bone marker PINP decreased significantly during clodronate treatment reflecting reduced bone turnover.

3. Tamoxifen treatment after adjuvant chemotherapy had opposite effects on BMD depending on menstrual status. Tamoxifen caused bone loss in patients who continued to menstruate after adjuvant chemotherapy. Contrarily, tamoxifen decreased bone loss in those women who developed chemotherapy-induced amenorrhea.

4. Changes in serum lipid levels during the chemotherapy correlated significantly with menstrual function. Only patients who developed signs of ovarian failure had marked elevations in serum total and LDL cholesterol. Tamoxifen treatment reversed the adverse effects of chemotherapy-induced ovarian failure on total and LDL cholesterol and even lowered serum levels below the baseline.

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