Bone mineral density loss during adjuvant chemotherapy in pre-menopausal women with early breast cancer: is it dependent on oestrogen deficiency?

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Abstract

Purpose

Pre-menopausal women given adjuvant chemotherapy for breast cancer experience both premature ovarian failure and loss of bone mineral density (BMD), and this study was designed to see if these observations are causally linked.

Methods

Chemotherapy was administered to 41 pre-menopausal women with early breast cancer enrolled prospectively in a study of ovarian function and BMD in such women given systemic therapy. After giving written informed consent, all patients underwent baseline and regular on-treatment measurements of BMD by dual-energy x-ray absorptiometry (DXA) scan, bone turnover and ovarian function by analysis of serum hormone levels and self-reported menstrual diaries.

Results

Baseline lumbar spine BMD in the 41 women given chemotherapy was higher than the normal population (Z score 0.28±0.14 (mean±sem), p=0.047), and fell significantly over the first six months from a mean of 1.05 to 1.01 g/m^2, p <0.0001, and similar but smaller changes were demonstrated in hip BMD. This fall was independent of age at diagnosis, type of chemotherapy, development of amenorrhoea or either baseline or on-treatment estradiol concentration. During the six months after completion of adjuvant chemotherapy, BMD fell further only in those women with low estradiol or experiencing amenorrhoea during the first six months, although all groups showed evidence of increased bone turnover.

Conclusions

This study demonstrates loss of both spine and hip BMD in pre-menopausal women during six months' adjuvant systemic chemotherapy to be independent of changes in ovarian function. Ovarian function was however related to BMD changes after chemotherapy ceased.

Introduction

It has long been recognised that treatment of pre-menopausal women with breast cancer induces loss of bone mineral density (BMD), with a consequent increased fracture risk. There are a number of studies that link the use of adjuvant cyclophosphamide, methotrexate and fluorouracil (CMF), amenorrhoea and loss of BMD [1,2]. For example, Saarto et al [1] showed that CMF induced ovarian failure and rapid bone loss in pre-menopausal breast cancer patients, with women over 40 years at particular risk. BMD changes at 2 years in the lumbar spine and femoral neck were -5.9% and -2.0% respectively. In a longer term CMF study, Vehmanen et al [2] found significant differences in 5-year BMD change between women with preserved menstruation and those who were amenorrhoeic (-1.3% and -10.4% respectively in lumbar spine and -0.3% and -5.8% respectively in the femoral neck). The majority of women with early breast cancer are now offered adjuvant anthracycline and/or taxane-based chemotherapy, which similarly has the potential to induce ovarian failure, but it is unclear whether this is the main or sole cause of the associated bone loss.

It has also been reported by Powles et al that the use of tamoxifen in pre-menopausal healthy women (who remained pre-menopausal for the duration of a 3-year study) caused an annual loss of BMD of 1.44% at the lumbar spine, compared with a small gain of 0.24% for women on placebo [3]. These data were confirmed in a study by Vehmanen et al in breast cancer patients who continued to menstruate following CMF [4]. These patients experienced a loss of 4.6% of baseline BMD value over 3 years, almost certainly because of the partial oestrogen antagonist activity of tamoxifen [4].

A prospective study by Petrek et al [5] used daily bleeding records to assess ovarian function in 595 breast cancer patients aged 20-45. In the month following completion of chemotherapy, approximately 16% patients had monthly bleeding on a standard course of doxorubicin and cyclophosphamide (whether or not followed by paclitaxel or doxetaxel) compared with 48% patients who received CMF. Tamoxifen use decreased bleeding between 12 – 24 months following chemotherapy. This study therefore concluded that age, the specific chemotherapy regimen received and tamoxifen use all impact ovarian function although effects on bone density were not assessed.

As the survival for patients with early breast cancer continues to improve, due to a combination of earlier detection and more effective therapy, the relevance of the long-term effects of adjuvant therapy is being increasingly recognised. Whilst this has been studied in some detail with respect to long-term cardiac toxicity and leukaemogenesis, there have been fewer studies that have looked at the pattern of ovarian function and BMD in pre-menopausal women, and only one of which we are aware that has sought any link between them [6].

In a prospective study of 49 North American pre-menopausal women undergoing adjuvant chemotherapy, Shapiro et al sought factors that were associated with premature loss of ovarian function, as defined by at least 3 months amenorrhoea and elevated serum FSH concentration. They concluded that, in addition to the known risk factor of age at time of chemotherapy, higher baseline BMD predicted for a greater chance of premature ovarian failure, with a 6-fold increase in chemotherapy-induced ovarian failure for every 0.1 g/cm^2 increase in baseline BMD [6].

In contrast, we are not aware of any study that has addressed the reverse question, namely whether loss of ovarian function is the cause of the known loss of BMD seen in pre-menopausal women undergoing adjuvant systemic therapy, and that is the subject of this report.

Materials and Methods

We have previously reported on the ovarian endocrine changes that occurred during systemic therapy for early breast cancer in a cohort of 56 pre-menopausal women who were recruited to a 5-year prospective study from the Edinburgh Breast Unit between March 2001 and April 2003 [7]. The study purpose is to document changes in endocrine function and bone properties during adjuvant systemic therapy for early breast cancer. In summary, there were decreases in ovarian hormone serum concentrations (estradiol, inhibin B, anti-Mullerian hormone) and increases in gonadotrophin concentrations during chemotherapy, and a decrease in ovarian volume and antral follicle count as assessed by ultrasound. These women represent about half of all pre-menopausal women diagnosed with invasive breast cancer in the unit over that period. Patients were excluded from consideration from that study if they had a pre-existing diagnosis of osteoporosis and/or were receiving bisphosphonate medication or prolonged systemic steroids at a dose

equivalent to at least 10mg prednisolone daily. All had operable breast cancer, and reported regular menses in the absence of hormonal contraception. Patients were recruited to the study after giving informed consent, usually before undergoing definitive loco-regional surgery for their breast cancer. Enrolment in the study did not in any way alter pre-operative diagnostic and staging tests, treatment decisions, timing or recruitment to other therapeutic trials.

Full details of the 5-year study will be published separately. This report is confined to the data on the 41 of these women administered systemic chemotherapy, and focuses on the BMD, bone turnover, and menstrual and ovarian function in the first year of follow-up. The emphasis here is that we are reporting data largely before any endocrine therapy.

Bone mineral density

All patients underwent baseline BMD and hormone measurements prior to starting any adjuvant systemic therapy. Lumbar spine and total hip BMD was measured by DXA scan (Hologic QDR 4500A) before any systemic therapy, and at 6 and 12 months [8]. The T-score measure of bone density is defined as the number of standard deviations away from the mean bone density of a young adult with peak bone density, whereas the Z-score is the number of standard deviations away from the mean bone density of a person of the same age, race and gender.

Serum estradiol

Serum estradiol (E2, early follicular phase where appropriate) was measured by radioimmunoassay pre-chemotherapy treatment and at 3 month intervals [7]. A detailed menstrual diary was kept by the patients: amenorrhoea was defined as no menstrual bleeding in the preceding 6 months. Bone marker analyses were carried out on fasting morning serum samples, collected at outpatient attendance at baseline, 6 and 12 months and stored at –20ºC. They were analysed in a randomised order by staff blinded to patient status. Measurements were performed in a single batch for each marker.

Bone turnover biomarkers

The bone formation marker bone specific alkaline phosphatase (Bone-ALP) was determined using the Access Ostase assay (Beckman Access, Beckman Coulter Inc, Fullerton, California, USA). This is a one-step immunoenzymatic assay procedure which

uses a mouse monoclonal antibody specific to Bone-ALP. The intra-assay CV, measured over a concentration range of 7.9 – 86.9 μg/l, was 1.7% - 2.6%.

Serum C-terminal telopeptide of collagen (S-CTX), a marker of bone resorption, was measured using an electrochemiluminescence immunoassay with the Roche serum β-Crosslaps reagent kit, analysed on a Roche Elecsys immunoassay analyser (Roche Diagnostics GmbH, D-68298 Mannheim), with a measuring range from 10 – 6000 ng/l. The intra-assay CV, measured over a concentration range of 80 – 3,600 ng/l was 1.0% - 4.6%.

Statistics

All comparisons of BMD, and serum levels of S-CTX and Bone-ALP were done using Student t-tests in Minitab (version 12.1). Using the Kolmogorov-Smirnov test, of all the variables assessed in this report, only S-CTX had any significant degree of non-normality. Longitudinal changes in BMD, E2, S-CTX and Bone-ALP were analysed by ANOVA with post-hoc Student's t test and proportions of women with amenorrhoea by Fisher's exact test. A Cox regression model was used to determine factors predicting for BMD during this first year, using the following variables: age, weight, type of chemotherapy, use of hormonal therapy, baseline and 6 month levels of E2, Bone-ALP, and S-CTX, and presence or not of menses. There were no formal calculations for sample size as this was an exploratory study and no corrections were made for missing data

Results

Patient demographics

Data are presented on a total of 41 women who were treated with chemotherapy including 3 who received it pre-operatively, and 33 (80%) of these women were treated with adjuvant endocrine therapy after completion of all chemotherapy. No patient received concomitant endocrine therapy with their chemotherapy. Three women were treated with cyclophosphamide/methotrexate/5-fluorouracil, 27 with an anthracycline plus cyclosphosphamide-based regimen, and 11 with anthracycline plus a taxane (Table 1). There was one additional patient on chemotherapy who failed to attend for subsequent DXA scans, and this patient has been omitted from all analyses. Post chemotherapy adjuvant endocrine treatment consisted of tamoxifen alone (n=24), tamoxifen plus

goserelin (n=8), or arimidex (n=1), started after 6-9 months. Median age at study entry was 41 yrs (mean 41, range 29-53), median baseline weight was 65kg (mean 67kg, range 48 – 94). In 37 women the tumor was ER positive, and ER negative/poor in 4.

Baseline lumbar spine BMD was significantly greater than in the DXA scanner reference population (Z score 0.34±0.15 (mean ± sem), p= 0.02). There was no relationship between baseline BMD and weight, body mass index, age or baseline E2 concentration. One woman had a T-score of <-2.5 (ie osteoporosis) at the hip at diagnosis; 8 had a T-score of <-1 (ie osteopenia) at the lumbar spine and 15 at the hip.

Bone mineral density

Mean lumbar spine BMD fell significantly from 1.05 ± 0.02 to 1.01 ± 0.02g/m^2 during the first 6 months of chemotherapy ($p < 0.0001$) and to 1.00 ± 0.02g/m^2 ($p < 0.001$) in the subsequent 6 months (Figure 1). During this time hormonal treatment was initiated in 33 women, with no difference in the later fall between the women who did, and did not receive subsequent hormonal therapy. Indeed, the only patient in whom BMD did not fall during chemotherapy, who was also the heaviest woman at 94Kg, apparently had a 3% rise during chemotherapy and no change thereafter. Total hip BMD also fell over each 6-month period (Figure 1), with broadly similar but smaller changes than those observed in the spine,

In contrast, mean serum E2 was unchanged in the women given chemotherapy over the first 6 months, although the median fell from 321 to 147 pmol/l ($p = 0.04$ by Mann-Whitney). There was a fall in lumbar spine BMD both in those women with E2 below 150pmol/l (the lower limit of the normal range for early follicular phase) at 6 months (n=21, 1.02 ± 0.03 to 0.99 ± 0.03 g/m^2, $p<0.0001$) and in those whose E2 was maintained (n=20, 1.09 ± 0.03 to 1.05 ± 0.03 g/m^2. $p<0.0001$) (Figure 2A and B). In addition, there was no relationship between changes in E2 over the first six months and either changes in the lumbar spine BMD or absolute value at 6 months. Hip BMD also fell in women with low E2 (0.95±0.02 to 0.93±0.02 g/m^2, p=0.001) and those with maintained E2 (0.97±0.03 to 0.96±0.03 g/m^2, p=0.006). However, over the second six months there was a further fall in lumbar spine BMD only in those women with a serum E2 < 150pmol/l (from 0.99 ± 0.03 to 0.95 ± 0.03 g/m^2, $p <0.0001$) with no change in those with maintained pre-menopausal concentrations of E2, where the mean lumbar spine BMD was 1.05 ± 0.03

g/m^2 at both 6 and 12 months (Figure 2A). There was no correlation between the changes in an individual's E2 level and the changes in her lumbar spine BMD, at either 6 or 12 months. In the 14 patients whose E2 level rose during chemotherapy from an average of 313 pmol/l at baseline to 702 pmol/l at 6 months, the average lumbar spine BMD fell over the first six month period from a mean of 1.07 g/m^2 to 1.03 g/m^2 ($p < 0.001$). Equally, in the 11 women whose E2 was higher at 12 months than at baseline (from an average of 345 pmol/l to 1405 pmo/l), average lumbar spine BMD fell from 1.05 g/m^2 to 1.02 g/m^2 ($p = 0.02$). In the one patient who had a rise of lumbar spine BMD during chemotherapy, the E2 level fell from a baseline level of 339 pmol/l to 87 pmol/l at 3 months, and thereafter never rose above 150 pmol/l. Total hip BMD showed a similarly greater change in women with low E2 (-1.4%), compared with -0.63% in those with maintained E2.

16 women maintained or returned to regular menses within 12 months following chemotherapy. Lumbar spine BMD fell in this group in the first 6 months only (during chemotherapy: 1.05 ± 0.03 to 1.02 ± 0.03 g/m^2, $p<0.0001$) (Figure 2C) despite the observation that these women showed no change in E2 at either 3 or 6 months, and an increase thereafter associated with tamoxifen therapy (Figure 3D). 25 women became amenorrhoeic: lumbar spine BMD fell in these women over both the first and second six month periods (1.04 ± 0.03 to 1.00 ± 0.03 to 0.97 ± 0.03 g/m^2, $p < 0.0001$, Figure 2C); in these, E2 also fell within 3 months and remained low (Figure 2D). A very similar pattern of changes was found in hip BMD: this fell only during chemotherapy in those women who maintained menses ((0.96±0.03 to 0.94±0.03 to 0.94±0.03 g/m^2, $p<0.01$ at 6 months vs baseline) but fell during both 6 months periods in those women who became amenorrhoeic (0.96±0.02 to 0.94±0.02 to 0.93±0.02 g/m^2, $p<0.0001$).

There was a clear fall in lumbar spine BMD during chemotherapy in both younger (<40) and older women in the first six months of treatment, (n=18, 1.08 ± 0.02 to 1.05 ± 0.02 and n=23, 1.02 ± 0.03 to 0.98 ± 0.03 g/m^2, respectively, both $p < 0.001$), but a further fall during the second six months was only seen in the 23 women aged 40 and above (to 0.95 ± 0.03 g/m^2, $p < 0.001$), associated with a greatly increased risk of amenorrhoea in these older women (20/23, vs 5/18 in younger group, $p<0.001$).

There was no evidence of differential effects on lumbar spine or hip BMD between anthracycline-CMF and taxane-based chemotherapy regimens (Table 2).

Bone turnover markers

Mean S-CTX and Bone-ALP levels are shown over the first 12 months in Figure 3 and Tables 2 and 3. It is clear that both S-CTX and Bone-ALP rise during and following chemotherapy, also with a rise in Bone-ALP in the second six months in the group of patients administered taxane-based chemotherapy (Table 3). In those women with normal E2 concentrations at 6 months or still menstruating at 12 months, whilst there was a clear rise in both markers over the first 6 months (during the administration of chemotherapy), there was no net change over the second six months (Table 3). However the levels of both markers at 12 months are still higher than at baseline, indicating persistent increased bone turnover in these women a year after commencing adjuvant chemotherapy. Women with low E2 or amenorrhoea at 6 months however showed a continuing rise in Bone-ALP. S-CTX did not change in the first 6 months in women with normal E2, but rose in those with low E2 or who became amenorrhoic at 6 months but showed no change in the second 6 months. There was no relationship between FSH levels, or changes in FSH levels, and either BMD, changes in BMD or the absolute levels or the change in level of either Bone-ALP or S-CTX (data not shown). Changes in an individual's E2 level did not correlate with changes in either Bone-ALP or S-CTX.

A regression model was run to establish which variables might predict lumbar spine BMD at 6 months: only the baseline level of BMD ($p < 0.001$) and the Bone-ALP concentration at 6 months were significant ($p = 0.017$ for change in Bone-ALP and $p < 0.02$ for the absolute level of Bone-ALP). No correlation was seen with S-CTX levels, or changes in S-CTX levels.

Discussion

These are the first data to demonstrate that a fall in both lumbar spine and hip BMD in pre-menopausal women induced by adjuvant chemotherapy occurs independently of the effect of that therapy on ovarian function, as evidenced by systemic E2 levels and menstruation. During the period of chemotherapy (6 months), the reductions in BMD at the lumbar spine and total hip in this study were approximately 4% and 2% respectively. These data are consistent with those of previous studies for pre-menopausal women

receiving adjuvant chemotherapy [1, 9-11]. Although this change is relatively small, given that a reduction of 1 in T-score is approximately equivalent to a 10% reduction in BMD, it may be seen that the direct chemotherapy-induced reduction in BMD could be sufficient to push osteopenic patients into the osteoporotic range and may be clinically significant in terms of increased fracture risk in the longer term in these relatively young women.

It has often been assumed that chemotherapy-induced BMD loss is related to its known damaging effect on ovarian function, and whilst that cannot be excluded in some women, these data clearly demonstrate that the fall in BMD was not significantly greater in women with evidence of loss of ovarian function, as determined by serum levels of E2 and menstrual diary. However once the chemotherapy had been completed, further falls in BMD over the next 6 months were clearly related to ovarian function (which in most cases was influenced by endocrine treatments) and did not occur in women with retained ovarian function, as indicated by either pre-menopausal levels of E2, or normal menstruation being maintained or returning after starting chemotherapy. The thesis that the BMD changes during chemotherapy are predominantly the result of the chemotherapy rather than ovarian function are supported by the observation that changes in an individual's E2 level, irrespective of her menstrual function, did not correlate with changes in her BMD, or her markers of bone metabolism, at either 6 or 12 months.

Many studies have shown that both bone formation markers and bone resorption markers increase with menopause, whether this be natural eg Rosenbrock et al [12], chemical eg Hadji et al [13] or surgical eg Peris et al [14,15]. In these studies, S-CTX increased by 33-100% and Bone-ALP by 37-50%. In the present study, bone resorption and bone synthesis both increased during the 6 months of chemotherapy, as evidenced by the rises in S-CTX of 60% and Bone-ALP of 33%. These findings are therefore consistent with the earlier studies above. We found increases in bone turnover in both the group with persisting E2 levels and in the group with low E2 levels (Table 3), consistent with the reduced BMD in both groups. For CTX, the increase was non-significant in the group with persisting E2 levels and this may reflect some return towards baseline CTX levels after the completion of chemotherapy (often 18 weeks), before the 6 month measurement. However during the second six month period, it is notable that there is no further increase in either marker in women with maintained E2 concentrations, and/or a return of

menstruation by 12 months: a time at which no patient is still receiving chemotherapy. Thus it is clear that the fall in BMD relates to the changes in bone turnover, and when the chemotherapy stops, if normal ovarian function returns, there is no further loss of bone – though little evidence of a reactive increase in synthesis either. Conversely, when there is evidence of lack of E2/ovarian activity following chemotherapy, there is ongoing loss of BMD.

These data are also consistent with a small study of 11 men and women (median age 39) undergoing high-dose chemotherapy, in which mean spine BMD fell from 1.13 to 1.08 g/m^2 over the six months of chemotherapy, but remained static over the subsequent 18 months [16]. That study however, did not relate these changes to possible changes in endocrine function. Similar losses during adjuvant chemotherapy have been previously reported, although the only other prospective study did not link changes to E2 concentrations, only amenorrhoea [10].

In a study of this size, it is possible that there are other confounding factors on BMD changes, such as the amount of exercise taken. This cannot be excluded: but given that a fall in BMD is seen in 40/41 patients irrespective of ovarian function, it is unlikely that differing amounts of exercise taken during chemotherapy are responsible for the lack of any relationship between ovarian function during chemotherapy and changes in BMD. Similarly, this study enrolled a little over half of all eligible patients seen in the Edinburgh breast unit over this period. Whilst one can only speculate the reasons that some women chose not to enrol in this observational study, there was no evidence that those who did consent were in any way unrepresentative of all the women seen: some women expressed reluctance because of the intention to carry out transvaginal ultrasounds as part of the study of ovarian function.

If this loss of BMD is not due to reduced stimulation by estrogen, what could be its aetiology? A recent study suggested that FSH might directly regulate bone mass [17], but despite the changes that occur in FSH in the patients in the present study [7], there was no correlation between either BMD or markers of bone resorption or synthesis and FSH levels. This does not rule out a role for FSH, but suggests that it alone cannot be the explanation and its apparent effects on bone may reflect that it is an indirect marker of ovarian function [18].

A more likely explanation is the known direct cytotoxic effect of chemotherapy agents on bone cells [19]. This has been demonstrated particularly in the growing skeleton [20-21], but there appears to be little data on the mechanisms of the direct toxic effects of chemotherapy agents on bone especially for the newer regimens [22]. There are limited data in experimental animals, eg a demonstration in rats that methotrexate had both short-term (30 days) and long-term (170 days) significant adverse effects on cancellous bone compared to a control group [23], but this study was phenomenological rather than mechanistic.

Supportive medication could be at least in part responsible: all patients in this study given chemotherapy also received as anti-emetic prophylaxis, both HT-3 antagonists (almost always granisetron) and steroids (dexamethasone). Long-term use of oral glucocorticoids is known to induce loss of BMD, and there is evidence that short-term use suppresses bone formation at least in men, as shown in a study of prednisolone 20mg daily for 7 days [24], where osteocalcin and PICP were reduced by 35% and 26% respectively compared with a placebo group though, interestingly, this study found no change in Bone-ALP. Also there was no change in bone resorption (as measured by CTX) in the prednisolone-treated group, in agreement with other studies which showed no change in osteoclasts activity on exposure to corticosteroids [25,26].

Whatever the cause, are there strategies to reverse or prevent this loss? The most obvious candidate to prevent increased bone resorption would be a bisphosphonate, and data from a Lebanese randomised trial suggests that the use of the intravenous bisphosphonate pamidronate every 3 months can reverse the loss of BMD in women developing amenorrhoea during adjuvant chemotherapy, but interestingly, not in those with maintained menstruation [27]. A phase III randomised, placebo-controlled, double-blind, study showed that, in pre-menopausal women undergoing adjuvant chemotherapy for breast cancer treated with the more potent bisphosphonate zoledronic acid (4 mg 3 monthly for 1 year) BMD was stable, whereas women receiving placebo experienced the anticipated bone loss [11]. By contrast, a similar randomised, placebo-controlled, double-blind study by Hines et al [9] in premenopausal women receiving adjuvant chemotherapy found no reduction in bone loss in patients treated with oral risedronate (35 mg weekly) for 1 year. It seems likely that the different conclusions reached in these two recent

studies is due to the intrinsic difference in the potency/treatment schedules of the bisphosphonates used, though subtle differences in the patient populations and their chemotherapy cannot be excluded. Data presented from the Austrian Breast Cancer studies group confirms that intermittent administration of zoledronic acid can reverse the significant loss of bone mineral density in premenopausal women receiving an GnRH agonist [28]. However it is not certain that bisphosphonates will prevent fractures in these relatively young women, although data from post-menopausal women without breast cancer confirms a protective effect [29]. Alternative strategies including non-pharmacological approaches to maintain bone health in pre-menopausal women are also needed.

In conclusion, these data demonstrate that adjuvant systemic chemotherapy has a deleterious effect on BMD in pre-menopausal women with early breast cancer, independent of any effect on ovarian function. Ovarian function was however confirmed to be important in determining further BMD loss after completion of chemotherapy. Given the ever-improving outcome for women with breast cancer, the potential long-term effect of this on fracture risk needs to be considered, and strategies put in place to minimise the loss.

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References

1. Saarto T, Blomqvist C, Valimaki M, Makela P, Sarna S, Elomaa I (1997) Chemical castration induced by adjuvant cyclophosphamide, methotrexate, and fluorouracil chemotherapy causes rapid bone loss that is reduced by clodronate: a randomized study in premenopausal breast cancer patients. J Clin Oncol 15:1341-1347.

2. Vehmanen L, Saarto T, Elomaa I, Makela P, Valimaki M, Blomqvist C (2001) 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 37:2373-2378.

3. Powles TJ, Hickish T, Kanis J (1996) Effect of tamoxifen on bone mineral density measured by dual-energy x-ray absorptiometry in healthy premenopausal and postmenopausal women. J Clin Oncol 14:78-84.

4. Vehmanen L, Elomaa I, Blomqvist C, Saarto T (2006) Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status. J Clin Oncol 24:675-680.

5. Petrek JA, Naughton MJ, Case LD et al (2006) Incidence, time course, and determinants of menstrual bleeding after breast cancer treatment: a prospective study. J Clin Oncol 24: 1045-1051.

6. Shapiro CL, Phillips G, van Poznak CH et al (2005) Baseline bone mineral density of the total lumbar spine may predict for chemotherapy-induced ovarian failure. Breast Cancer Res Treat 90: 41-46.

7. Anderson RA, Themmen AP, Al-Qahtani A, Groome NP, Cameron DA (2006) The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. Human Reproduction 21:2583-2592.

8. Tothill P, Hannan WJ (2007) Precision and accuracy of measuring changes in bone mineral density by dual-energy X-ray absorptiometry. Osteoporosis International18:1515-1523.

9. Hines SL, Mincey BA, Sloan JA et al (2009) Phase III randomised, placebo-controlled, double-blind trial of risedronate for the prevention of bone loss in premenopausal women undergoing chemotherapy for primary breast cancer. J Clin Oncol 27: 1047-1053.

10. Shapiro CL, Manola J, Leboff M (2001) Ovarian failure after adjuvant chemotherapy is associated with rapid bone loss in women with early-stage breast cancer. [see comments]. J Clin Oncol 19: 3306-3311.

11. Hershman DM, McMahon DJ, Crew KD et al. Zoledronic acid prevents bone loss in premenopausal women undergoing adjuvant chemotherapy for early-stage breast cancer. J Clin Oncol 2008;26:4739-45.

12. Rosenbrock H, Seifert-Klauss V, Kaspar S et al (2002). Changes of biochemical bone markers during the menopausal transition. Clin Chem Lab Med 40: 143-151.

13. Hadji P, Ziller M, Maskow C et al (2009). The influence of chemotherapy on bone mineral density, quantitative ultrasonometry and bone turnover in pre-menopausal women with breast cancer. Eur J Cancer 45: 3205-3212.

14. Peris P, Alvarez L, Monegal A et al (2002). Effect of surgical menopause and Paget's disease of bone on the isomerization of type I collagen carboxyterminal telopeptide: evolution after antiresorptive therapy. J Bone Miner Metab 20: 116-120.

15. Peris P, Alvarez L, Monegal A et al (1999). Biochemical markers of bone turnover after surgical menopause and hormone replacement therapy. Bone 25: 349-353.

16. Zekri JM, Brown JE, Gutcher S, Ellis S, Coleman R (2007) Effects of High Dose Chemotherapy and Autologous Peripheral Stem Cells Transplantation on Skeletal

Health. Clinical Oncology (Royal College of Radiologists)16, S27, Abstract P2.01

17. Sun L, Peng Y, Sharrow AC et al (2006) FSH directly regulates bone mass.[see comment]. Cell 125: 247-260.

18. Seibel MJ, Dunstan CR, Zhou H, Allan CM, Handelsman DJ (2006) Sex steroids, not FSH, influence bone mass. Cell 127:1079.

19. Banfi A, Podesta M, Fazzuoli L et al (2001) High-dose chemotherapy shows a dose-dependent toxicity to bone marrow osteoprogenitors: a mechanism for post-bone marrow transplantation osteopenia. Cancer 92: 2419-2428.

20. Van Leeuwen BL, Verkerke GJ, Hartel RM et al (2003) Chemotherapy decreases epiphyseal strength and increases bone fracture risk. Clinical Orthopaedics & Related Research 4 :243-254.

21. Van Leeuwen BL, Kamps WA, Jansen HW, Hoekstra HJ (2000) The effect of chemotherapy on the growing skeleton. Cancer Treat Rev 26: 363-376.

22. Bjarnason NH, Hitz M, Jorgensen NR (2008) Adverse bone effects during pharmacological breast cancer therapy. Acta Oncol 47: 747-754.

23. Wheeler DL, Vander Griend RA, Wronski TJ, Miller GJ, Keith EE, Graves JE (1995) The short- and long-term effects of methotrexate on the rat skeleton. Bone 16: 215-221.

24. Gram J, Junker P, Nielsen HK, Bollerslev J (1998) Effects of Short-Term Treatment With Prednisolone and Calcitriol on Bone and Mineral Metabolism in Normal Men. Bone 23: 297-302.

25. Geusens P, Vanderschueren D, Verstraeten A et al (1991). Short-term course of $1,25(OH)_2D_3$ stimulates osteoblasts but not osteoclasts in osteoporosis and osteoarthritis. Calcif Tissue Int 49: 168-173.

26. Prummel MF, Wiersinga WM, Lips P et al (1991). The course of biochemical parameters of bone turnover during treatment with corticosteroids. J Clin Endocrin Metab 72: 382-386.

27. Fuleihan GE-J, Salamoun M, Mourad YA et al (2005) Pamidronate in the Prevention of Chemotherapy-Induced Bone Loss in Premenopausal Women with Breast Cancer: A Randomized Controlled Trial. J Clin Endocrinol Metab 90: 3209-3214.

28. Gnant MF, Mlineritsch B, Luschin-Ebengreuth G et al (2007) Zoledronic acid prevents cancer treatment-induced bone loss in premenopausal women receiving adjuvant endocrine therapy for hormone-responsive breast cancer: a report from the Austrian Breast and Colorectal Cancer Study Group. J Clin Oncol 25: 820-828.

29. Black DM, Delmas PD, Eastell R et al (2007). Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med 356: 1809-1822.

Figure Captions

Fig. 1

Lumbar spine and total hip BMD in 41 premenopausal women with early breast cancer treated with chemotherapy.

Time 0 is prior to any treatment. Filled symbols, lumbar spine; open symbols, total hip. N=41, mean ± SEM. * $p<0.001$ vs previous measurement.

Fig. 2

BMD in women separated by serum E2 concentrations (A and B) and menstrual function (C and D). Note that 33 women started tamoxifen after 6-9 months.

(A) Lumbar spine BMD (B) serum E2 in women by oestradiol level at 6 months: normal (n=20, red) vs those with E2 concentration < 150 pmol/l at 6 months (n=21, blue). * $p<0.001$ vs previous measurement for BMD. E2 concentrations were significantly different between groups overall ($p<0.0001$) and at each time point after baseline ($p<0.05$). Mean ± sem.
(C) Lumbar spine BMD and (D) serum E2 in women with regular menses at 12 months of treatment (n=16, red) vs those who became amenorrhoeic (n=25, blue). BMD: * $p<0.001$ vs previous measurement. E2 concentrations were significantly different between groups overall ($p<0.0001$) and at each time point after baseline ($p<0.02$). Mean ± sem.

Fig. 3

(A) Bone-ALP and (B) S-CTX in women having chemotherapy (n=41).

Note that 33 women started tamoxifen after 6-9 months. **$p<0.01$, * $p<0.05$ vs previous measurement.

Table 1 Chemotherapy regimens given to patients.

Regimen	Number of women	Duration (weeks)	Number of cycles anthracycline	Cyclophosphamide dose & duration	Number of cycles Taxane
Non-trial					
AC (pre-op)	3	18	6	3 600 mg/m^2 over 18 weeks	0
CMF	3	18	0	4 500 mg/m^2 over 18 weeks	0
A-CMF (Bonadonna)	7	36	4	4 800 mg/m^2 over 24 weeks	0
E-CMF	9	24	4	3 000 mg/m^2 over 12 weeks	0
Trial					
E-CMF (TACT)	8	28	4	4 800 mg/m^2 over 16 weeks	0
FEC-T (TACT)	9	24	4	2 400 mg/m^2 over 12 weeks	4
EC-T (TANGO)	2	24	4	2 400 mg/m^2 over 12 weeks	4

CMF	cyclophosphamide, methotrexate and 5-fluorouracil.
AC	doxorubicin and cyclophosphamide
A	doxorubicin
E	epirubicin
FEC-T	5-fluorouracil, epirubicin and cyclophosphamide, followed by Docetaxel
EC-T	epirubicin, cyclophosphamide followed by paclitaxel
TACT	Taxotere as Adjuvant Chemotherapy trial [20]
TANGO	tAnGo trial (A phase III randomised trial addressing the role of gemcitabine in paclitaxel-containing, epirubicin-based, adjuvant chemotherapy for higher risk, early stage, breast cancer)

Measurement	**Regimen**					
BMD (lumbar spine) g/m^2	AC (pre-op) (N=3)	CMF (N=3)	A-CMF (Bonadonna) (N=7)	E-CMF (inc TACT trial) (N=17)	Taxane based* (N=11)	All (N=41)
Baseline BMD*	1.03 (0.09)	1.08 (0.02)	1.03 (0.07)	1.08 (0.03)	1.01 (0.03)	1.05 (0.02)
6-month BMD*	0.96 (0.08)	1.05 (0.01)	0.99 (0.07)	1.05 (0.04)	0.99 (0.03)	1.01 (0.02)
Mean % BMD change (6 months) (p value)	-6.60 (0.08)	-2.48 (0.2)	-4.06 (0.001)	-3.50 (<0.00001)	-2.70 (0.02)	-3.8 (<0.0001)
12-month BMD*	0.94 (0.06)	1.00 (0.02)	0.99 (0.07)	1.04 (0.03)	0.94 (0.03)	1.00 (0.02)
Mean % BMD change (12 months) (p value)	-8.03(0.09)	-6.65 (0.07)	-4.32 (<0.02)	-4.02 (<.0001)	-7.00 (<0.005)	4.8 (<0.001)

BMD (total hip) g/m^2						
Baseline BMD*	0.91 (0.05)	0.97 (0.02)	0.93 (0.06)	1.00 (0.03)	0.93 (0.03)	0.96 (0.02)
6-month BMD*	0.88 (0.05)	0.95 (0.02)	0.91 (0.06)	0.98 (0.03)	0.91 (0.03)	0.94 (0.02)
Mean % BMD change (6 months) (p value)	-2.56	-1.61	-2.11	-2.00 (0.004)	-1.83 (<0.01)	_1.98 (<0.0001)
12-month BMD*	0.87 (0.05)	0.95 (0.02)	0.91 (0.06)	0.97 (0.03)	0.89 (0.02)	0.93 (0.02)
Mean % BMD change (12 months) (p value)	-4.07	-1.56	-1.85	-2.66 (0.0004)	-4.11 (<0.0001)	-2.93 (<0.0001)
Bone-ALP (μg/ml)						
Baseline Bone-ALP*	6.26 (0.96)	5.41 (0.90)	5.33 (0.71)	5.28 (0.38)	5.10 (0.30)	5.32 (0.23)
6-month Bone-ALP*	6.10 (1.01)	5.63 (0.72)	8.47 (1.31)	6.66 (0.43)	6.67 (0.64)	6.86 (0.35)
Mean % Bone-ALP change (6 months) (p value)	6.1 (0.9)	6.2 (0.9)	70 (0.02)	29 (0.002)	31 (0.01)	33 (<0.0001)
S-CTX (ng/ml)						
Baseline S-CTX*	0.15 (0.02)	0.18 (0.03)	0.20 (0.03)	0.19 (0.03)	0.31 (0.05)	0.18 (0.01)
6-month S-CTX*	0.22 (0.09)	0.27 (0.06)	0.31 (0.09)	0.20 (0.03)	0.31 (0.05)	0..26 (0.02)
Mean % S-CTX change (6 months) (p value)	48 (0.6)	23 (0.8)	75 (0.3)	31 (0.7)	96 (0.02)	60 (0.01)

* Data are mean values (sem)

CMF	cyclophosphamide, methotrexate and 5-fluorouracil.
AC	doxorubicin and cyclophosphamide
A	doxorubicin
E	epirubicin
FEC-T	5-fluorouracil, epirubicin and cyclophosphamide, followed by Docetaxel
EC-T	epirubicin, cyclophosphamide followed by paclitaxel
FEC-T (TACT)	Taxotere as Adjuvant Chemotherapy trial [20]
Or EC-T (TANGO)	tAnGo trial (A phase III randomised trial addressing the role of gemcitabine in paclitaxel-containing, epirubicin-based, adjuvant chemotherapy for higher risk, early stage, breast cancer)

Table 2 – Changes in bone mineral density, bone ALP and S-CTX by regimen

	Patient treatment group							
	Chemo-therapy	Chemo-therapy + hormone therapy	Chemo-therapy alone	Taxane-based	No fall in E2 at 6 months	With fall in E2 at 6 months	Menstruat-ing at 12 months	Amen-orrhoeic at 12 months
Number of women	41	33	8	11	20	21	16	25
Baseline Bone ALP (μg/ml)*	5.36 (0.23)	5.42 (0.26)	5.10 (0.30)	5.10 (0.30))	5.08 (0.28)	5.63 (0.35)	5.24 (0.40)	5.44 (0.28)

Bone ALP at 6 months (μg/ml)*	6.86 (0.35)	7.22 (0.38)	5.37 (0.68)	6.67 (0.64)	6.69 (0.43)	7.01 (0.56)	6.60 (0.49)	7.02 (0.49)
Mean % change in bone ALP at 6 months (p value)	33 (<0.0001)	39 (<0.0001)	48 (n.s.)	31 (0.01)	39 (<0.002)	27 (<0.01)	35 (0.02)	33 (<0.001)
Bone ALP at 12 months (μg/ml)*	7.58 (0.40)	7.78 (0.46)	6.73 (0.78)	8.46 (0.44)	6.74 (0.52)	8.38 (0.57)	6.59 (0.58)	8.21 (0.51)
Mean % change in bone ALP 6 -12 months (p value)	15 (0.03)	9.9 (ns)	35 (ns)	37 (0.02)	1.3 (ns)	28 (0.1)	0.5 (ns)	24 (0.1)
Baseline S-CTX (ng/ml)*	0.16 (0.01)	0.18 (0.02)	0.16 (0.03)	0.17 (0.02)	0.19 (0.03)	0.18 (0.02)	0.18 (0.02)	0.19 (0.02)
S-CTX at 6 months (ng/ml)*	0.26 (0.02)	0.25 (0.02)	0.28 (0.05)	0.31 (0.05)	0.21 (0.03)	0.30 (0.04)	0.21 (0.03)	0.29 (0.04)
Mean % change in S-CTX at 6 months (p value)	60 (0.01)	57 (0.04)	74 (n.s.)	96 (0.02)	45 (n.s.)	75 (<0.01)	41 (n.s.)	73 (0.01)
S-CTX at 12 months (ng/ml)*	0.29 (0.03)	0.28 (0.03)	0.31 (0.09)	0.38 (0.08)	0.25 (0.02)	0.32 (0.05)	0.21 (0.02)	0.34 (0.04)
Mean % change in S-CTX 6 -12 months (p value)	29 (ns)	33 (ns)	13 (ns)	26 (ns)	44 (ns)	16 (ns)	10 (ns)	2 (ns)

* Data are mean values (sem)

Table 3 – Serum bone ALP and S-CTX changes by patient treatment group

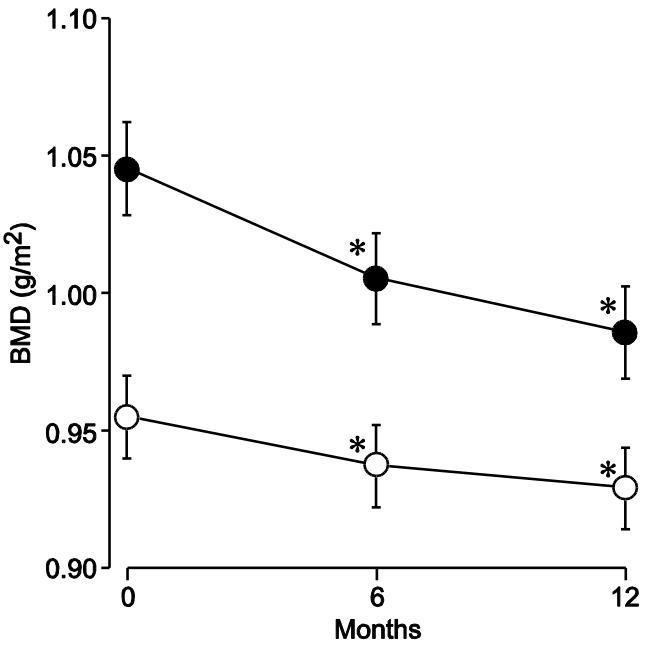

Figure 1

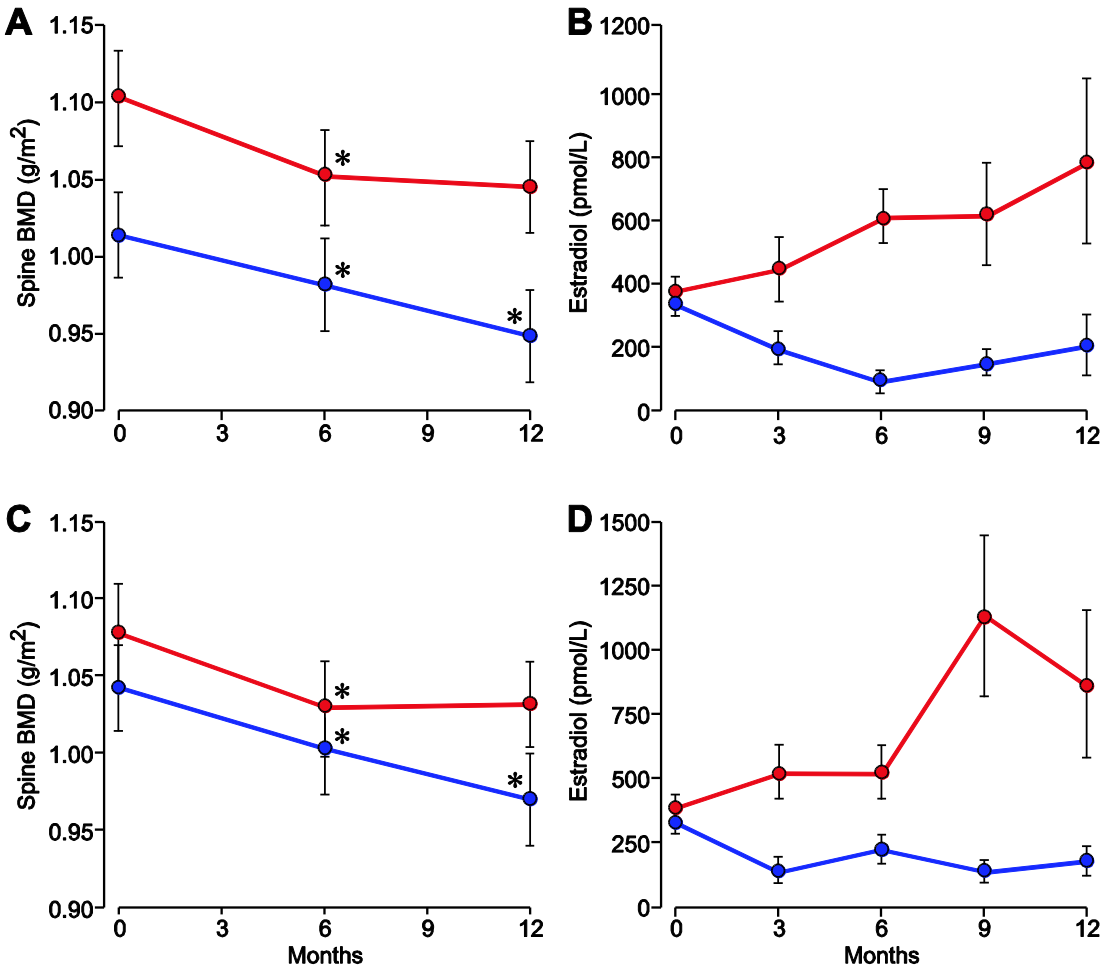
A
Spine BMD (g/m2)
1.15
1.10
1.05
1.00
0.95
0.90
0
3
6
9
12
*
*
*
B
Estradiol (pmol/L)
1200
1000
800
600
400
200
0
C
Spine BMD (g/m2)
Months
D
Estradiol (pmol/L)
1500
1250
1000
750
500
250
Months

Figure 3

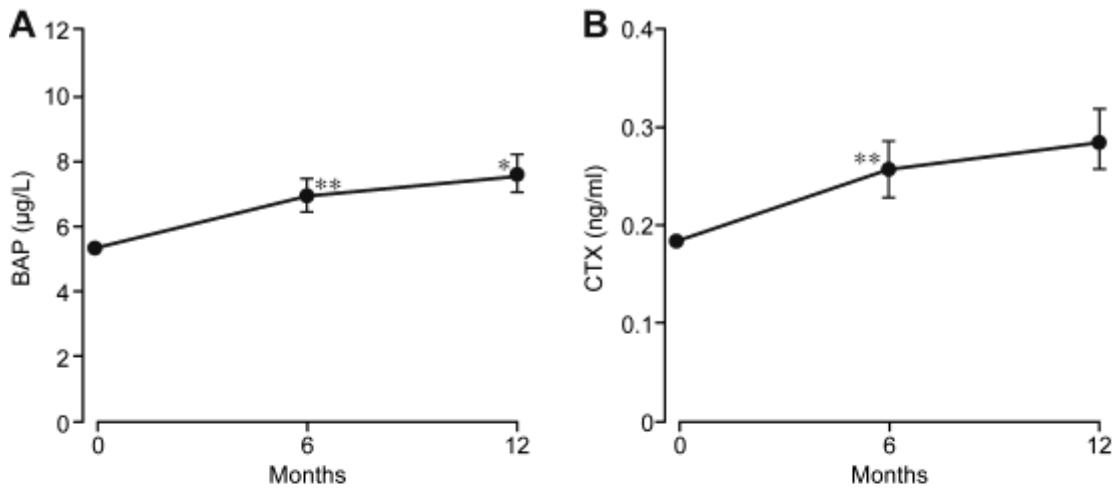
A
BAP (μg/L)
12
10
8
6
4
2
0
**
*
0
6
12
Months
B
CTX (ng/ml)
0.4
0.3
0.2
0.1
0
**
0
6
12
Months

Figure 3