

Serum Bone Markers in Breast Cancer Patients During 5-Fluorouracil, Epirubicin and Cyclophosphamide (FEC) Therapy

MARITA E.H. KILAJÄRVI¹, EEVA K. SALMINEN², OUTI M.M. PAIJA³,
ARJA M. VIRTANEN^{1,4}, AILA E. LEINO¹ and KERTTU A. IRJALA¹

¹TUCH LABORATORIES and ²Department of Oncology, Turku University Central Hospital, POB 52, FIN-20521 Turku;

³Department of Pharmacology and Clinical Pharmacology, University of Turku and Clinical Pharmacology Unit, Turku University Central Hospital, POB 52, FIN-20521 Turku;

⁴Research and Development Center of the Social Insurance Institution, Peltolantie 3, FIN-20720 Turku, Finland

Abstract. *Background:* Chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC) is now indicated for adjuvant therapy of breast cancer. Its effects on serum bone markers and bone metabolism are unclear. *Patients and Methods:* The bone formation marker serum osteocalcin, the bone resorption marker serum carboxyterminal telopeptide of type I collagen (CTx), serum parathyroid hormone (PTH), 25-hydroxyvitamin D (25(OH)D) and calcium concentrations were assessed in nine premenopausal breast cancer patients with no distant metastases at baseline, before the fourth cycle and after the ninth cycle of FEC therapy. All patients became amenorrheic during chemotherapy. *Results:* Individual values of bone markers remained within the reference ranges. The mean concentrations increased slightly. The only significant changes from baseline were observed in serum osteocalcin; concentrations were 17.6 ± 4.9 µg/l (mean \pm SD), 17.5 ± 4.2 µg/l, 22.8 ± 6.4 µg/l ($p=0.003$). Serum CTx concentrations were 998 ± 605 pmol/l, 886 ± 562 pmol/l and 1473 ± 1102 pmol/l at baseline, before the 4th and after the 9th cycle ($p=ns$). Serum 25(OH)D concentrations were all very low (mean concentrations were 26.6 ± 10.1 mmol/l, 29.9 ± 6.5 mmol/l and 27.7 ± 10.6 mmol/l) and remained stable as did mean serum PTH and calcium concentrations. *Conclusion:* The finding of slight increases of the bone markers suggests early bone loss in premenopausal women. The independent effects of estrogen deprivation on bone cannot be separated from the effects of FEC therapy on bone.

In breast cancer, combination chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC) is now indicated not only for treatment of metastatic disease (1,2)

Correspondence to: Eeva Salminen, MD, Department of Oncology, Turku University Hospital, BOP 52, 20521 Turku, Finland. Tel: +358-2-313 2817, Fax: +358-2-313 2809, e-mail: eevsal@utu.fi

Key Words: Breast cancer, chemotherapy, bone metabolism markers, 5-fluorouracil, epirubicin, cyclophosphamide.

but also for adjuvant therapy (3). FEC, like other anthracycline-containing regimens, may prove to be more beneficial in the treatment of breast cancer than the currently more favoured cyclophosphamide, methotrexate and 5-fluorouracil (CMF) chemotherapy (4). Consequently, with the increasing incidence of breast cancer, more women will receive FEC therapy in the future.

With regard to other breast cancer therapies, hormonal therapy with tamoxifen is known to preserve lumbar spine and femoral bone mineral density. This effect, seen in both breast cancer patients and healthy women, is also reflected in serum bone marker concentrations (5-7). The effects of breast cancer chemotherapy on bone metabolism have been studied with biochemical bone markers during anthracycline-containing combination therapy with cyclophosphamide, doxorubicin and 5-fluorouracil (CAF), epirubicin therapy and various other therapies (8-10). In patients without bone metastases, bone metabolism maintained its earlier level during both CAF and epirubicin therapy, as measured by serum carboxyterminal telopeptide of type I collagen (ICTP), a bone resorption marker, and serum bone-specific alkaline phosphatase and osteocalcin, both bone formation markers. However, in patients with bone metastases, these serum bone markers have been reported to increase following both progressive disease and partial responses to therapies (8-10).

In an earlier study, we observed a significant increase of serum parathyroid hormone (PTH) in some breast cancer patients with bone metastases treated with FEC (11). Hypocalcaemia is known to increase PTH secretion, which in turn increases the release of calcium from the bone. However, the increase of PTH was accompanied by only a slight decrease of serum calcium in our study. On the other hand, vitamin D increases the transfer of serum calcium to the bone. In addition, low vitamin D concentration, a risk factor for osteoporosis, is known to increase serum PTH (12,13). Vitamin D was not measured in the earlier study.

Table I. Clinical and histopathological characteristics of the breast cancer patients at study entry.

Patient	Age (years)	TNM stage	Histological grade	Receptor status	WHO PS	Concomitant medication
1	41	T3N1M0	2	Er-Pr-	0	-
2	46	T2N1M0	2	Er-Pr-	1	-
3	46	T3N1M0	2	Er-Pr-	0	-
4	52	T3N1M0	2	Er+Pr-	1	Amitriptyline, diclofenac
5	47	T1N1M0	3	Er-Pr-	1	Paracetamol
6	49	T3N1M0	3	Er-Pr-	0	-
7	44	T3N1M0	2	Er-Pr+	1	Temazepam, ibuprofen, ferrous glycine sulphate
8	47	T2N1M0	3	Er+Pr-	1	-
9	37	T1N2M0	3	Er+Pr+	1	-

Er = estrogen receptor, Pr = progesterone receptor, WHO PS: performance status

The aim of this study was to investigate whether possible changes in bone metabolism are detectable in sera of breast cancer patients during FEC therapy.

Patients and Methods

Patients. The study group consisted of nine premenopausal women who had high-risk breast cancer and no distant metastases and were scheduled for FEC therapy. The inclusion criteria were: age under 60 years, histologically verified and radically operated stage T1-T3 breast cancer, either ≥ 8 metastatic axillary nodes or ≥ 5 metastatic axillary nodes with negative estrogen or progesterone receptors or nuclear grade 2-3 (or histological grade 2-3) and no distant metastases. The patient characteristics and histopathological features of the tumours are presented in Table I.

The FEC regimen consisted of 5-fluorouracil 600 mg/m², epirubicin 60 mg/m² and cyclophosphamide 600 mg/m² given intravenously every three weeks. A total of 8 or 9 cycles were given. Before each cycle, after blood specimens had been collected, the patient was also given standard antiemetic therapy. After chemotherapy, radiotherapy was scheduled and, following radiotherapy, tamoxifen was initiated for 5 years. As shown in Table I, three patients had continuous concomitant medication, which was unchanged during the study period.

The study was conducted at Turku University Central Hospital and approved by the Joint Commission on Ethics of Turku University and Turku University Central Hospital, Finland. Written informed consent was obtained from all the patients.

Methods. Blood samples were collected before initiating chemotherapy, before the 4th cycle and after the 8th or 9th cycle, *i.e.*, after approximately 6 months of FEC therapy. The specimens were stored at -20°C and analysed in one batch. The analyses of serum osteocalcin concentrations were performed on ELSA-OSTEO immunoradiometric assay (CIS Bio International, Gif-sur-Yvette, France). Serum CTx was measured with Serum CrossLaps™ enzyme-linked immunosorbent assay (Osteometer BioTech A/S, Herlev, Denmark). Serum PTH was analysed with the immunoradiometric assay N-tact PTH SP kit (Incstar Co, Stillwater, Minnesota, USA), and serum 25(OH)D with a ¹²⁵I radioimmunoassay kit (DiaSorin, Stillwater, Minnesota, USA). Serum calcium concentrations were measured photometrically with Hitachi 917® (Hitachi, Tokyo, Japan; reagents from Boehringer Mannheim).

Statistical analyses. The statistical analysis of all assessments was performed with an analysis of variance model for repeated measures, with structured covariance matrices. Comparisons between the baseline and follow-up values were made by contrasts with Dunnett-Hsu correction. Logarithmic transformations were applied when appropriate. All statistical calculations were performed with SAS V8 for Windows (SAS Institute, Cary, NC, USA).

Results

The results of individual serum osteocalcin, CTx, PTH and 25(OH)D concentrations at baseline and during treatment are presented in Figure 1. Both osteocalcin and CTx showed a minor increase in mean serum concentrations. The mean concentrations at baseline, before the 4th cycle and after the 8-9th cycle were 17.6±4.9 µg/l (mean±SD), 17.5±4.2 µg/l and 22.8±6.4 µg/l ($p=0.003$, as compared with baseline) for serum osteocalcin and 998±605 pmol/l, 886±562 pmol/l and 1473±1102 pmol/l for serum CTx, respectively (statistically nonsignificant changes). Although some patients had relatively large increases in bone marker concentrations, the values remained well within the reference ranges of premenopausal patients (8-36 mg/l for osteocalcin and 85-4525 pmol/l for CTx). All patients became amenorrheic during FEC therapy.

The mean serum PTH and 25(OH)D remained at the baseline concentrations (PTH concentrations were 14.3±5.9 ng/l, 11.1±8.9 ng/l and 14.4±5.6 ng/l and 25(OH)D concentrations were 26.6±10.1 mmol/l, 29.9±6.5 mmol/l and 27.7±10.6 mmol/l at consecutive time points, $p=ns$). The concentrations of serum 25(OH)D were low: none of the patients had 25(OH)D concentrations consistently ≥ 37 nmol/l, and five patients had 25(OH)D concentrations always below 37 nmol/l. The mean concentrations of serum calcium remained at baseline (2.32±0.06 mmol/l, 2.30±0.09 mmol/l, 2.33±0.05 mmol/l).

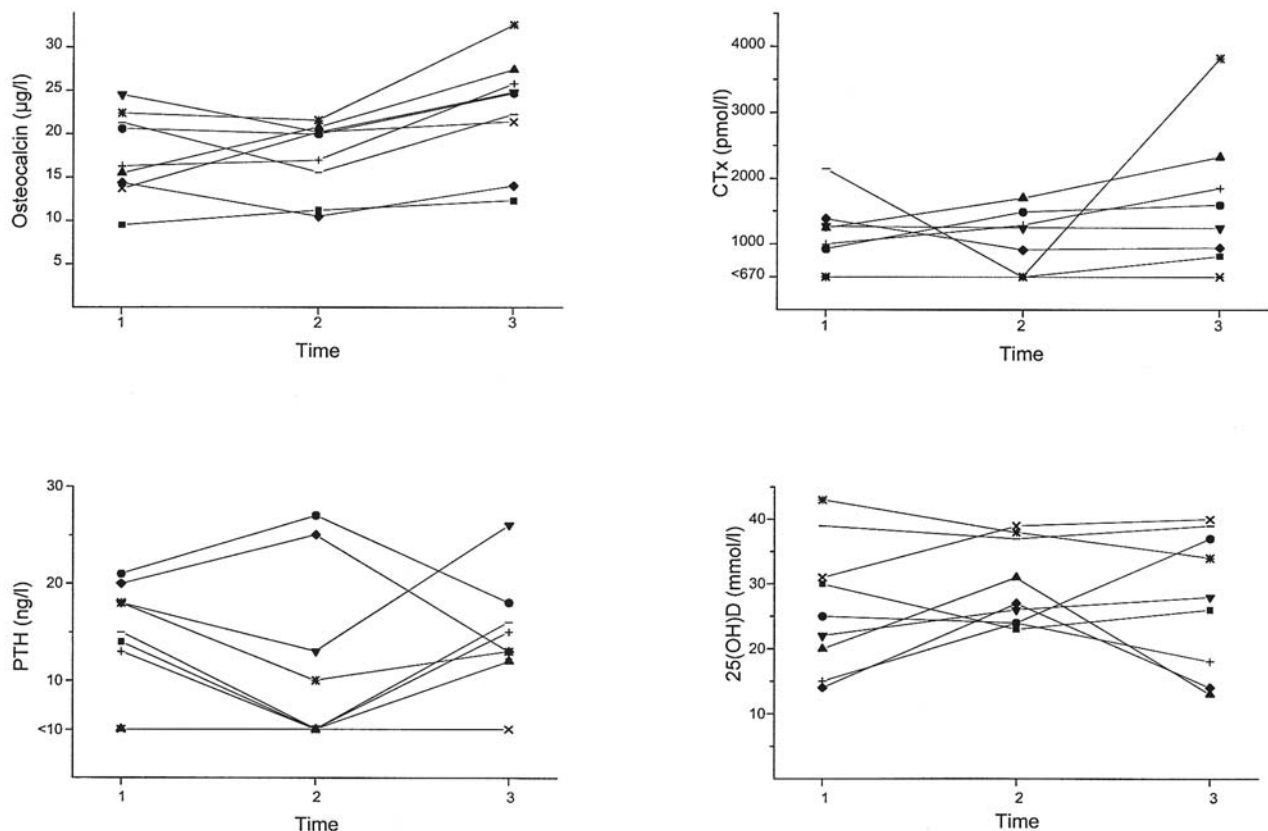


Figure 1. Results of serum osteocalcin, carboxyterminal telopeptide of type I collagen 'CrossLaps' (CTx), parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) in breast cancer patients during and after 5-fluorouracil, epirubicin and cyclophosphamide (FEC) therapy. (1 = at baseline, 2 = before 4th cycle, 3 = after 8th or 9th cycle, cycles repeated every 21 days).

Discussion

In the present study, serum osteocalcin concentrations increased significantly during the 6-month FEC treatment. The slight increase in CTx concentrations was not statistically significant. All the individual values remained well within the reference ranges. In addition, the changes occurred in low concentrations susceptible to analytical difficulties (14).

Chemotherapy-induced amenorrhea is known to cause relatively rapid bone loss in breast cancer patients (15-18). In premenopausal patients with chemotherapy-induced ovarian failure, decreased bone mineral density, as measured by dual-energy X-ray absorptiometry (DXA) and serum aminoterminal propeptide of type I procollagen (PINP), serum osteocalcin concentrations and bone-specific alkaline phosphatase activities, have been reported as early as 6 and 12 months after commencing chemotherapy (16-18). In our study, all patients became amenorrheic and therefore the independent effects of estrogen deprivation on bone cannot be separated from the effects of FEC therapy on bone.

Breast cancer patients with bone metastases seem to have increased serum osteocalcin and urinary CTx concentrations (19,20). In an earlier study with breast cancer patients without bone metastases, no changes were reported in serum osteocalcin concentrations during an anthracycline (epirubicin) therapy (9). Similarly, we did not observe major changes in serum CTx and osteocalcin concentrations in breast cancer patients without bone metastases during FEC therapy. The analytical and biological variations of bone markers should be taken into account when evaluating the use of markers in detection of skeletal changes (14, 21).

We earlier observed a significant increase of PTH in some patients before the 3rd and 5th cycle of FEC, as compared with baseline (11). In the present study, serum PTH concentrations remained stable. The patients in our previous study had bone metastases, which may have affected the results.

The serum 25(OH)D concentrations were low even though the summer months of June, July and August were included: none of the patients had concentrations consistently over 37 nmol/l and five patients had 25(OH)D concentrations always below 37 nmol/l, the level considered minimally adequate to

maintain bone mineral density (12). Seasonal variation was not evident, which is consistent with an earlier study in postmenopausal women (22) but inconsistent with a study in 9- to 15-year-old girls (23). The low serum concentrations are in line with the recent evidence of common hypovitaminosis D in the Finnish population (23,24).

In conclusion, adjuvant FEC therapy appears to slightly increase serum osteocalcin concentrations in breast cancer patients. However, the therapy frequently causes amenorrhea, the effect of which cannot be separated from the effect of FEC therapy on bone.

Acknowledgements

This study was supported by the Cancer Society of Finland.

References

- 1 A prospective randomized phase III trial comparing combination chemotherapy with cyclophosphamide, fluorouracil, and either doxorubicin or epirubicin. French Epirubicin Study Group. *J Clin Oncol* 6: 679-688, 1998.
- 2 Phase III randomized study of fluorouracil, epirubicin, and cyclophosphamide v fluorouracil, doxorubicin, and cyclophosphamide in advanced breast cancer: an Italian multicentre trial. Italian Multicentre Breast Study with Epirubicin. *J Clin Oncol* 6: 976-982, 1988.
- 3 Coombes RC, Bliss JM, Wils J *et al*: Adjuvant cyclophosphamide, methotrexate, and fluorouracil versus fluorouracil, epirubicin, and cyclophosphamide chemotherapy in premenopausal women with axillary node-positive operable breast cancer: results of a randomized trial. The International Collaborative Cancer Group. *J Clin Oncol* 14: 35-45, 1996.
- 4 Polychemotherapy for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet* 352: 930-942, 1998.
- 5 Kenny AM, Prestwood KM, Pilbeam CC and Raisz LG: The short term effects of tamoxifen on bone turnover in older women. *J Clin Endocrinol Metab* 80: 3287-3291, 1995.
- 6 Love RR, Barden HS, Mazess RB, Epstein S and Chappell RJ: Effect of tamoxifen on lumbar spine bone mineral density in postmenopausal women after 5 years. *Arch Intern Med* 154: 2585-2588, 1994.
- 7 Ward RL, Morgan G, Dalley D and Kelly PJ: Tamoxifen reduces bone turnover and prevents lumbar spine and proximal femoral bone loss in early postmenopausal women. *Bone Miner* 22: 87-94, 1993.
- 8 Koizumi M, Matsumoto S, Takahashi S, Yamashita T and Ogata E: Bone metabolic markers in the evaluation of bone scan flare phenomenon in bone metastases of breast cancer. *Clin Nucl Med* 24: 15-20, 1999.
- 9 Neri B, Cecchetti M, Pacini P, Bartalucci S, Gemelli MT and Giorgi F: Osteocalcin as a biological marker in the therapeutic management of breast cancer bone metastases. *Cancer Invest* 7: 551-555, 1989.
- 10 Coleman RE, Whitaker KB, Moss DW, Mashiter G, Fogelman I and Rubens RD: Biochemical prediction of response of bone metastases to treatment. *Br J Cancer* 58: 205-210, 1988.
- 11 Kailajärvi M, Ahokoski O, Virtanen A, Salminen E and Irjala K: Early effects of 5-fluorouracil, epirubicin and cyclophosphamide therapy on common laboratory tests. *Anticancer Res* 21: 2867-7282, 2001.
- 12 Thomas MK, Lloyd-Jones DM, Thadhani RI *et al*: Hypovitaminosis D in medical inpatients. *N Engl J Med* 338: 777-783, 1998.
- 13 Guillemant J, Taupin P, Le HT *et al*: Vitamin D status during puberty in French healthy male adolescents. *Osteoporos Int* 10: 222-225, 1999.
- 14 Watts NB: Clinical utility of biochemical markers of bone remodeling. *Clin Chem* 45: 1359-1368, 1999.
- 15 Bruning PF, Pit MJ, de Jong-Bakker M, van den Ende A, Hart A and van Enk A: Bone mineral density after adjuvant chemotherapy for premenopausal breast cancer. *Br J Cancer* 61: 308-310, 1990.
- 16 Saarto T, Blomqvist C, Välimäki M, Mäkelä P, Sarna S and Elomaa I: 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, 1997.
- 17 Shapiro CL, Manola J and Leboff M: Ovarian failure after adjuvant chemotherapy is associated with rapid bone loss in women with early-stage breast cancer. *J Clin Oncol* 19: 3306-3311, 2001.
- 18 Saarto T, Blomqvist C, Risteli J, Risteli L, Sarna S and Elomaa I: Aminoterminal propeptide of type I procollagen (PINP) correlates to bone loss and predicts the efficacy of antiresorptive therapy in pre- and post-menopausal non-metastatic breast cancer patients. *Br J Cancer* 78: 240-245, 1998.
- 19 Coleman RE, Mashiter G, Fogelman I *et al*: Osteocalcin: a potential marker of metastatic bone disease and response to treatment. *Eur J Cancer Clin Oncol* 24: 1211-1217, 1988.
- 20 Berruti A, Piovesan A, Torta M *et al*: Biochemical evaluation of bone turnover in cancer patients with bone metastases: relationship with radiograph appearances and disease extension. *Br J Cancer* 73: 1581-1587, 1996.
- 21 Christgau S, Bitsch-Jensen O, Hanover Bjarnason N *et al*: Serum CrossLaps for monitoring the response in individuals undergoing antiresorptive therapy. *Bone* 26: 505-511, 2000.
- 22 Villareal DT, Civitelli R, Chines A and Avioli LV: Subclinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. *J Clin Endocrinol Metab* 72: 628-634, 1991.
- 23 Lehtonen-Veromaa M, Möttönen T, Irjala K *et al*: Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. *Eur J Clin Nutr* 53: 746-751, 1999.
- 24 Kauppinen-Mäkelin R, Tähtelä R, Löyttyniemi E, Kärkkäinen J and Välimäki MJ: A high prevalence of hypovitaminosis D in Finnish medical in- and outpatients. *J Intern Med* 249: 559-563, 2001.

Received November 7, 2003

Accepted February 5, 2004