SHORT REPORT

Serum Testosterone Levels and Breast Cancer Recurrence

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Prospective studies show that high serum levels of androgens and estrogens are associated with increased incidence of postmenopausal breast cancer. The aim of the present analysis was to study the prognostic value of serum testosterone, estradiol and related factors in postmenopausal breast cancer patients. One hundred and ten patients without clinical recurrence were included in the study. After 5.5 years of follow-up, 31 patients developed distant metastasis (16), local relapse (4), or contralateral breast cancer (11). The risk of adverse events in relation to hormone level was examined by Cox' proportional hazard modeling, adjusting for hormone receptor status and stage at diagnosis. Body mass index and serum levels of testosterone, estradiol and glucose were significantly higher in patients who recurred than those who did not. The hazard ratios were 1.8 (95% CI = 0.5–6.3) for the middle and 7.2 (95% CI = 2.4–21.4) for the upper tertiles of baseline testosterone distribution. Other hormones had only minor influence on prognosis. High testosterone predicts breast cancer recurrence. Further studies are required to determine whether dietary or other medical intervention to reduce testosterone can reduce the recurrence of breast cancer.

Key words: *breast cancer; recurrences; testosterone*

Prospective cohort studies on healthy volunteers who donated a blood sample at recruitment have shown beyond reasonable doubt that, after menopause, women with high serum levels of steroid sex hormones (both androgens and estrogens) are at increased risk of subsequent breast cancer. Testosterone and estradiol have similar predictive values, with relative risks of the order of 2–3 for women in the highest quintile compared to those in the lowest quintile of the hormone concentration.[1] Several anthropometric and metabolic determinants of high sex hormone availability have also been found associated with breast cancer risk, including obesity, especially abdominal obesity,[2] low serum levels of sex hormone-binding globulin (SHBG),[1] high levels of insulin,[3] fasting glucose and bioavailable insulin-like growth factor-I (IGF-I).[4] Oophorectomy and antiestrogenic treatment reduce breast cancer incidence[5,6] and the incidence of recurrence in breast cancer patients.[7,8] Several studies have also suggested that overweight,[2,9] weight gain during adjuvant treatment,[10–12] hyperinsulinemia[13] and increased androgenic activity[14–16] are associated with increased breast cancer recurrences. In the present study, we followed up women enrolled in a previous dietary intervention trial aimed at reducing sex hormone levels in postmenopausal breast cancer patients[17] to examine the relationship between serum hormone levels and cancer recurrence.

Material and methods

One hundred and fifteen postmenopausal women who were operated for breast cancer at least a year previously, not undergoing chemotherapy and with no clinical evidence of disease recurrence volunteered to participate in Diet and Androgens Trial-2 (DIANA-2), a dietary intervention study that required radical modification of the usual diet over a year and the collection of blood samples for hormone measurements as well as anthropometric measurements at the beginning and end of the intervention year. Participants were recruited with the help of breast cancer patients'

associations and advertising in the media and senology departments of hospitals in the Milan area (northern Italy). Patients had to be postmenopausal for at least 2 years, not on a special diet, not undergoing treatment for metabolic or endocrine disease and have at least one ovary. All patients signed an informed consent and the study was approved by the Institutional Review Board and Ethical Committee of the Milan National Cancer Institute. The results of the trial on the reduction of hormone levels have been published elsewhere.[17] In the present analysis, 5 women were excluded either because of premenopausal hormone profile or because metastases were detected at baseline examination or soon afterward. Analysis of the predictive value of baseline hormonal levels on subsequent breast cancer recurrence was therefore carried out on 110 women of age 56.8 ± 5.6 years. In this group, the mean time between breast cancer diagnosis and recruitment to the study was 4.6 ± 4.4 years. Forty-two women (38%) receiving tamoxifen treatment at enrolment continued this treatment during the intervention year. At diagnosis, 73 cancers were less than 2 cm (pT1), 63 were node-negative, 62 were classified as positive for estrogen receptors, 51 as positive for progesterone receptors, with 8 not examined for receptor status. The pathology report could not be traced in 8 patients for whom disease stage at diagnosis was classified as unknown. Three patients could not be reexamined a year after recruitment, so only 107 patients were available to study the effect of changes in hormone levels on recurrence. Two patients were lost to follow-up and censored at 3 years (date of their last clinical check-up); all the others were followed for 5.5 years after recruitment. The dietary intervention lasted 9 or 12 months, as participants were randomized at recruitment to either intervention or control diet for a period of 3 month to assess the short-term metabolic effects of dietary change.[17] Differences between hormone levels at baseline and a year later were closely similar in the 2 groups, so in the present study both groups were analyzed together.

The dietary intervention, described in detail elsewhere,[17,18] was characterized by reduced intake of high glycemic index and insulinemic index foods, such as white bread, white flour, potatoes, cakes, white rice and sugar; reduced intake of all sources of saturated fat, including red meat, milk and dairy products; increased intake of fiber-rich foods, such as whole grain cereals, wholemeal bread and flour, pulses and fresh vegetables; increased intake of soy products and other phytoestrogen-rich foods; increased intake of foods containing n-3 fatty acids, such as fish, seaweed and flax seeds. The diet was *ad libitum* and there was no recommendation to reduce calorie intake. However, the food rec-

ommended was satiating and most women lost weight (mean, 3.2 kg over the study period, corresponding to a reduction in consumption of about 60 kcal per day). Compliance was monitored through repeated 24-hr dietary diaries.

Laboratory measurements

To reduce intraindividual variation in hormone levels, blood samples were collected between 8 and 9 a.m., after overnight fasting. Serum hormone levels were determined using commercially available kits: radioimmunoassay kits from Orion Diagnostic (Turku, Finland) for testosterone and estradiol, immunoradiometric assay kits from Farmos (Oulunsalo, Finland) for SHBG and microparticle enzyme immunoassay kits from Abbot (Abbot Park, IL) for insulin. For insulin, samples were analyzed within 2 weeks of collection. For testosterone, estradiol and SHBG, baseline and 12-month samples were stored at $-20°C$ and analyzed in a single batch to reduce interassay variation. The coefficients of intra- and interassay variation in 8 replicates were, respectively, 4.2% and 12.2% for a testosterone concentration of 0.37 ng/ml, 10.6% and 17.6% for an estradiol concentration of 5.4 pg/ml, 3.5% and 6.9% for an SHBG concentration of 48.5 nmol/l and 2.5% and 5.1% for an insulin concentration of 8.1 μIU/ml.

Statistical analysis

The analysis focused on the risk of breast cancer recurrence (local relapse, distant metastasis, contralateral breast cancer) in relation to baseline hormone levels and changes in hormone concentrations over the dietary period. Hormone values were log-transformed to obtain approximately normal distributions. Body mass index (BMI) was computed as body weight/squared height (kg/m^2). The means of baseline hormone levels in patients who recurred were compared with those of patients who did not. The effect of the measured variables on risk of recurrence was assessed by hazard ratios and 95% confidence intervals with the Cox proportional hazards model. Given the small number of cases, the analysis was based on the distribution of hormonal values in tertiles. Analysis by quartile, however, did not substantially differ.

The following covariates were considered as potential confounders: age, extent of disease at diagnosis, as size of the primary (T1, T2 or more, or not available) and axillary node status (N^-, N^+, or not available), estrogen and progesterone receptor status (positive, negative, or not available), current tamoxifen treatment (yes or no), time between diagnosis and recruitment (1–2 years, 3–5 years, more than 5 years) and whether the patient had been assigned to intervention or control during the first 3 months of the study. T, N and hormone receptor status proved to be significant confounders and were retained in all models. A second set of analyses was carried out excluding 16 patients for whom T, N, or

receptor status was not available. As the results were closely similar, only the first analyses are presented here. Testosterone levels were slightly lower in women under tamoxifen treatment (4.10 *vs.* 4.24 ng/ml). The association of testosterone with prognosis, however, was similar in women with or without tamoxifen treatment and including tamoxifen in the model did not change the results.

The proportional hazards assumption was checked for each model by scaled Shoenfeld residuals analysis. The distribution of disease outcomes between categories of hormone concentrations was also tested by Fisher's exact test. The analyses were carried out using the Stata statistical package.

Results

Sixteen patients developed distant metastasis, 4 local relapses and 11 contralateral breast cancers (1 patient who developed both local relapse and contralateral cancer was counted only once, in the latter category). Unless otherwise specified, these adverse events are subsequently considered together as recurrences. Patients who recurred showed significantly greater serum values of testosterone, estradiol, glucose and BMI than patients who did not (0.52 *vs.* 0.38 ng/ml, $p = 0.00$; 8.06 *vs.* 5.52 pg/ml, $p = 0.02$; 96 *vs.* 91 mg/dl, $p = 0.02$; 27.1 *vs.* 25.4 kg/m,^{2} $p = 0.05$, respectively). Furthermore, fasting insulin and body weight were higher, and SHBG lower, in the recurrence group, but differences were not significant.

Table I shows hazard ratios of recurrence according to baseline hormone levels, metabolic and anthropometric variables, categorized into tertiles. Only testosterone levels were strongly and significantly associated with recurrence. Hazard ratios were 1.8 (95% CI = 0.5–6.3) and 7.2 (95% CI = 2.4–21.4) for the middle (0.34–0.49 ng/ml) and upper tertiles (0.5–0.93 ng/ml) of the distribution, respectively, compared to the lower tertile (0.16–0.33 ng/ml). Risk estimates for testosterone changed only marginally following adjustment for the other study variables (data not shown). Because of this strong prognostic effect, all other variables included in the study are also presented after adjustment for baseline testosterone levels. Body weight, insulin and glucose levels were also associated with risk of recurrence with a borderline statistical significance [hazard ratios for the upper tertile were, respectively, 2.9 (CI = 1.1–8.0), 2.4 (CI = 0.9–6.7) and 2.4 (CI = 0.9–6.5)]. The risk of recurrence also increased with increasing levels of estradiol, but this association disappeared upon adjusting for testosterone levels.

When we restricted the analysis to the 66 patients whose cancers were positive for estrogen or progesterone receptors, or both, the association of baseline estradiol levels with breast cancer recur-

TABLE I – RELATIVE RISK OF RECURRENCE ACCORDING TO BASELINE HORMONE LEVELS AND OTHER VARIABLES ($n = 110$)

	Tertiles	Crude hazard ratio	Adjusted hazard ratio[1]	Adjusted hazard ratio[2]
Testosterone	2nd 0.34–0.49	1.87 (0.55–6.37)	1.80 (0.52–6.25)	
(ng/ml)	3rd 0.50–0.93	6.92 (2.35–20.38)	7.19 (2.42–21.35)	
Estradiol	2nd 4.2–7.1	1.66 (0.64–4.29)	1.64 (0.63–4.31)	1.33 (0.50–3.56)
(pg/ml)	3rd 7.2–31.2	1.93 (0.76–4.91)	1.84 (0.70–4.83)	0.94 (0.32–2.73)
SHBG	2nd 47.1–76.1	0.76 (0.36–1.74)	0.69 (0.28–1.69)	0.59 (0.23–1.48)
(nmol/l)	3rd 76.2–159.8	0.52 (0.21–1.30)	0.38 (0.14–1.00)	0.50 (0.18–1.40)
Insulin	2nd 5.8–9.1	1.90 (0.76–4.76)	1.44 (0.56–3.74)	1.09 (0.40–2.93)
(μUI/ml)	3rd 9.2–31.5	1.67 (0.64–4.40)	1.91 (0.71–5.18)	2.42 (0.87–6.74)
Glucose	2nd 89–95	1.63 (0.63–4.21)	1.40 (0.52–3.72)	1.23 (0.46–3.30)
(mg/dl)	3rd 96–144	2.24 (0.88–5.68)	2.46 (0.94–6.44)	2.42 (0.90–6.53)
Weight	2nd 62–70	1.00 (0.39–2.60)	1.46 (0.52–4.07)	2.04 (0.71–5.92)
(kg)	3rd 71–88	1.85 (0.79–4.32)	2.38 (0.95–5.97)	2.91 (1.14–7.95)
BMI	2nd 25–27	1.78 (0.70–4.47)	1.95 (0.76–5.00)	1.45 (0.55–3.84)
(kg/m^2)	3rd 28–37	1.86 (0.78–4.42)	2.16 (0.86–5.42)	1.86 (0.76–4.54)

[1]Adjusted for hormone receptors T and N.–[2]Adjusted for hormone receptors T and N and tertiles of baseline testosterone.

TABLE II – SERUM TESTOSTERONE LEVELS BEFORE AND A YEAR AFTER THE DIETARY INTERVENTION IN 107 BREAST CANCER PATIENTS IN RELATION TO RECURRENCES OVER 5.5 YEARS OF FOLLOW-UP

Testosterone		Number of patients	Disease-free	Metastasis	Local relapse	Second cancer	Any recurrence	Hazard ratio[3] (95% CI)
Baseline	1-Year							
High[1]	High	39	19	10	3	7	20	1 (reference)
High	Low	13	11	1		1	2	0.15 (0.03–0.71)
Low[2]	High	3	2	1			1	1.01 (0.13–7.81)
Low	Low	52	46	2	1	3	6	0.15 (0.06–0.39)

[1]Testosterone concentration above median of 0.4 ng/ml.–[2]Testosterone concentration up to median of 0.4 ng/ml.–[3]For any type of recurrence, adjusted for T, N and hormone receptor status.

rence became stronger (hazard ratio for the upper tertile = 2.8; 95% CI = 1.0–8.3). After adjustment for baseline testosterone, however, the association was no longer significant (hazard ratio = 2.0; 95% CI = 0.6–6.4).

Separate analyses for women who developed contralateral cancer and those who developed local relapse plus distant metastasis produced similar results. After adjusting for hormonal receptors T and N, the hazard ratio for the upper tertile of testosterone was 6.1 (95% CI = 1.2–31.2) for contralateral cancer and 9.9 (95% CI = 2.2–45.2) for local relapse or metastasis.

We also report data of disease progression in relation to serum testosterone levels at baseline and after 1 year (Table II). In 39 patients whose testosterone levels were above the median of 0.4 ng/ml both at baseline and after the dietary intervention (1 year), the recurrence rate was very high (10 distant metastasis, 3 local relapses, 7 contralateral breast cancers). The 52 patients with testosterone levels lower or equal to 0.4 ng/ml both at baseline and after 1 year experienced only 6 recurrences: 2 metastases, 1 local relapse and 3 contralateral breast cancer (p = 0.0001, one-sided Fisher exact test with respect to patients with high testosterone at both examinations). The 13 patients whose testosterone levels reduced from above to below the median suffered only 2 recurrences (1 distant metastasis and 1 contralateral cancer); comparing these patients with patients whose testosterone levels remained above the median value, the recurrence rate was significantly lower (p = 0.02, one-sided Fisher). In 3 patients, testosterone levels increased from below to slightly above the median; 1 developed metastases.

After adjustment for tumor stage and hormone receptor status at diagnosis, the hazard ratio of the 13 patients whose testosterone levels decreased from above to below the median baseline value compared to those with persistently high testosterone levels was 0.15 (95% CI = 0.03–0.71), suggesting that reduction of testosterone by dietary intervention can reduce the recurrence rate in breast cancer patients with high testosterone levels. The hazard ratio for patients with low testosterone before and after the dietary intervention was also 0.15 (95% CI = 0.06–0.39, comparing to those with persistently high testosterone). Similar analyses for the other hormones showed only minor effects.

Discussion

In the present study on the risk of breast cancer recurrence during 5.5 years of follow-up, high baseline levels of serum testosterone emerged as a strong prognostic factor for contralateral breast cancer, distant metastasis and local relapse. Our analysis also suggests that a decrease in serum testosterone levels may reduce the risk of these unfavorable outcomes.

The suggestion that androgens play a key role in the development and progression of breast cancer dates back 30 years[14] and is supported by data indicating that high urinary testosterone levels are associated with poor outcome in breast cancer patients.[15,16] Insulin stimulates the synthesis of androgens in the ovary.[19] Recently, high serum insulin levels have also been implicated,[13] with relative risks of the same magnitude as in the present study in which, however, the association was not statistically significant, possibly due to the small study size. Several studies have indicated that overweight patients, often characterized by hyperinsulinemic insulin resistant status and high sex hormones levels, have poorer survival.[2,9] The observation that breast cancer patients who gain weight during chemotherapy are at increased risk of recurrence[10–12] is also consistent with the present results.

The present study deals with small numbers of patients (110) and adverse events (31). Its results therefore require confirmation by larger studies; however, they were not unexpected, since serum testosterone levels have been found associated with breast cancer risk in many case-control and cohort studies.[1,16] From the results of our study, it is not clear whether testosterone per se is directly responsible for promoting breast cancer cell proliferation and metastatic spread or is just a marker of a complex metabolic syndrome, linked to overnutrition and the Western lifestyle, and characterized by overweight, high fasting glucose levels, insulin resistance and high bioavailability of sex hormones and other growth factors. However, the relatively weak association between breast cancer prognosis and serum estradiol levels suggests that the risk of testosterone is unlikely to be mediated by its physiologic conversion to estradiol in adipose tissue.[20] Thus, a more plausible hypothesis is that serum androgens increase the risk either directly or because they are aromatized into estrogens in breast tissue and in breast cancer cells.[21]

Results of the present study have indicated the potential effect of serum steroids, in particular testosterone, on breast cancer progression. Our previous studies have shown that levels of testosterone and its related metabolic and endocrine variables can be favorably modified by a comprehensive change in diet in which the consumption of refined carbohydrates and saturated fats is reduced and the consumption of whole-grain cereals, pulses and vegetables increased.[17,18]

The present study suggests that the hormone changes induced by such a diet may favorably influence the prognosis for breast cancer, justifying further studies on larger series of patients. If the predictive value of serum testosterone will be confirmed in larger studies, its measurement should become part of the standard diagnostic workup of breast cancer patients, and dietary or other medical intervention to reduce testosterone levels should be considered.

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