Circulating Hormone Levels in Breast Cancer Patients. Correlation with Serum Tumor Markers and the Clinical and Biological Features of the Tumors

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Abstract. *Background: Breast cancer grows in a hormone-rich environment which influences its biological features and thus, ultimately, its clinical behavior. Circulating hormone levels were measured in premenopausal and postmenopausal breast cancer patients prior to surgery, and correlated with all available clinical and biological features of the tumors and with serum tumor marker levels. Materials and Methods: FSH, LH, 17-beta-estradiol, progesterone and prolactin were measured in 112 previously untreated breast cancer patients (54 premenopausal and 58 postmenopausal). Serum tumor markers (CEA, CA 15.3, CA-125 and Ca19.9) were measured at the same time. All tumors were studied after surgery for hormone receptor (ER and PR), Ki67, c-erb-B2 and p53 expression by means of immunohistochemistry, and for DNA-ploidy by means of flow cytometry. Results: In premenopausal patients, high gonadotropin levels correlated directly with c-erb-B2 overexpression by the tumors (FSH: p=0.02; LH: p=0.05). High estradiol levels correlated inversely (p=0.009) with Ki67 expression. In postmenopausal patients, high estradiol levels were inversely related to c-erb-B2 expression by the tumors (p=0.03), and high progesterone levels were also inversely related to Ki67 expression by the tumors (p=0.05). FSH levels correlated inversely (p=0.02) with circulating carcinoembryonic antigen (CEA) levels. Conclusion: Circulating estradiol levels seem to be associated with a less proliferative breast cancer phenotype. FSH and LH levels, on the other hand, seem to exert dual actions in premenopausal and postmenopausal breast cancer patients.*

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Breast cancer is the paradigm of hormone-dependent cancer. Both premenopausal and postmenopausal women secrete steroid hormones thoroughout their lives, albeit with a different pattern, which is regulated mainly by the ovary in the former, and the adrenal gland in the latter. When women develop breast cancer, therefore, the tumor grows within a hormonal milieu which has a decisive influence upon its development. It is therefore surprising that so few studies have tried to investigate this fact, although the issue of the ideal timing of breast cancer surgery in relationship to the menstrual phase of premenopausal women periodically arises (1-4), and has still not been settled.

In the present study, circulating hormone levels were measured in premenopausal and postmenopausal breast cancer patients prior to surgery and correlated with all available clinical and biological features of the tumors. Additionally, they were correlated, for the first time, with circulating tumor marker levels. The purpose was to investigate whether there is a connection between the hormonal environment of the host and breast cancer, to aid us in a better understanding of the biology and the clinical behavior of these tumors.

Materials and Methods

Circulating hormone levels (FSH, LH, 17-beta-estradiol, progesterone and prolactin) were measured in 112 patients prior to surgery for breast cancer. According to their menstrual history and circulating gonadotropin levels, 54 patients were premenopausal and 58 postmenopausal. The distribution of histological varieties, pTNM stage, histological and nuclear grade of the tumors was homogeneous in both groups.

Tumor marker levels were measured at the same time in all patients (CEA, CA 15.3, CA-125 and CA19.9). All tumors were studied after surgery for hormone receptor (ER and PR), Ki67, c-erb-B2 and p53 expression by means of immunohistochemistry, and for DNA-ploidy by means of flow cytometry, according to protocols previously described by us (5,6). Clinical parameters, such as tumor size and axillary node status, were also included into the final analysis.

For the comparison with dichotomic variables, hormone levels were divided into "high" and "low" using the median value of the distribution for each group (premenopausal and postmenopausal) as cut-off. The median of the different hormone level values for premenopausal patients were as follows: FSH 6.0 mIU/ml, LH 5.5 mIU/ml, estradiol 91 pg/ml, progesterone 1.6 ng/ml and prolactin 10 ng/ml. The corresponding values for postmenopausal patients were: FSH 58.0 mIU/ml, LH 25.5 mIU/ml, estradiol 15 pg/ml, progesterone 0.5 ng/ml and prolactin 7.5 ng/ml. Odds' ratios and 95% confidence intervals of each relative distribution were then calculated.

For the correlation of hormone levels with continuous variables we used Spearman's test, because none of the latter showed a Gaussian distribution.

Results

In premenopausal patients, high gonadotropin levels showed a direct correlation with c-erb-B2 overexpression by the tumors (FSH: $p=0.02$; LH: $p=0.05$). High estradiol levels, in their turn, showed a very marked inverse correlation ($p=0.009$) with the percentage of Ki67-expressing tumor cells.

In postmenopausal patients, FSH levels showed an inverse ($p=0.02$) correlation with circulating carcinoembryonic antigen (CEA) levels. Both gonadotropins (FSH and LH) showed the same trend ($p=0.08$) towards an inverse correlation with axillary node invasion. High estradiol levels were inversely related to c-erb-B2 expression by the tumors ($p=0.03$) and showed a trend ($p=0.08$) towards an inverse correlation with Ki67 expression by them. High progesterone levels, finally, were again inversely related to Ki67 expression by the tumors ($p=0.05$), and directly associated with estrogen receptor positivity of the latter ($p=0.03$).

All results are summarized in Tables I and II.

Table I. *Correlation of circulating hormone levels with circulating tumor marker levels and with clinical and biological fetaures of the tumors in premenopausal breast cancer patients. In order to appreciate trends towards a significant correlation, p values < 0.1 are displayed, although only p values < 0.5 are considered significant.*

	FSH	LH	Estradiol	Progesterone	Prolactin
Tumor size	n.s.	n.s.	n.s.	n.s.	n.s.
Axillary nodes Metastasis	n.s.	n.s.	n.s.	n.s.	n.s.
ER	n.s.	n.s.	n.s.	n.s.	n.s.
PR	n.s.	n.s.	n.s.	n.s.	n.s.
Ki67	n.s.	n.s.	0.009*	n.s.	n.s.
p53	n.s.	n.s.	n.s.	n.s.	n.s.
c-erb-B2	0.02	0.05	n.s.	n.s.	n.s.
CEA	n.s.	n.s.	n.s.	n.s.	n.s.
CA 15.3	n.s.	n.s.	n.s.	n.s.	n.s.
CA-125	n.s.	n.s.	n.s.	n.s.	n.s.
CA 19.9	n.s.	0.09*	n.s.	n.s.	n.s.

* inverse correlation; n.s = not significant
ER = estrogen receptors; PR = progesterone receptors

Table II. *Correlation of circulating hormone levels with circulating tumor marker levels and with clinical and biological fetaures of the tumors in postmenopausal breast cancer patients. In order to appreciate trends towards a significant correlation, p values < 0.1 are displayed, although only p values < 0.5 are considered significant.*

	FSH	LH	Estradiol	Progesterone	Prolactin
Tumor size	n.s.	n.s.	n.s.	n.s.	n.s.
Axillary nodes Metastasis	0.08*	0.08*	n.s.	n.s.	n.s.
ER	n.s.	n.s.	n.s.	0.03	n.s.
PR	n.s.	n.s.	n.s.	n.s.	n.s.
Ki67	n.s.	n.s.	0.08*	0.05*	n.s.
p53	n.s.	n.s.	n.s.	n.s.	n.s.
c-erb-B2	n.s.	n.s.	0.03*	n.s.	n.s.
CEA	0.02*	n.s.	n.s.	n.s.	n.s.
CA 15.3	n.s.	n.s.	n.s.	n.s.	n.s.
CA-125	n.s.	n.s.	n.s.	n.s.	n.s.
CA 19.9	n.s.	n.s.	n.s.	n.s.	n.s.

* inverse correlation; n.s = not significant
ER = estrogen receptors; PR = progesterone receptors

Discussion

Circulating hormone levels in premenopausal breast cancer patients were studied previously by Pujol *et al.* (7). Their study had a decisive advantage over our own one, concerning just the subgroup of premenopausal patients, in that they were able to make the blood extraction for analysis on the same day as the surgery, whereas we obtained our sample a mean of four days before surgery. This allowed them to identify the precise date of the menstrual phase of their patients at the time of surgery.

In spite of this theoretical drawback of our study, the obtained results were almost exactly the same: Pujol *et al.* did not find any relationships between the menstrual phase at surgery and tumor size, cathepsin-D level, histological grade, axillary metastasis and progesterone receptors. They only found a phase-dependent increase in

estrogen receptor content of the tumors in favor of the follicular phase. We also found no correlation with the above-mentioned parameters, excepting cathepsin-D, which we did not study. Furthermore, Pujol *et al.* found that high FSH and LH levels (above the median, which was also our cut-off) were associated with a significantly worse prognosis, in terms of shorter disease-free survival and overall survival. Although we have no survival data due to insufficient follow-up, our results are in full agreement with the just cited ones, for we found a significant association of high FSH and LH levels with c-erb-B2 overexpression of the tumors, which is an accepted molecular marker of a significantly worse prognosis of breast cancer. In this same group of premenopausal patients, furthermore, we found a very strong inverse correlation between estradiol levels and Ki67 expression or, being the same, proliferation of the tumors. The same trend was observed in postmenopausal patients, although not reaching statistical significance. This is at first sight surprising, since breast cancer incidence is directly related to circulating estrogens. However, one aspect is the incidence of breast cancer, while an entirely different one is the biological behavior and even prognosis of those same tumors, once cancer is established. In fact, it has been repeatedly reported that breast cancers arising in women under hormone replacement therapy with estrogens have a better prognosis than others, although hormone replacement therapy is, in its turn, associated with an increase in the incidence of those same tumors (8,9). It is, therefore, very possible that estrogens induce breast cancer on the one hand, but that these estrogen-induced cancers present a less aggressive tumor phenotype, on the other.

We are not aware of any previous studies comparing circulating hormone levels with the clinical and biological features of breast cancers from postmenopausal women. Our results in this subgroup are particularly interesting. Circulating estradiol showed the same trend towards an inverse correlation with tumor proliferation (Ki67) already mentioned for premenopausal patients, although not reaching statistical significance. This association, with a less aggressive phenotype, is reinforced by the finding that estradiol also showed an inverse and significant correlation with c-erb-B2 overexpression by the tumors. FSH and LH, on the other hand, seemed to show a completely opposite trend to the one described in premenopausal patients. In fact, the FSH and LH levels were inversely associated with the most ominous clinical prognostic parameter, axillary node invasion, and this association almost reached statistical significance. Along this same line, FSH levels were again inversely associated with circulating CEA levels, this time in a statistically significant way. Progesterone levels, finally, were significantly associated with less proliferative activity of the tumors (Ki67) and showed a direct association with the estrogen receptor content of the latter but, somewhat surprisingly, the same was not the case for estradiol levels. Since in postmenopausal patients progesterone obviously does not stem from gonadal sources, this association with such an important biological feature as estrogen receptor content merits further study.

Serum tumor markers, finally, have never before been studied in connection with circulating hormones in breast cancer. There are studies on menstrual cycle-dependent variability of serum tumor markers in healthy women, which show that such a dependence may exist, but is very scant. So, Erbagci *et al.* (10) found that only CA 15.3 showed significantly higher levels in the luteal phase, the rest of the tested markers remaining unchanged throughout the menstrual cycle. Our study seems to corroborate that serum tumor marker levels are little influenced by circulating hormones, with only the cited exception of CEA in postmenopausal patients.

In conclusion, circulating estradiol levels seem to be associated with a less proliferative breast cancer phenotype. FSH and LH levels, on the other hand, seem to exert dual actions in premenopausal patients, where they are associated with oncogenic activation (c-erb-B2), and in postmenopausal women, where they are inversely related to axillary node invasion and CEA serum levels. Progesterone levels, finally, are associated with lower proliferation and estrogen receptor expression by the tumors in postmenopausal patients.

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