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Circulating estradiol defines the tumor phenotype in menopausal breast cancer patients

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ABSTRACT

Objective: To correlate circulating hormone levels with the clinical and biological features of the tumors in menopausal breast cancer patients.
Design: Circulating hormone levels were measured in 161 previously untreated menopausal breast cancer patients within 72 h of their planned surgery. The obtained hormone levels were correlated with tumor size, histological and nuclear grade, histological score, axillary nodal status, DNA-ploidy and Ki67-, c-erb-B2-, p53, Bax-, VEGF- and Nup88-expression.
Results: The only statistically significant correlations found between circulating hormone levels and all tested variables were an inverse one between estradiol and the expression of the apoptosis-associated *Bax* gene ($p = 0.009$), and again an inverse correlation between estradiol and the expression of c-erb-B2 ($p = 0.04$). When comparing hormone levels with each other, a significant correlation between estradiol and progesterone ($p < 0.0001$), an inverse one between estradiol and FSH ($p = 0.04$) and a direct one between LH and prolactin ($p = 0.001$) were found.
Conclusion: Higher circulating estradiol levels in postmenopausal breast cancer patients are associated with molecular features usually defining a biologically less aggressive tumor phenotype.

1. Introduction

Although breast cancer is subject to hormonal influence, the hormonal environment in which it grows is markedly different in premenopausal and postmenopausal women. Whereas in premenopausal women hormone levels fluctuate due to physiological variations during the menstrual cycle, hormone levels are rather constant in the postmenpausal patient, and tend only to vary over long time spans. It is plausible to think that this prolonged cross-talk between tumor and host hormones may have lasting effects on the biology of the tumor. There are many studies on the correlation between circulating steroid hormone levels and the incidence of breast cancer. However, surprisingly, the correlation between circulating hormone levels and the biological and clinical features of breast cancer, once established, has been the subject of very few ones, among which a small pilot study from our own group [1]. In a previous report by Pujol et al., high FSH and LH levels have been associated with a significantly worse prognosis in premenopausal breast cancer patients [2]. One inherent difficulty in many of these studies on premenopausal patients is to date the menstrual phase adequately, something which is not always possible using the menstrual history alone, in the absence of serial hormone measurements or endometrial biopsy. The timing of the blood extraction for hormone analysis in relationship to the date of surgery is also crucial. These problems disappear with postmenopausal patients, because, as has been said above, their physiological hormone levels are not subject to the extreme variations found in premenopausal women. Therefore, we have devised the present study, restricted to postmenopausal carriers of breast cancer. In it, we have correlated circulating hormone levels, determined at the time of surgery, with the clinical and biological features of the tumors, in an intent to verify in a sufficiently large series the initial results of our above-mentioned pilot study.

2. Patients and methods

Data from 161 patients were studied. They were operated upon at Centro de Patología de la Mama, Madrid, Spain, between January 2000 and December 2007. Menopausal status was ascertained by means of the following parameters: age, amenorrhea of at least 12 months' duration and elevated gonadotropin levels. When all of them were concordant, the corresponding patient data were

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included into the study. These strict criteria were followed in order to exclude perimenopausal patients from it. Accordingly, the mean age of the patients was 60.1 years, the mean FSH level 69.32 mU/ml and the mean LH level 27.81 mU/ml. The histological classification of the tumors was as follows: ductal infiltrating 137; lobular infiltrating 19; tubular 3; medullary 1; anaplastic 1. Of them, 112 were T1 tumors, 39 T2 tumors, 2 T3 tumors and the remaining 8 were diffuse, unclassifiable tumors encompassing the whole breast with minute foci.

All patients were previously untreated for their breast cancer, and were receiving no kind of hormonal treatment, or of any other treatment liable to interact with circulating hormones at the time of blood sampling. This was performed within 72 h of initial surgery.

Hormone levels were determined in a central laboratory facility, employing the same reactants and the same autoanalyzer in all cases.

Standard pathological workup included the determination of tumor size, nodal invasion, histological and nuclear grade, histological score, as well as the immunohistochemical assessment of hormone receptors (ER and PR), c-erb-B2, p53 and Ki67 expression, according to protocols extensively described in previous papers [3,4]. The tumors were considered positive for hormone receptors, c-erb-B2 and p53, when they showed more than 10% reactive cells. The Ki67 score consisted of the percentage of reactive tumor cell nuclei.

DNA-ploidy of the tumors was determined by means of flow-cytometry, using the protocols previously described by us [5,6]. The tumors were considered diploid, when their DNA-index was 1.0, and aneuploid for any diverging value.

The expression of the angiogenesis-associated *VEGF* gene, the apoptosis-associated *Bax* gene, as well as of the *Nup88* gene, previously shown by us to be associated with a significantly more aggressive tumor phenotype in breast cancer, was determined by means of immuno-flow-cytometry, as described extensively elsewhere [5,6].

The correlation between continuous variables was studied by means of Sperman's rank correlation test, because the tested variables did not show individually a Gaussian distribution. The association of hormone receptor levels with dichotomic variables was studied by means of contingency tables and Fisher's exact test, after dichotomising the circulating hormone levels, using the median value as cutoff. The results were considered significant, when the corresponding p value was <0.05.

3. Results

The only statistically significant correlations found between circulating hormone levels and all tested variables were an inverse one between estradiol and the expression of the apoptosis-associated *Bax* gene ($r = -0.26$; $p = 0.009$), and again an inverse correlation between estradiol and the expression of c-erb-B2 ($p = 0.04$). In larger, aggressive tumors, the core tends to show a predominance of apoptotic cells, whereas highly proliferating cells tend to be located at the periphery. In order to exclude a possible bias in our results due to this fact, the statistical analysis on the correlation of *Bax* expression with circulating hormone levels was carried out separately for the subgroup of small T1 tumors, where the distribution of cells according to their biology is much more uniform, and the sample obtained for flow-cytometry is a cross-section of the whole tumor. The inverse correlation between estradiol levels and *Bax* expression was even stronger than for the whole cohort ($r = -0.33$; $p = 0.007$), thus excluding in principle a bias introduced by sampling at the core or the periphery of larger tumors.

When comparing hormone levels with each other, finally, we found a significant correlation between estradiol and progesterone ($p < 0.0001$), an inverse one between estradiol and FSH ($p = 0.04$) and a direct one between LH and prolactin ($p = 0.001$).

The results are summarized in Tables 1 and 2.

Table 1
Correlation of circulating hormone levels with continuous variables.

	Estradiol	Progesterone	FSH	LH	Prolactin
Tumor size	$r = 0.04$ $p = 0.66$	$r = 0.10$ $p = 0.20$	$r = 0.002$ $p = 0.97$	$r = 0.04$ $p = 0.61$	$r = 0.12$ $p = 0.13$
Histological grade	$r = 0.03$ $p = 0.71$	$r = -0.16$ $p = 0.07$	$r = 0.000$ $p = 1.00$	$r = 0.08$ $p = 0.39$	$r = -0.06$ $p = 0.47$
Nuclear grade	$r = 0.09$ $p = 0.27$	$r = -0.07$ $p = 0.45$	$r = -0.05$ $p = 0.58$	$r = 0.09$ $p = 0.29$	$r = 0.03$ $p = 0.74$
Histological score	$r = -0.02$ $p = 0.79$	$r = -0.13$ $p = 0.12$	$r = -0.12$ $p = 0.14$	$r = -0.09$ $p = 0.31$	$r = -0.01$ $p = 0.93$
Nodal invasion	$r = 0.05$ $p = 0.47$	$r = 0.02$ $p = 0.75$	$r = 0.04$ $p = 0.61$	$r = -0.04$ $p = 0.64$	$r = 0.12$ $p = 0.13$
Ki67	$r = -0.07$ $p = 0.39$	$r = -0.12$ $p = 0.11$	$r = -0.04$ $p = 0.56$	$r = 0.03$ $p = 0.70$	$r = 0.10$ $p = 0.20$
Bax	$r = 0.26$ $p = 0.009$	$r = 0.01$ $p = 0.88$	$r = 0.15$ $p = 0.11$	$r = 0.02$ $p = 0.86$	$r = 0.006$ $p = 0.52$
Nup88	$r = 0.01$ $p = 0.88$	$r = -0.04$ $p = 0.71$	$r = -0.15$ $p = 0.13$	$r = 0.03$ $p = 0.79$	$r = 0.05$ $p = 0.60$
VEGF	$r = 0.04$ $p = 0.70$	$r = 0.007$ $p = 0.93$	$r = 0.03$ $p = 0.70$	$r = 0.05$ $p = 0.57$	$r = 0.17$ $p = 0.07$
DNA-index	$r = 0.04$ $p = 0.58$	$r = 0.006$ $p = 0.93$	$r = 0.09$ $p = 0.24$	$r = 0.004$ $p = 0.80$	$r = 0.09$ $p = 0.25$

Spearman's rank correlation test.

4. Discussion

The results from this study largely confirm those reported in our previous, pilot study addressing the same issue [1]. Again, estradiol levels were inversely and significantly correlated with the expression of the most widely studied oncogene product in breast cancer, c-erb-B2. Very recently, Sieri et al. found a similar association following a case–control study on the relationship between serum sex hormone levels and breast cancer risk in postmenopausal women [7]. They report that among 165 postmenopausal women developing breast cancer within the ORDET cohort, testosterone (not estrogen) levels were associated with breast cancer risk, whereas estrogen levels tended towards an association with c-erb-B2-negative cancer.

Table 2
Association of circulating median hormone levels with dichotomic variables.

	Median value	c-erb-B2 (p value)	p53 (p value)	ER (p value)	PR (p value)
Estradiol	16 pg/ml	0.04[a]	0.43	0.70	0.43
Progesterone	0.7 pg/ml	0.22	0.32	0.70	0.75
FSH	62.9 mU/ml	0.42	0.17	0.35	1.00
LH	25.7 mU/ml	0.54	0.44	0.85	0.87
Prolactin	7.8 ng/ml	0.42	0.55	0.85	0.75

Fisher's exact test.
[a] Inverse association.

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In the present study we found additionally that estradiol levels are inversely and significantly correlated with the expression of the apoptosis-associated *Bax* gene, something not reported before. In vitro, estrogen has been reported to inhibit UV radiation-induced apoptosis in MCF-7 breast cancer cells through binding to mitochondrial estrogen receptors, thus preventing, among other things, the translocation of Bax to the mitochondria [8]. The results of our study might indicate that this is possibly due to a downregulation of Bax expression by estradiol, since the levels of both were inversely correlated. From previous studies by our own group [9] we know that the expression of the Bax protein, measured by means of immuno-flow-cytometry, is in its turn significantly associated with clinical and biological features indicative of a worse prognosis. The general conclusion to be derived from the present study, therefore, is that higher circulating estradiol levels in postmenopausal breast cancer patients are associated with a biologically less aggressive breast cancer phenotype. This should reflect itself in a correlation with a histologically less aggressive phenotype, which was not the case, or with a lower proliferation rate of the tumors. There was also no correlation whatsoever with *Nup88* expression, a gene shown by us for the first time to be associated with all clinical and biological features defining a more aggressive breast cancer phenotype [4]. The precise role of *Nup88* in breast cancer is still unknown, which makes it difficult to further speculate why no association with the expression of this gene was found, although one was expected at the start of the study, and therefore it was included into it. On the other hand, what is apparent from our results is that circulating estrogens are in no way associated with a higher biological aggressiveness of breast cancer in postmenopausal women. If anything, the available evidence points in the opposite direction. This is also indirectly in agreement with the findings of Pujol et al. [2], who correlated high circulating FSH and LH levels with a significantly worse prognosis of breast cancer patients. As is evident from our own results, FSH levels in postmenopausal patients are still subject to the negative feed-back exerted by estradiol, whatever its origin, so that high FSH and LH levels merely reflect low estradiol levels.

Our results only apparently contradict the innumerable reports linking especially estrogens with an elevated incidence of breast cancer. Because one thing is the incidence of a given tumor, i.e., the new cases appearing in the population, and quite another the biology of those same tumors in relationship with the promoting agent. In the case of breast cancer, it may well be that elevated circulating steroid hormones may promote the passage of latent precursor lesions to overt cancer, and even promote its growth. At the same time, it is also well known that breast cancers arising in menopausal women under hormone replacement therapy have a better prognosis than the rest, among other things because estrogen-induced breast cancers are by definition hormone-sensitive, and hormone sensitive breast cancers have the best inherent prognosis [10,11].

A surprising finding from the present study was the highly significant correlation found between circulating LH and prolactin levels in our cohort of patients, something never reported before, to our knowledge. The only similar finding was reported more than three decades ago by Sheth et al. [12], who studied circulating levels of prolactin in breast cancer patients and normal controls. In their normal controls, prolactin levels were higher during the luteal phase than during the early follicular phase, i.e., when LH levels are highest. Intriguingly, we found the reported correlation between LH and prolactin levels only in our cohort of postmenopausal breast cancer patients. We carried out an analysis of 50 randomly chosen age-matched, confirmed postmenopausal healthy controls, and in this subset of healthy women LH levels did not correlate with prolactin levels ($r = 0.17$; $p = 0.23$). It is difficult to venture an explanation for these findings. In a study by Silva et al. [13], LH levels were measured in the sera and tissues from breast cancer patients and normal controls, and were found to be significantly higher in the tissues of cancer carriers, with a gradient from tumor tissue to normal tissue in the vicinity of the tumor to normal tissue far away from it. It might be that endogenous LH produced by the tumor tissue and shed into the bloodstream partly alters circulating LH levels. However, the mean LH levels in our breast cancer carrier and healthy control population were identical (27.53 vs. 27.83). This, in principle, excludes another possible explanation for the association, derived from experimental evidence produced by our own group [14]: dopamine represses both LHRH and prolactin secretion, and stress in its turn depresses dopamine levels, so that stress, indirectly, might concomitantly increase circulating LH and prolactin. The finding of the high correlation between LH and prolactin might thus be explained by stress in women just informed of a breast cancer diagnosis, within 72 h of their planned surgery, if compared to healthy women. However, in this case, both LH and prolactin levels should be raised, in principle, so that probably another, still unknown effect must be involved in order to explain the association between LH and prolactin in breast cancer patients.

The highly significant correlation found between estradiol and progesterone levels, finally, does merely reflect that, in postmenopausal women, estradiol synthesis takes place in a linear fashion mainly in the adrenal gland, progesterone being one of the first (in fact, the third) necessary precursor links of the chain.

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