Testosterone and Biological Characteristics of Breast Cancers in Postmenopausal Women

Giorgio Secreto,1 Elisabetta Venturelli,1 Elisabetta Meneghini,1 Marco Greco,2 Cristina Ferraris,2 Massimo Gion,3 Matelda Zancan,3 Aline S.C. Fabricio,3 Franco Berrino,1 Adalberto Cavalleri,1 and Andrea Micheli1

1Department of Preventive and Predictive Medicine and 2Breast Surgery, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy and 3Association for Application of Biotechnologies in Oncology and Centre for the Study of Biological Markers of Malignancy, Regional Hospital, Local Health Unit (AULSS) No. 12, Venice, Italy

Abstract

Androgens are involved in the development of breast cancer, although the mechanisms remain unclear. To further investigate androgens in breast cancer, we examined the relations between serum testosterone and age, body mass index (BMI), tumor size, histologic type, grade, axillary node involvement, estrogen receptor status, progesterone receptor status, and HER2 overexpression in a cross-sectional study of 592 postmenopausal breast cancer patients. Mean testosterone differences according to categories of patient and tumor characteristics were assayed by Fisher's or Kruskall-Wallis test as appropriate; adjusted odds ratios (OR) of having a tumor characteristic by testosterone tertiles were estimated by logistic regression. Testosterone concentrations were significantly higher in women with BMI ≥30 versus BMI <25. ORs of having a tumor ≥2 cm increased significantly with increasing testosterone tertiles, and the association was stronger in women ≥65 years. The OR of having infiltrating ductal carcinoma was significantly higher in the highest compared with the lowest testosterone tertile. ORs of having estrogen receptor−and progesterone receptor−negative versus estrogen receptor− and progesterone receptor−positive tumors decreased significantly with increasing testosterone tertiles. In women ≥70 years, those with high testosterone had a significantly greater OR of HER2-negative cancer than those with low testosterone. These results support previous findings that high-circulating testosterone is a marker of hormone-dependent breast cancer. The age-related differences in the association of testosterone with other disease and patient characteristics suggest that breast cancers in older postmenopausal women differ markedly from those in younger postmenopausal women. The relationship between testosterone and HER2 status in the oldest patients merits further investigation. (Cancer Epidemiol Biomarkers Prev 2009;18(11):2942–8)

Introduction

The androgen excess theory (1), based on studies done by our group, suggests that androgen excess is a marker of a complex hormonal disorder that involves estrogens as well as other hormones and metabolic factors and that facilitates the development of breast cancer. Prospective studies (2-5) have in fact repeatedly found a strong association between high-circulating androgens and increased breast cancer risk. However, the role of androgens in breast cancer is still debated, and some authors maintain that androgens are protective through their action in inhibiting the stimulatory effect of estrogens on breast epithelium (6-9).

To further investigate the role of androgens in breast cancer, we did a cross-sectional study on a cohort of postmenopausal breast cancer patients, examining the association between serum testosterone concentrations and age, body mass index (BMI), tumor size, histologic type, grade, axillary node involvement, estrogen receptor status, progesterone receptor status, and HER2 overexpression. Our aim was to provide further evidence for the androgen excess theory, to thereby obtain a more complete characterization of the patient and the disease that may have implications for prognosis and treatment.

Materials and Methods

Patients. Candidate patients were those treated surgically for primary breast cancer at the Senology Unit of our institute from December 2003 to December 2006. Inclusion criteria were histologically confirmed nonmetastatic breast carcinoma (any T, any N, M0). Exclusion criteria were nonepithelial cancer, in situ breast cancer, previous diagnosis of cancer (except in situ cervical cancer or nonmelanoma skin cancer), and neoadjuvant chemotherapy or hormone therapy. A total of 944 patients (296 premenopausal, 56 perimenopausal, and 592 postmenopausal) were identified. Written informed consent was obtained from all included patients. The study was approved by the Scientific and Ethical Committee of our institute.

Here, we consider only postmenopausal women, defined as those (a) with last menstruation ≥12 mo before...
enrollment (510 patients), (b) who had undergone bilateral oophorectomy (34 patients), or (c) who had undergone hysterectomy without oophorectomy or monolateral oophorectomy and were ≥50 y old (47 patients).

It is much more difficult to analyze premenopausal patients due to the very large intra-individual variations in sex hormone levels, including testosterone, over the menstrual cycle. Testosterone data in premenopausal subjects would also need to be adjusted for circulating levels of other hormones (namely, luteinizing hormone, follicle-stimulating hormone, and progesterone) as well as the day of the cycle on which the blood sample was taken, as done in our previous study on premenopausal women (5). Furthermore, in view of the sophisticated statistical modeling implied by such adjustments, a large number of premenopausal cases, all with information on the timing of blood sampling in relation to phase of cycles, would have to be considered.

The women provided a fasting blood sample before surgery. The samples were processed, divided in aliquots, and stored at −80°C pending analysis for serum testosterone (and other variables not reported here). Patient and tumor characteristics were extracted from patients’ clinical records and entered into a specifically designed database.

The 592 postmenopausal patients were of mean age of 66.1 y, SD ± 9.1 (range, 41-97 y). Weight and height were available for 531 of these. Eleven patients had missing data for tumor size, 8 for grade, 16 for axillary status, 1 for estrogen receptor, 3 for progesterone receptor, and 163 for HER2.

### Statistical Methods

Testosterone concentrations were square root–transformed, as the distribution of concentrations was not normal. Fisher’s test or the nonparametric Kruskal-Wallis test (if the criterion of homoscedasticity was not met) was used to assess differences between mean testosterone levels according to categories of patient and tumor characteristics. Trends across categories were investigated by the Cuzick test. Differences in tumor characteristics according to BMI categories (<25, 25-30, ≥30 kg/m², as recommended by WHO; ref. 10) and age categories (<65 and ≥65 y) were investigated by the χ² test.

Multivariable analyses were next used to assess associations between testosterone and tumor characteristics taking potential confounders into account, with the women divided into tertiles of testosterone distribution.
Reference categories were tumor size <2 cm, histology other than pure invasive ductal carcinoma (IDC), grade 1 disease, positivity for estrogen receptor, positivity for progesterone receptor, positivity for HER2, and axillary involvement. For each tumor characteristic, the odds of being in a given tumor characteristic category, compared with the reference category, was compared between testosterone tertiles, with odds ratios (OR) estimated by binomial or multinomial logistic regression. Age and BMI were assessed as potential confounders by entering them as continuous and categorical variables and checking the linearity assumption on the logit scale. Age, as a continuous variable, was linear on the logit scale for all tumor characteristics and was included as an adjustment in all models. BMI, dichotomized as <25 versus ≥25 kg/m², had some influence on OR estimates only for tumor size and histology and was included as an adjustment only in models estimating the odds of size and histology being related to testosterone.

Ninety-five percent confidence intervals (95% CI) were estimated. The likelihood ratio test was used to assess linear trend in ORs with increasing testosterone as a three-level categorical variable. All P values refer to two-sided statistical tests; differences with \( P \leq 0.05 \) were considered significant. The analyses were done with the Stata statistical package, release 9.0 (2005; Stata Corporation).

**Results**

Table 1 shows mean testosterone levels by categories of patient and tumor characteristics. Women ≥70 years had slightly higher (nonsignificant) mean testosterone levels than younger patients. Testosterone levels increased significantly with increasing BMI and increasing tumor size and were significantly higher in women with estrogen receptor–positive or progesterone receptor–positive tumors (compared with estrogen receptor and progesterone receptor negative). Mean testosterone levels ranged from 0.383 ± 0.164 ng/mL for BMI <25 kg/m² to 0.469 ± 0.219 ng/mL for BMI ≥30 kg/m² (\( P \) trend = 0.001), from 0.390 ± 0.203 ng/mL for tumors <1 cm to 0.445 ± 0.191 ng/mL for tumors ≥2 cm (\( P \) trend = 0.001), and were 0.346 ± 0.158 ng/mL in estrogen receptor– and progesterone receptor–negative cancers compared with 0.420 ± 0.197 ng/mL for cancers that were estrogen receptor–positive or progesterone receptor–positive, or both (\( P = 0.001 \)). Only 12 women had nonlobular or nonductal carcinoma and were characterized by particularly low testosterone levels. Mean testosterone levels did not change significantly with tumor grade, axillary lymph node status, or HER2 status.

Table 2 shows mean testosterone levels by tumor characteristics for two age categories (<65 years and ≥65 years): by this analysis, the significant association of higher testosterone levels with tumors ≥2 cm (present when all women were considered together) was confined to women of ≥65 years. For both age classes, testosterone remained significantly lower in estrogen receptor– and progesterone receptor–negative tumors compared with estrogen receptor– or progesterone receptor–positive tumors. In the ≥65-year age class, patients with HER2-negative tumors had higher (nonsignificant) testosterone levels than those with HER2-positive tumors.

Table 3 shows ORs (variously adjusted) for women with various tumor characteristics being in a given testosterone tertile compared with the lowest tertile (reference). The odds of having tumor ≥2 cm instead of tumor <2 cm increased with increasing testosterone tertiles: 1, 1.84 (95% CI, 1.14-2.98), and 2.47 (95% CI, 1.54-3.95; \( P \) trend < 0.001). Women in the highest testosterone tertile were twice as likely to have IDC as women in the lowest tertile (OR, 2.07; 95% CI, 1.18-3.62). Women in the lowest testosterone tertile were significantly more likely to have estrogen receptor– and progesterone receptor–negative tumors than estrogen receptor– and progesterone receptor–positive tumors, with ORs of 1, 0.43 (95% CI, 0.24-0.76), and 0.42 (95% CI, 0.23-0.75; \( P \) trend = 0.002). Low-testosterone

### Table 2. Mean serum levels (SD) of testosterone by tumor characteristics in postmenopausal breast cancer women according to age (<65 and ≥65 y)

<table>
<thead>
<tr>
<th>Tumor size, cm</th>
<th>Age &lt;65 y</th>
<th>Age ≥65 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>n (%)</td>
<td>Mean ± SD, ng/mL</td>
</tr>
<tr>
<td>≥2</td>
<td>200 (71.9)</td>
<td>0.398 ± 0.166</td>
</tr>
<tr>
<td>Histology</td>
<td>78 (28.1)</td>
<td>0.407 ± 0.172</td>
</tr>
<tr>
<td>IDC</td>
<td>227 (79.9)</td>
<td>0.405 ± 0.167</td>
</tr>
<tr>
<td>Other infiltrating carcinoma</td>
<td>57 (20.1)</td>
<td>0.373 ± 0.165</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20 (7.1)</td>
<td>0.413 ± 0.211</td>
</tr>
<tr>
<td>2</td>
<td>146 (52.1)</td>
<td>0.400 ± 0.170</td>
</tr>
<tr>
<td>Axillary nodal status</td>
<td>114 (40.8)</td>
<td>0.397 ± 0.158</td>
</tr>
<tr>
<td>Negative</td>
<td>171 (60.8)</td>
<td>0.403 ± 0.173</td>
</tr>
<tr>
<td>Positive</td>
<td>110 (39.2)</td>
<td>0.389 ± 0.160</td>
</tr>
<tr>
<td>ER/PR status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>47 (16.6)</td>
<td>0.348 ± 0.163</td>
</tr>
<tr>
<td>Positive</td>
<td>236 (83.4)</td>
<td>0.409 ± 0.166</td>
</tr>
<tr>
<td>HER2 status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>136 (48.9)</td>
<td>0.397 ± 0.167</td>
</tr>
<tr>
<td>Positive</td>
<td>142 (51.1)</td>
<td>0.402 ± 0.169</td>
</tr>
</tbody>
</table>

\(^1\)Fisher’s test.

\(^2\)Nonparametric Kruskal-Wallis test (criterion of homoscedasticity not met).

\(^3\)Negative, ER negative and PR negative; positive, ER positive and PR positive or ER negative and PR positive or ER positive and PR negative.
women were also more likely (nonsignificant) to have tumors that were estrogen receptor negative and progesterone receptor positive than estrogen receptor-positive and progesterone receptor-positive tumors, whereas the distribution of estrogen receptor-positive and progesterone receptor-negative tumors did not vary with testosterone level. Thus, the relation between testosterone and hormone receptor status was essentially confined to estrogen receptors; this was further illustrated by our finding that age- and BMI-adjusted ORs of having an estrogen receptor-negative tumor rather than an estrogen receptor-positive tumor decreased with increasing testosterone as follows: 1, 0.43 (95% CI, 0.23-0.82), and 0.49 (95% CI, 0.26-0.93; \( P \) trend = 0.018). The distribution of tumor grade, nodal status, and HER2 status did not vary significantly with testosterone level.

When the logistic regression analysis was restricted to women age \( \geq 65 \) years, the association of testosterone with BMI and hormone receptor status was essentially confined to estrogen receptor status; this was further illustrated by our finding that age- and BMI-adjusted OR of having a tumor \( \geq 2 \) cm rather than <2 cm tumor was 1.65 (95% CI, 1.13-2.40), with age- and testosterone-adjusted OR of 1.61 (95% CI, 1.10-2.36). The age-adjusted OR of IDC rather than other histology was 1.65 (95% CI, 1.08-2.52) with age- and testosterone-adjusted OR of 1.55 (95% CI, 1.01-2.38).

We also found significant associations of BMI with tumor size, histology, and hormone receptor status, which were only in part attributable to BMI and testosterone association. Thus, women with BMI \( \geq 25 \) kg/m\(^2\) more frequently had large tumors and IDC histology than women with BMI <25 kg/m\(^2\). The age-adjusted OR of having a tumor \( \geq 2 \) cm rather than <2 cm tumor was 1.65 (95% CI, 1.13-2.40), with age- and testosterone-adjusted OR of 1.61 (95% CI, 1.10-2.36). The age-adjusted OR of IDC rather than other histology was 1.65 (95% CI, 1.08-2.52) with age- and testosterone-adjusted OR of 1.55 (95% CI, 1.01-2.38). The relation of BMI to hormone receptor status is shown in Table 4 for all women and for those of age \( \geq 65 \) years. In all women, high BMI was related to progesterone receptor positivity but not to estrogen receptor status. The association was stronger for women age \( \geq 65 \) years and, in contrast to the situation with testosterone, was most evident for progesterone receptor. In all women, progesterone receptor positivity was significantly related to low BMI in the estrogen receptor positivity and progesterone receptor positivity comparison; in women age \( \geq 65 \) years, progesterone receptor positivity was significantly related to low BMI in both comparisons. The age-, testosterone-, and estrogen receptor status–adjusted OR of having a progesterone receptor–negative rather than progesterone receptor–positive tumor was 0.58 (95% CI, 0.31-0.93).
0.37-0.93) in all women with BMI ≥25 kg/m² compared with those with BMI <25 kg/m², and the corresponding OR in women ≥65 years and BMI ≥25 kg/m² was 0.50 (95% CI, 0.26-0.96).

Discussion
In the present study, we further explored the role of androgens in breast cancer by evaluating associations between serum testosterone levels—determined on blood samples taken before surgery—and age, BMI, and selected tumor characteristics, in postmenopausal women diagnosed with the disease. Our main findings are that high testosterone levels were significantly associated with high BMI, larger tumor size, and positive estrogen receptor status. We also found that testosterone levels were significantly higher in HER2-negative cancers, but only in patients of ≥70 years of age. There was no association between testosterone and age.

We comment first on the heterogeneity of our postmenopausal cohort. Although the majority (510 of 592; 86%) were in physiologic menopause, 34 (6%) women had an oophorectomy and 47 (8%) had a hysterectomy or unilateral oophorectomy. The small subgroups had significantly lower (P = 0.002) mean testosterone levels (0.35 ± 0.15 and 0.33 ± 0.17 ng/mL, respectively) than women in physiologic menopause (0.42 ± 0.20 ng/mL), in agreement with published data (11, 12). However, when these subgroups were excluded or when postmenopausal type was included in the analysis, adjusted ORs did not change substantially (data not shown).

Table 4. ORs (95% CI) of hormone receptor status by BMI categories in all postmenopausal and those of age ≥65 y

<table>
<thead>
<tr>
<th>BMI (kg/m²) categories</th>
<th>All postmenopausal women</th>
<th>Women ages ≥65 y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;25</td>
<td>≥25</td>
</tr>
<tr>
<td>ER/PR status*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER− and PR−/ER+ and PR+</td>
<td>40/154</td>
<td>37/208</td>
</tr>
<tr>
<td>Age-adjusted OR (95% CI)</td>
<td>1.00 (0.42-1.12)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>Age- and testosterone-adjusted OR (95% CI)</td>
<td>0.68 (0.41-1.13)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>ER+ and PR−/ER+ and PR+</td>
<td>48/154</td>
<td>32/208</td>
</tr>
<tr>
<td>Age-adjusted OR (95% CI)</td>
<td>1.00 (0.42-1.12)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>Age- and testosterone-adjusted OR (95% CI)</td>
<td>0.49 (0.29-0.81)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>ER status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative/positive</td>
<td>47/202</td>
<td>40/240</td>
</tr>
<tr>
<td>Age-adjusted OR (95% CI)</td>
<td>1.00 (0.42-1.12)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>Age- and testosterone-adjusted OR (95% CI)</td>
<td>0.72 (0.45-1.14)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>Age, testosterone−, and PR status−adjusted OR (95% CI)</td>
<td>1.00 (0.42-1.12)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>PR status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative/positive</td>
<td>88/161</td>
<td>69/211</td>
</tr>
<tr>
<td>Age-adjusted OR (95% CI)</td>
<td>1.00 (0.42-1.12)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
<tr>
<td>Age- and testosterone−adjusted OR (95% CI)</td>
<td>0.59 (0.33-0.94)</td>
<td>0.59 (0.33-0.94)</td>
</tr>
<tr>
<td>Age, testosterone−, and ER status−adjusted OR (95% CI)</td>
<td>1.00 (0.42-1.12)</td>
<td>1.00 (0.42-1.12)</td>
</tr>
</tbody>
</table>

*Multinomial logistic regression; the ER positive and PR negative category had too few subjects to produce reliable OR estimates and was not considered in this analysis.
progression was greater in patients with high urinary androgens compared with those with normal levels (28) and, more recently, that use of diet to lower circulating testosterone reduced the frequency of disease progression (29).

Based on the findings of the present study and those reviewed above, we propose that testosterone levels should be routinely determined during both the work-up of newly diagnosed postmenopausal breast cancer patients and during follow-up. At present, tumor estrogen receptor status is the main criterion for selecting breast cancer patients for endocrine treatment. We believe, however, that this selection could be improved by serial measurements of circulating testosterone. In patients with persistently high testosterone, medical oophorectomy might be considered associated with antiestrogens or aromatase inhibitors in women with estrogen receptor–positive disease, or with chemotherapy in estrogen receptor–negative patients. The exact treatment would, of course, depend on other risk factors such as grade, nodal status, and HER2 overexpression.

Another major finding of our study is that testosterone concentrations did not decline with age, consistent with reports in healthy postmenopausal women that testosterone levels remain constant with advancing age (11, 12, 30). The ovary seems to be an important source of testosterone and other androgens in postmenopausal women (12, 31, 32). Synthesis takes place in the stromal tissue and is likely to be luteinizing hormone driven (11). In predisposed women, excessive levels of gonadotropins after menopause can lead to stromal cell hyperplasia and hence increased levels of circulating testosterone. Ovarian stromal cell hyperplasia was recognized over 50 years ago as often characterizing women who had died of breast cancer (33), whereas studies by our group (24, 25) have documented high testosterone levels in patients with breast cancer and ovarian interstitial cell hyperplasia, prompting us to suggest that oophorectomy should be considered for hyperandrogenemic postmenopausal breast cancer patients.

Finally, our study revealed that testosterone levels were significantly higher in women with HER2-negative breast cancer than those with HER2-positive breast cancer, but only in patients ≥70 years old, and that the association persisted after adjusting for estrogen receptor and progesterone receptor status. Thus, for most of our patients, testosterone levels were unrelated to HER2 status. Kahán et al. (23) also found no significant association between testosterone and HER2 status in postmenopausal breast cancer patients in the age range of 45 to 65 years (23). The relation of circulating testosterone to HER2 status in our oldest patients is intriguing and requires confirmation by further studies. It has been found that activation of the androgen pathway in prostate cancer cells cultured without androgens is induced by HER2 overexpression, whereas addition of androgens to the medium blocks HER2 overexpression (34). If such an interaction were to occur in breast cancer cells, it might explain the negative association of testosterone with HER2 overexpression.

To sum up, we have found that high testosterone is associated with hormone-dependent breast cancers in postmenopausal patients, in further support of the androgen excess theory of breast cancer development (1). Testosterone is therefore a marker of hormone-dependent disease, at least in postmenopausal patients independently of whether testosterone is responsible for the increased breast cancer risk, whether it acts after conversion to estrogen or whether it is simply an indicator of a general metabolic imbalance that provides a milieu favoring the development of cancer (1). Our view is that high androgen and high estrogen cooperate to promote breast cancer development (2–5). Nevertheless, we should not lose sight of the implication of the marker status of testosterone: It can be reliably measured in patients and healthy women to provide a practical indicator of prognosis or risk, respectively. By contrast, circulating levels of estrogen are too profoundly affected by changes in dietary habits, body weight, and lifestyle (5) to provide such an indicator. To further explore the relation of androgenic activity to breast cancer, we are planning to measure levels of the receptors for epithelial growth factor and androgens in breast cancers because both mediate the effect of androgenic steroids and at the same time measure circulating levels of dihydrotestosterone and estradiol.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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