The influence of hormone replacement therapy on the pathology of breast cancer

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Abstract
Aim of the study: To assess whether the pathological characteristics of breast carcinomas arising in post-menopausal women who ever used hormonal replacement therapy (HRT) differ from those of post-menopausal patients who never used HRT.

Materials and methods: Six hundred and forty three consecutive breast cancer patients were entered in a case control-study. Cases were represented by 111 breast cancer patients who had used or were using HRT at the time of diagnosis, while the remaining 532 patients who never used HRT were chosen as controls.

Results: Tumour diameter was smaller in HRT users (17.6 vs 22.1 mm; \( p = 0.002 \)) and tumours of lobular histology were almost twice more frequent among HRT users as in 'never users' (21 vs 12%; \( p = 0.01 \)). No differences were found in grading, hormonal receptor status and axillary nodal status. The expression of c-erb B-2, p53, Ki67 and PS2 measured by immunohistochemistry was similar in the two groups.

Conclusions: Our findings suggest that HRT use may modify the pathological presentation of breast cancer. Further studies are indicated, while other clinical-pathological characteristics did not differ according to HRT use.

Introduction
Results from observational studies and randomized controlled trials have shown that current and recent long-term use of HRT after the menopause increases the risk of breast cancer.\(^1\)\(^-\)\(^3\) Recent data suggest that the use of preparations containing estrogens plus progestins may be associated with a greater risk of breast cancer than estrogens alone.\(^2\)\(^-\)\(^4\)

The effect of HRT on mortality from breast cancer is still unclear. More than 20 years ago Burch et al. had already observed that the prognosis was better for women developing breast cancer either during or after HRT as compared to never users.\(^5\)

This finding was confirmed by many observational...
studies recently reviewed by Nanda, who suggested that the reduction of breast cancer mortality observed since 1990 may be at least in part attributable to the widespread use of HRT. Actually, this survival advantage is likely related to earlier diagnosis as women on HRT generally undergo more frequent clinical and mammographic examinations than women who do not take hormones. Nevertheless, a direct effect of HRT on tumour growth cannot be excluded and data are accumulating to suggest that breast cancers arising during or after HRT show less aggressive biological features.

On the contrary, the recent One Million Women study reported that current users of HRT had significantly increased mortality from breast cancer, although the relative risk (RR) estimates was of borderline significance. Also in the Women’s Health Initiative trial breast cancers diagnosed in women allocated to HRT were significantly larger than those diagnosed in the placebo arm, further challenging the validity of claims that mortality from breast cancer is lower in HRT users.

In this case-control study, we have compared the pathological characteristics of a series of breast tumours from post-menopausal women in order to assess whether carcinomas arising in hormone users (cases) differ from those of women who had never used HRT (controls). In order to limit the biases inherent to the study design, both cases and controls were chosen among patients diagnosed and treated at the same institution. Recall bias, unreliable memories and unreliable recording were minimized since information on HRT use was obtained by doctors at the time of hospital admittance for primary surgery and not by mail questionnaires or telephone calls. Nevertheless, interview bias could not be excluded since different doctors could have obtained this information with different accuracy.

**Patients and methods**

All post-menopausal patients who underwent surgery for primary breast carcinoma at Department of Gynaecological Oncology, Mauriziano ‘Umberto I’ Hospital of Turin and Institute for Cancer Research and Treatment of Candio, since the 1st of January 1998 to the 31st of December 2002 were identified in the institutional database. The following groups of patients were considered post-menopausal: patients who had previously undergone oophorectomy and patients who had their last menstrual period since at least 12 months, or since at least 3 months and FSH and LH serum levels above a cut-off value of 20 IU/l.

All patients with previous neoplastic disease were excluded from the study: data on age at menopause, age at diagnosis, body mass index (BMI) were recorded. In order to be considered HRT users, patients had to have used hormonal compounds for at least six consecutive months, either by the oral or transdermal routes of administration. Due to the relatively small number of HRT users, current and past users were grouped together. Histological examination of the surgical samples was carried out on 10-18 paraffin blocks including the tumour, the skin, the nipple and the remaining parenchyma as well. The histotype of breast carcinoma was classified according with the criteria suggested by WHO 2003. The histologic grade was based on semi-quantitative methods (tubule/gland formation, nuclear pleomorphism and mitotic count) according to Elston-Ellis scoring system. A careful search of lymph-nodes was performed clearing the axillary fat in a graded alcohol scale and each paraffin block was examined on two sections at different level of depth.

Hormonal receptor status was assessed according to the criteria suggested at the NIH Consensus Conference in 2000 and at the Consensus Meeting of St Gallen in 2001. C-erb B-2 status was assessed by the FDA approved Herceptest (score 1-3 + ). The MIB 1 index was categorized as low or high. Each case was reviewed by two pathologists in order to guarantee consistency of results.

**Statistical analysis**

Nominal variables were analysed by means of bivariate or multidimensional contingency tables. Potential association were evaluated by the chi square test and the Fisher exact test when requested for ordinal data and quantitative variables, associations were assessed by the Pearson’s correlation coefficient. The mean values of subgroup with dependent variables were compared by the Students’ t-test and variance analysis. The normal distribution of variables was assessed by the Kolmogorov-Smirnov’s test. For variables with non-normal distribution, a non-parametric analysis was performed by using the U Mann-Whitney test. The relative risk was determined by calculating the odds ratio and the 95% confidence interval.

**Results**

Medical records of 643 patients were identified and
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...divided into two groups according to whether they had or had not used HRT after the menopause. HRT had never been used by 532 patients, while 111 women had ever used HRT, 38 of which were current users at the time of cancer diagnosis.

Age at menopause, age at diagnosis and BMI were found to be significantly different in HRT users and non-users. The mean age at menopause was 47.7 years (SD 5.56) in HRT users and 50.1 years (SD 4.26) in never users (p < 0.0001). The mean age at diagnosis was lower in HRT users (59.9 years, SD 8.9) as compared to never-users (65.8 years; SD 9.1) (p < 0.0001). The BMI was lower in HRT users than in never users (24.1 vs 25.1; p = 0.027) (Table 1).

Breast carcinomas arising in the two groups of patients had different histopathological characteristics. The mean tumour diameter was lower in hormone users (17.6 mm; SD 0.61) than in never users (22.1 mm; SD 0.61) (p = 0.002).

Invasive lobular carcinoma (ILC) was almost twice as frequent in women who had ever used HRT compared to non-users (21 vs 12%). There was a trend toward a lower frequency of invasive ductal carcinomas (IDC) and a higher frequency of 'in situ' lesions in HRT users, but the difference among the two groups was not significant. The distribution of grading and receptor status was not statistically different in the two groups (Table 2). The evaluation of the lymph nodal status was available in 499 patients who underwent sentinel node biopsy or radical axillary dissection. Surgery on the axilla was not performed (Nr = 144) in patients with 'in situ' carcinomas, in patients older than 65 years with PT la, low-grade invasive cancer, or in patients with severe comorbidity. Overall, there was no difference in the lymph-node status between the two groups. By performing a subgroup analysis according to the number of involved nodes (1-3 and > 3), less women using HRT were found in the subgroup with > 3 involved nodes, although this difference did not reach statistical significance (31.4 vs 47.7%; p = 0.07) (Table 2).

The evaluation of other pathological variables was performed in a subset of patients for whom the complete dataset was available. No difference was observed between the two groups with respect to oncogene c-erb B-2 expression and the proliferative index Ki-67. The apoptotic marker p53 and the marker of estrogenic signal transduction PS2 were less frequently expressed among HRT users, although the difference was not significant (Table 3).

Discussion

Our findings confirm that differences do exist between post-menopausal women developing breast cancer depending if they have ever used HRT or not. It is well recognized that HRT duration, time since last HRT and type of HRT (estrogens alone vs estrogens plus progestins) may influence breast cancer risk; nevertheless, due to the limited size of the sample, we have been not be able to conduct meaningful subgroup analyses to disentangle their effect on the variables studied.

Age at menopause

HRT users experienced the menopause at a younger age and were also younger at the time of breast cancer diagnosis as compared to women who had never used estrogens. This findings are consistent with other published data.12–15

Tumour diameter

We observed that tumour diameter at diagnosis was significantly smaller in HRT users as compared to non-users. Most of the available evidence is consistent with our data,14,16–24 with the exception

Table 1  Clinical characteristics of 643 consecutive breast cancer patients by hormone replacement therapy (HRT) user/non-user status

<table>
<thead>
<tr>
<th>HRT use</th>
<th>Mean (years)</th>
<th>Standard error mean</th>
<th>p valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>No</td>
<td>65.9</td>
<td>.3976</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>59.9</td>
<td>.8476</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>No</td>
<td>50.1</td>
<td>.20</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>47.7</td>
<td>.61</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>No</td>
<td>25.2</td>
<td>.2004</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>24.1</td>
<td>.0387</td>
</tr>
</tbody>
</table>

BMI, body mass index.

a Student’s t-test.
of the WHI trial,7 in which HRT users have larger tumours as compared to the placebo group, and few other studies that did not find any difference in tumour size.25,26

Tumour histology

We found an almost two-fold increase in the frequency of invasive lobular cancers among HRT users as compared to non-users, while ductal carcinomas were not increased in our series. Six recent case-control studies showed a relative risk of 2.0–2.9 of developing invasive lobular carcinoma in current or past hormone users.27–32 Conversely, only two studies31,33 reported a significant increase of the risk of developing ductal tumours in HRT users. Data from the surveillance, epidemiology, and end results (SEER) of the National Cancer Institute34 clearly demonstrate a progressive increase of lobular carcinomas since 1999 in the USA, whereas the incidence of ductal carcinomas has remained virtually stable during the same time-period. The increase of lobular carcinomas has been observed among women of all ages, but is more pronounced

![Table 2 Tumour characteristic by HRT use](image_url)

![Table 3 Prognostic factors in breast cancer by HRT user/non-user status](image_url)

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NC/CLIS, ductal carcinoma in situ/lobular carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor.

a Pearson’s χ² test.
among post-menopausal women. The SEER data do not provide any information on HRT use, but there is evidence of a temporal coincidence between the increase of lobular carcinomas and the widespread use of HRT among post-menopausal women in the USA.\(^{34}\)

The increased frequency of invasive lobular carcinomas associated to HRT use, if confirmed, might have impact on current screening strategies; in fact, lobular carcinoma is more difficult to detect, both at clinical and mammographic evaluation. In spite of this, most of the literature data and also our own study suggest that tumour diameter at diagnosis is smaller among HRT users. As already pointed out, this is in contrast with the results of the WHI study, where the increased breast density associated with HRT use was deemed responsible for the reduced sensitivity of screening and, therefore, for the more advanced stage at diagnosis. Yet, in this study the distribution of invasive ductal, lobular and tubular carcinomas was similar in the HRT vs placebo groups.\(^{7}\)

### Tumour grading

We did not detect difference in the distribution of tumour grading according to HRT use. Literature data are inconsistent: many studies show higher frequencies of grade 1 tumours in women treated with HRT,\(^{17,18,20,22,24,34}\) while others did not find any difference.\(^{7,14,21,26}\)

### Nodal status

In contrast with many authors,\(^{7,15-17,20,23}\) and according to a few others,\(^{14,21,25}\) we did not find any difference in the rate of axillary positive nodes in the two groups of patients. Of interest, there was a non-significant lower percentage of patients with more than three involved nodes among HRT users as compared to controls; this finding is consistent with the literature data.\(^{18,21,34,35}\)

### Receptor status

No difference of ER and PgR expression in the tumours was found according to previous HRT use, as reported by others.\(^{14,17,18,20,25}\) Nevertheless, information on this issue are inconsistent, with some authors reporting lower ER expression among users,\(^{18,36}\) others higher PgR expression among users\(^{35}\) and others the highest expression of ER in women taking continuous combined regimens.\(^{16,34}\)

### Other prognostic tumour markers

We did not find significant differences in the expression of c-erbB-2, Ki 67, p53 and PS2 among HRT users and non-users, but the small sample size of this study and the lack of comparative data in the literature do not allow to draw any conclusion on this specific finding.

### Conclusions

Data are accumulating to support the hypothesis that breast cancers of women who ever used HRT show distinctive pathological characteristics. The most relevant finding is the almost two-fold increase of lobular carcinomas among HRT users. Further studies are warranted to elucidate whether differences of tumour characteristics, adherence to screening or type and length of HRT actually influence the prognosis of HRT users who develop breast cancer.

### References


