Association of Hormone Replacement Therapy to Estrogen and Progesterone Receptor Status in Invasive Breast Carcinoma

Wendy Y. Chen, M.D., M.P.H.[1,2]
Susan E. Hankinson, Sc.D.[2,3]
Stuart J. Schnitt, M.D.[4]
Bernard A. Rosner, Ph.D.[2]
Michelle D. Holmes, M.D., Dr. PH.[2]
Graham A. Colditz, M.D., Dr. PH.[2]

[1] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts.

[2] Channing Laboratory, Department of Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts.

[3] Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts.

[4] Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts.

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Address for reprints: Wendy Y. Chen, M.D., M.P.H., Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115; Fax: (617) 525-2008; E-mail: wychen@partners.org

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BACKGROUND. Observational studies and randomized trials have demonstrated that hormone replacement therapy (HRT) increases the recipient's risk of developing breast carcinoma. Because it is known that some breast malignancies are hormonally responsive and that others are not, it has been hypothesized that HRT may be associated with the development of estrogen receptor (ER)-positive/progesterone receptor (PR)-positive breast carcinoma more so than with the development of ER-negative/PR-negative breast carcinoma.

METHODS. The Nurses' Health Study is a prospective cohort study that enrolled 121,700 female registered nurses ages 30–55 years in 1976. In the current study, the authors analyzed 2548 malignancies that developed among eligible postmenopausal women in that cohort between 1980 and 2000 and for which data on ER and PR status were available.

RESULTS. Compared with women who had never used HRT, current long-term users of HRT were more likely to develop ER-positive/PR-positive breast carcinoma (multivariate risk ratio [RR], 1.80; 95% confidence interval [CI], 1.52–2.12) but were not any more likely to develop ER-negative/PR-negative disease (multivariate RR, 1.00; 95% CI, 0.72–1.39). This effect grew stronger with increasing duration of current HRT use and was also more pronounced among women with body mass index < 25 kg/m^2. Furthermore, the association between HRT use and ER-positive/PR-positive disease was stronger among patients receiving combined HRT (CHRT) regimens, which included estrogen and progesterone, than among users of estrogen alone (ERT). In addition, tumors tended to develop more quickly in current users of CHRT than in ERT users.

CONCLUSIONS. The finding that current users of HRT were more likely to develop ER-positive/PR-positive tumors than they were to develop ER-negative/PR-negative ones suggests that both endogenous and exogenous hormonal factors may influence breast tumor characteristics. In analyses of the effects of hormonal factors on breast tumor development, ER-positive/PR-positive tumors and ER-negative/PR-negative tumors should be considered separately from each other. ***Cancer*** **2004;101:1490–500.** © *2004 American Cancer Society.*

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In observational studies and randomized controlled trials, hormone replacement therapy (HRT) has been shown to increase breast carcinoma risk; furthermore, breast carcinoma risk has been shown to increase with increasing duration of HRT use.[1,2] Nonetheless, it remains controversial as to whether HRT use is more strongly associated with hormone receptor–positive breast carcinoma than with hormone receptor–negative breast carcinoma. Findings made in pre-

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vious studies have been inconsistent, with some studies indicating that HRT use only increases the risk of hormone receptor–positive tumor development[3–7] and others reporting no difference between receptor-positive and receptor-negative tumors in terms of the HRT-induced risk increase.[8–11] Many of these studies were hampered by limited numbers of cases or by a lack of complete information regarding other breast carcinoma risk factors. In addition, several studies grouped past and current users of HRT into a single group, a practice that may have obscured important interactions, as the effects of current HRT use are much stronger than the effects of past use.[1,12] Finally, most studies examined estrogen receptor (ER) status and progesterone receptor (PR) status separately and did not consider tumors with mixed receptor status (i.e., ER-positive/PR-negative and ER-negative/PR-positive tumors) separately from those that had uniformly positive or uniformly negative hormone receptor status. Tumors with mixed receptor status may exhibit an intermediate response to exogenous hormonal interventions (e.g., tamoxifen therapy),[13] and the influence of HRT on the development of such tumors is not well understood. Furthermore, combination HRT (CHRT) regimens, which include both estrogen and progesterone, appear to increase breast carcinoma risk more dramatically compared with estrogen replacement therapy (ERT) alone,[2,14–16] although it is not known whether these two interventions differ with respect to the hormone receptor status of the resulting tumor.

The purpose of the current analysis was to evaluate the relation between HRT use and hormone receptor status in breast tumors found in postmenopausal women enrolled in the Nurses' Health Study, a prospective cohort study initiated in 1976.

MATERIALS AND METHODS

The Nurses' Health Study cohort was recruited in 1976, when 121,700 female registered nurses ages 30–55 years who resided in one of 11 U.S. states (California, Florida, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, Pennsylvania, or Texas) completed a baseline questionnaire that included items addressing risk factors for the development of malignancy and cardiovascular disease. Every 2 years since then, follow-up questionnaires have been sent out with the goal of updating information on risk factors and the development of disease. The institutional review board at Brigham and Women's Hospital (Boston, MA) approved the current study protocol. The study population was predominantly Caucasian (84.1%); of the remaining participants, 1.6% were of African descent, 0.7% were of Hispanic descent, 0.9% were of Asian descent, and 12.7% were of other or unknown ethnic origin. This distribution reflected the demographic characteristics of registered nurses in the U.S. in 1976. No additional women were enrolled in the study after 1976. The participation rate was extremely high, with less than 6% of all person-time being lost to follow-up. (Participants who actively withdrew from the study or who had not returned a questionnaire in more than 10 years were considered to be lost to follow-up.) Furthermore, because we routinely searched the National Death Index for the status of nonresponders, mortality data were even more complete (98%).[17]

To control for risk factors such as alcohol consumption, the current analysis was limited to postmenopausal women who completed the baseline dietary questionnaire in 1980. A total of 98,462 nurses completed and returned their initial dietary questionnaires. Follow-up dietary questionnaires were distributed in 1984, 1986, 1988, 1990, and 1994. A participant was considered to have begun contributing person-time to the current analysis at the time of her first report of menopause. Participants were classified as having postmenopausal status when they reported the occurrence of natural menopause or when they reported undergoing hysterectomy with bilateral oophorectomy. Self-reports of natural menopause and of the extent of ovarian surgery have been shown to be highly accurate and reproducible in the current cohort.[18] Women who reported cessation of their menses following hysterectomy without bilateral oophorectomy were considered to be hormonally premenopausal and thus were excluded from the analysis until the age at which natural menopause had occurred in 90% of the remainder of the study cohort (54 years for current cigarette smokers and 56 years for nonsmokers), at which time they were considered to have postmenopausal status. Menopausal status data were updated every 2 years. Therefore, the population of interest includes women who reported experiencing menopause in 1980 as well as those who reported experiencing menopause at any time between 1980 and the end of the follow-up period for the current analysis. Women for whom HRT-related data on a specific questionnaire were unavailable were excluded from the analysis beginning on the date of that questionnaire but were reentered at the next date on which complete information on HRT use was available.

All types of postmenopausal HRT, including ERT and CHRT, were considered in the current analysis. In analyses in which patients were stratified according to HRT type, ERT was defined as the oral administration of conjugated estrogens, whereas CHRT was defined as the sequential or concurrent oral administration of

estrogen and progesterone. Past HRT users were analyzed separately from current users, because the effect of current HRT use on breast carcinoma risk is stronger than the effect of past use. Current HRT users were further subdivided according to treatment duration, because breast carcinoma risk increases with increasing duration of current HRT use.[1,12]

The primary endpoint of the current study was the diagnosis of invasive breast carcinoma. Each questionnaire asked whether breast carcinoma had been diagnosed and, if so, when it was diagnosed. We asked participants who reported having been diagnosed with breast carcinoma (or, for those who died, their next of kin) for permission to review the pertinent medical records for confirmation. We also searched the National Death Index for reports of breast carcinoma–related death among nonresponders. Pathology reports were obtained in 96% of all cases, and physicians abstracted information regarding hormone receptor status from these reports. For the purposes of the analysis, tumors classified as having borderline-positive ER or PR status were considered to be positive for expression of the receptor in question. Central pathologic review was not performed. Among the hormone receptor assays performed were biochemical assays, which predominated during the early years of follow-up, and immunohistochemical (IHC) assays, which predominated in subsequent years. Carcinomas in situ were excluded from the analysis, because data on hormone receptor status in such tumors were not consistently available. Follow-up for the identification of breast carcinoma cases was estimated to be 95% complete.

The follow-up period for patients in the current analysis began in 1980 and ended with death or the diagnosis of any type of malignancy (whichever occurred first) or on June 1, 2000, if neither death nor a diagnosis of malignant disease was reported. HRT use status (*current*, *past*, or *never*) as reported on each biennial questionnaire was used to prospectively categorize patients for the 2-year period that followed. Polytomous logistic regression, which can be used to model multiple outcomes simultaneously, allowed us to compute age-adjusted and multivariable-adjusted risk ratios (RRs) with 95% confidence intervals (CIs). Covariates in the current model included age (continuous), age at menopause (continuous), type of menopause (natural, surgical with bilateral oophorectomy, or other), age at menarche (continuous), parity/age at first birth (nulliparous; 1–2 children and age $\leq$ 22 years at first birth; 1–2 children and age 23–25 years at first birth; 1–2 children and age $\geq$ 26 years at first birth; $\geq$ 3 children and age $\leq$ 22 years at first birth; $\geq$ 3 children and age 23–25 years at first birth; $\geq$ 3 children and age $\geq$ 26 years at first birth; or data on parity and/or age at first birth unavailable), weight gain of $>$ 10 kg since age 18 years (yes or no), family history of breast carcinoma in a first-degree relative (yes or no), average daily alcohol consumption (none, 0.1–4.9 grams per day, 5.0–9.9 grams per day, 10–19.9 grams per day, or $\geq$ 20 grams per day), questionnaire cycle, and self-reported benign breast disease (yes or no). Past oral contraceptive use was not included in the model, as it was not significantly associated with postmenopausal breast carcinoma risk in the current cohort.

Interactions were evaluated using a log–likelihood ratio test that included a cross-product interaction term and using a Wald test for that interaction term. Tests for trends involving duration of HRT use were performed using a continuous term to represent the length of HRT. All analyses were performed using SAS software (Version 8.0; SAS Inc., Cary, NC), except for comparisons within polytomous models (e.g., ER-positive/PR-positive vs. ER-negative/PR-negative), which were performed using a FORTRAN program that employed a log–likelihood ratio test to compare the goodness-of-fit of the various models. A two-sided P value of less than 0.05 was considered indicative of statistical significance.

RESULTS

In the current analysis, 27,701 women met the aforementioned criteria and were entered at the start of the initial follow-up period (1980–1982). By 1998, the first year of the final 2-year follow-up period, 59,481 participants were postmenopausal and thus eligible for the analysis. During follow-up, 3032 invasive breast tumors for which pathologic data were available were reported by eligible participants. Data on both ER and PR status were available in 2548 (84.0%) of these cases. Tumors diagnosed in women who had never received HRT were more likely to lack data on ER/PR status than were tumors diagnosed in current HRT users (18.5% vs. 14.2%; $P < 0.01$). Cases for which data on ER and/or PR status were unavailable were excluded from the analysis. Of the tumors included in the current analysis, 1546 (60.7%) had positive ER status and positive PR status, 461 (18.1%) had positive ER status and negative PR status, 83 (3.3%) had negative ER status and positive PR status, and 458 (18.0%) had negative ER status and negative PR status. This distribution was comparable to the corresponding distribution among postmenopausal women in the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database.[19]

Because of their rarity, ER-negative/PR-positive tumors were grouped together with ER-positive/PR-

TABLE 1
Breast Carcinoma Risk Factor Distributions According to HRT Use at Baseline[a]

Characteristic	Never used HRT (n = 14,510)	Currently use HRT (n = 5714)
Mean age (yrs)	53.8	51.4
Mean age at menopause (yrs)	48.2	43.5
Natural menopause (%)	86.3	26.4
Age at menarche (%)		
≤ 11 yrs	20.7	22.3
12–13 yrs	56.9	55.6
≥ 14 yrs	22.4	22.1
Age at first full-term birth (%)[b]		
Nulliparous	7.6	10.3
≤ 22 yrs	14.8	20.8
23–25 yrs	37.7	38.7
≥ 26 yrs	38.5	29.0
Parity (%)		
Nulliparous	7.6	10.3
1–2 births	29.4	36.5
≥ 3 births	62.9	53.3
BMI < 25 kg/m^2 (%)	57.3	68.9
Breast carcinoma in a first-degree relative (%)	7.8	6.7
Personal history of benign breast disease (%)	4.3	6.9

HRT: hormone replacement therapy; BMI: body mass index.

[a] 1980.

[b] Data on age at first full-term birth were unavailable for 1.4% of all nonusers and 1.2% of all current users.

negative tumors in the *mixed ER/PR status* category. The ER-positive/PR-positive, mixed ER/PR status, and ER-negative/PR-negative categories were selected a priori because they differ with respect to the probability of tumor response to hormonal interventions (e.g., tamoxifen therapy); this finding suggests that tumors in these categories may exhibit differential sensitivity to hormonal influences. Approximately half of all tumors were assayed using biochemical methods, whereas the remaining tumors were assayed using IHC methods. Tumors with mixed ER/PR status were more likely to have been assayed using biochemical methods than were ER-positive/PR-positive or ER-negative/PR-negative tumors (percentage of tumors in a given category assessed using biochemical methods: mixed status, 61.6%; ER-positive/PR-positive, 45.5%; ER-negative/PR-negative, 48.7%).

Table 1 compares the characteristics of women who had never used HRT with the characteristics of those who were using HRT at baseline (i.e., in 1980). At baseline, current HRT users were more likely to have a body mass index (BMI) < 25 kg/m^2, to have experienced surgically induced menopause, to have a personal history of benign breast disease, and to be nulliparous compared with women who had never received HRT.

Table 2 summarizes the relation between HRT use and hormone receptor status–specific breast carcinoma risk. HRT use was associated with a significant increase in the risk of developing ER-positive/PR-positive breast carcinoma, but not in the risk of developing ER-negative/PR-negative disease. Also notable was our finding that the association between HRT use and tumors with mixed ER/PR status was stronger than the association between HRT use and ER-negative/PR-negative disease but weaker than the association between HRT use and ER-positive/PR-positive disease. Among HRT recipients with < 5 years of current use, there was no significant relation between HRT use and the observed pattern of hormone receptor expression ($P = 0.36$ for overall comparison). In contrast, among women with ≥ 10 years of current HRT use, there was an elevated risk of developing ER-positive/PR-positive disease (RR, 1.80 [95% CI, 1.52–2.12]), but not ER-negative/PR-negative disease (RR, 1.00 [95% CI, 0.72–1.39]; $P = 0.06$ for overall comparison and 0.03 for comparison of ER-positive/PR-positive breast carcinoma risk with ER-negative/PR-negative breast carcinoma risk); a similar risk pattern was observed among women with 5–9.9 years of current HRT use. The risk of developing tumors with mixed ER/PR status was intermediate between the risk of developing hormone receptor–negative disease and the risk of developing hormone receptor–positive disease. Table 3 lists parameter estimates (with standard errors) obtained from the associated logistic regression model.

Because inaccuracies in the reporting of age at menopause can bias results,[20] and for the sake of replicating the eligibility criteria for the CHRT arm in the Women's Health Initiative study,[21] the current cohort was reanalyzed after excluding women who experienced surgically induced menopause (Table 4). Results were similar, with many of the observed effects being slightly stronger than in the original analysis. HRT use remained more likely to be associated with ER-positive/PR-positive tumors than with tumors exhibiting other patterns of hormone receptor expression.

Women receiving HRT may be more likely to comply with breast cancer screening recommendations and thus may have increased rates of breast carcinoma detection relative to women who have never received HRT; consequently, yet another reanalysis of the current cohort was limited to women who reported receipt of a screening mammogram or a clinical breast examination within 2 years before questionnaire return. Data on screening were available only from 1990 onward. Overall rates of screening mam-

TABLE 2
Relation between HRT Use and Breast Carcinoma Risk According to Hormone Receptor Status

	Multivariate RR (95% CI)[a] for breast tumor development (n = 2548)					
HRT use status	**ER−/PR−**	***n***	**Mixed ER/PR status[b]**	***n***	**ER+/PR+**	***n***
Never	1.0 (ref)	183	1.0 (ref)	202	1.0 (ref)	481
Past	0.69 (0.52–0.92)	76	1.01 (0.79–1.28)	113	1.08 (0.93–1.25)	313
Current						
< 5 yrs	0.96 (0.71–1.28)	67	1.33 (1.02–1.74)	85	1.34 (1.14–1.59)	218
5–9.9 yrs	1.11 (0.82–1.50)	65	1.16 (0.85–1.57)	57	1.67 (1.41–1.96)	283
≥ 10 yrs	1.00 (0.72–1.39)	67	1.59 (1.19–2.14)	87	1.80 (1.52–2.12)	301
P for trend in association with current HRT use	0.38		0.0015		< 0.0001	

RR: risk ratio; CI: confidence interval; HRT: hormone replacement therapy; ER: estrogen receptor; PR: progesterone receptor; −: negative; +: positive; ref: referent group.

[a] Adjusted for age, age at menopause, type of menopause, age at menarche, parity, age at first birth, weight gain since age 18 years, history of breast carcinoma in a first-degree relative, average daily alcohol consumption, questionnaire cycle, and personal history of benign breast disease.

[b] Includes estrogen receptor–positive/progesterone receptor–negative tumors and estrogen receptor–negative/progesterone receptor–positive tumors.

TABLE 3
Beta Coefficients for Breast Carcinoma Risk Factors

	Beta coefficient (standard error)		
	ER−/PR−	**Mixed ER/PR status[b]**	**ER+/PR+**
Intercept	−7.85 (0.82)[c]	−10.51 (0.78)[c]	−10.32 (0.50)[c]
Age (yrs)	−0.0011 (0.0090)	0.032 (0.0085)[c]	0.032 (0.0049)[c]
Age at menopause (yrs)	0.020 (0.010)	0.034 (0.0099)[c]	0.031 (0.0056)[c]
Age at menarche (yrs)	−0.024 (0.033)	−0.032 (0.031)	−0.031 (0.018)
Surgically induced menopause	0.11 (0.14)	−0.34 (0.14)[c]	−0.22 (0.082)[c]
Family history of breast carcinoma	0.50 (0.13)[c]	0.59 (0.12)[c]	0.42 (0.072)[c]
Personal history of benign breast disease	0.46 (0.11)[c]	0.47 (0.10)[c]	0.41 (0.06)[c]
History of large change in body weight	0.035 (0.096)	0.058 (0.088)	0.21 (0.053)[c]
Parity/age at first birth			
1–2 births, ≤ 22 yrs	−0.52 (0.29)	−0.32 (0.26)	−0.26 (0.15)
1–2 births, 23–25 yrs	−0.21 (0.21)	−0.10 (0.19)	−0.32 (0.12)[c]
1–2 births, > 25 yrs	−0.090 (0.19)	−0.018 (0.17)	−0.090 (0.11)
≥ 3 births, ≤ 22 yrs	−0.36 (0.20)	−0.50 (0.19)	−0.34 (0.11)[c]
≥ 3 births, 23–25 yrs	−0.17 (0.18)	−0.30 (0.17)	−0.24 (0.098)[c]
≥ 3 births, > 25 yrs	−0.15 (0.20)	−0.17 (0.18)	−0.017 (0.10)
Alcohol consumption (g/day)			
0.1–4.9	0.09 (0.11)	0.12 (0.10)	0.057 (0.06)
5.0–9.9	0.18 (0.16)	0.033 (0.15)	0.045 (0.093)
10.0–19.9	0.056 (0.16)	0.011 (0.14)	0.30 (0.079)[c]
≥ 20	0.084 (0.19)	0.24 (0.16)	0.34 (0.10)[c]
Hormone replacement therapy use			
Previous	−0.37 (0.14)	0.0094 (0.13)	0.078 (0.085)
Current, < 5 yrs	−0.41 (0.15)	0.29 (0.14)[c]	0.30 (0.085)[c]
Current, 5–9.9 yrs	0.10 (0.15)	0.15 (0.16)	0.51 (0.084)[c]
Current, ≥ 15 yrs	−0.0005 (0.17)	0.47 (0.15)[c]	0.59 (0.09)[c]
Per yr of current use	0.00073 (0.00083)	0.0024 (0.00074)[c]	0.0033 (0.00040)[c]

ER: estrogen receptor; PR: progesterone receptor; −: negative; +: positive.

[a] Note: Questionnaire cycle was not included in the table, as this variable was divided into 10 categories and therefore would make the table cumbersome without providing a significant amount of additional information.

[b] Includes estrogen receptor–positive/progesterone receptor–negative tumors and estrogen receptor–negative/progesterone receptor–positive tumors.

[c] $P < 0.05$.

TABLE 4
Relation between HRT Use and Breast Carcinoma Risk among Recently Screened Women and among Women Who Experienced Natural Menopause

	Multivariate RR (95% CI)[a]											
	Women with screening mammogram or clinical breast examination in preceding 2 yrs[b] (n = 2260)						Women who experienced natural menopause (n = 1659)					
HRT use status	ER−/PR−	n	Mixed ER/PR status[c]	n	ER+/PR+	n	ER−/PR−	n	Mixed ER/PR status[b,c]	n	ER+/PR+	n
Never	1.0 (ref)	156	1.0 (ref)	178	1.0 (ref)	394	1.0 (ref)	157	1.0 (ref)	167	1.0 (ref)	395
Past	0.69 (0.51–0.93)	68	0.94 (0.79–1.28)	97	1.08 (0.92–1.27)	268	0.60 (0.42–0.86)	39	1.01 (0.76–1.34)	71	1.11 (0.93–1.33)	195
Current												
< 5 yrs	0.97 (0.71–1.33)	62	1.25 (0.94–1.65)	79	1.39 (1.17–1.66)	207	1.04 (0.74–1.46)	49	1.35 (0.99–1.83)	63	1.40 (1.15–1.69)	166
5–9.9 yrs	1.14 (0.83–1.56)	62	1.03 (0.74–1.43)	50	1.71 (1.43–2.03)	218	0.92 (0.60–1.41)	27	1.39 (0.97–1.99)	39	1.88 (1.55–2.29)	147
≥ 10 yrs	0.99 (0.70–1.39)	64	1.48 (1.09–2.03)	80	1.79 (1.50–2.15)	277	0.91 (0.54–1.55)	17	2.04 (1.39–2.99)	37	1.69 (1.32–2.16)	90[a]
P for trend involving current HRT use	0.42		0.011		< 0.0001		0.81		0.001		< 0.0001	

RR: risk ratio; CI: confidence interval; HRT: hormone replacement therapy; ER: estrogen receptor; PR: progesterone receptor; −: negative; +: positive; ref: referent group.

[a] Adjusted for risk factors listed in Table 2.

[b] Analysis was limited to person-time associated with women who reported receiving a screening mammogram or clinical breast examination within the preceding 2 years (from the 1990 questionnaire cycle onward). Before 1990, data on mammographic screening and clinical breast examination were not available, and thus all person-time was included.

[c] Includes estrogen receptor–positive/progesterone receptor–negative tumors and estrogen receptor–negative/progesterone receptor–positive tumors.

TABLE 5
Relation between HRT Use and Breast Carcinoma Risk According to Tumor Hormone Receptor Status and HRT Type

	Multivariate RR (95% CI)[a] (n = 1891)					
HRT use status	ER−/PR−	n	Mixed ER/PR status[b]	n	ER+/PR+	n
Never	1.0 (ref)	183	1.0 (ref)	202	1.0 (ref)	481
Past	0.69 (0.52–0.92)	76	0.98 (0.77–1.25)	113	1.05 (0.91–1.23)	313
Current						
< 5 yrs						
Estrogen alone	0.70 (0.41–1.20)	16	1.13 (0.72–1.78)	22	1.02 (0.77–1.38)	46
Estrogen + progesterone	1.11 (0.74–1.67)	31	1.40 (0.97–2.01)	37	1.74 (1.40–2.17)	112
5–9.99 yrs						
Estrogen alone	0.97 (0.61–1.53)	24	0.93 (0.57–1.52)	19	1.37 (1.06–1.78)	73
Estrogen + progesterone	1.16 (0.73–1.82)	22	1.30 (0.84–2.03)	23	2.05 (1.64–2.57)	99

RR: risk ratio; CI: confidence interval; HRT: hormone replacement therapy; ER: estrogen receptor; PR: progesterone receptor; −: negative; +: positive; ref: referent group.

[a] Adjusted for risk factors listed in Table 2.

[b] Includes estrogen receptor–positive/progesterone receptor–negative tumors and estrogen receptor–negative/progesterone receptor–positive tumors.

mography use and receipt of clinical breast examination were relatively high within this subcohort. Again, the observed effects were similar to, but slightly stronger than, the effects documented in the original analysis (Table 4). A repeat analysis limited to women with mammographically detected breast carcinoma yielded similar results (data not shown).

Table 5 summarizes the relation between current HRT use and invasive breast carcinoma risk according to both the hormone receptor status of the tumor and the type of HRT used. Among women with ≥ 10 years of current HRT use, the mean duration of current ERT use (17.4 years [1998 questionnaire]) greatly exceeded the duration of current CHRT use (13.2 years [1998 questionnaire]) ($P < 0.0001$ [t test]). Therefore, to avoid biases caused by differences in the length of HRT, current HRT use was censored at 10 years. If open-ended categories (e.g., duration of HRT use ≥ 5 years) are used, the effects of ERT appear to be much stronger than the effects of CHRT, although much of

this difference can be attributed to the observed difference in average duration of use.

In addition to excluding women with > 10 years of current HRT use, we also excluded those who reported using other or unknown types of HRT; these exclusions left 1891 cases for the analysis of ERT versus CHRT. Irrespective of HRT type, HRT use was associated only with an increase in ER-positive/PR-positive (and not ER-negative/PR-negative) breast carcinoma risk. Again, an intermediate risk of developing tumors with mixed ER/PR status was noted. As in other studies, the overall breast carcinoma risk associated with CHRT use was greater than the risk associated with ERT use. Furthermore, ER-positive/PR-positive breast carcinoma risk appeared to increase more rapidly with increasing CHRT use; the RR associated with < 5 years of current ERT use was 1.02 (95% CI, 0.77–1.38), whereas the RR associated with the same duration of current CHRT use was 1.74 (95% CI, 1.40–2.17) ($P = 0.003$). Among women with 5–9.9 years of current HRT use, breast carcinoma risk was statistically significantly increased for both ERT users (RR, 1.37 [95% CI, 1.06–1.78]) and CHRT users (RR, 2.05 [95% CI, 1.64–2.57]), although again, the risk associated with CHRT was greater than the risk associated with ERT alone ($P = 0.01$).

Women who reported receiving unknown or other types of HRT ($n = 298$) were included in a separate analysis. In this analysis, no strong association between elevated breast carcinoma risk and < 5 years of current HRT use was found. Most of these 298 women were receiving alternative forms of estrogen-based therapy (e.g., estradiol, estropipate, or a combination of esterified estrogens and methyltestosterone), whereas a minority of women were receiving oral progesterone alone. Women with 5–9.9 years of current use of other or unknown types of HRT had an elevated ER-positive/PR-positive breast carcinoma risk (RR, 1.51 [95% CI, 1.16–1.97]) but did not have an elevated risk of developing tumors with mixed ER/PR status (RR, 1.07 [95% CI, 0.63–1.81]) or negative ER/PR status (RR, 1.14 [95% CI, 0.70–1.66]). As expected, these results were similar to those documented in women receiving unopposed estrogen.

Because the effects of HRT may vary according to BMI,[1,16,22,23] and because obesity is associated with higher levels of circulating endogenous estrogen,[24] additional analyses in which patients were stratified according to BMI were performed (Figure 1). Furthermore, to take into account the differential effect of obesity on premenopausal and postmenopausal breast carcinoma risk, the analysis also controlled for weight at age 18 years. The influence of HRT use on ER-positive/PR-positive breast carcinoma risk was

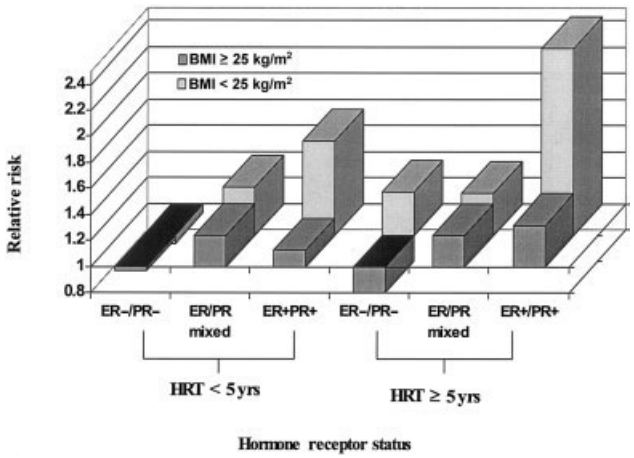

FIGURE 1. Breast carcinoma risk stratified according to body mass index (BMI), hormone receptor status, and duration of hormone replacement therapy (HRT). Women who have never used HRT serve as the referent group for both strata. ER: estrogen receptor; PR: progesterone receptor; −: negative; +: positive.

greatest for women with BMI < 25 kg/m^2; women with BMI < 25 kg/m^2 and ≥ 5 years of current HRT use had an RR of 2.43 (95% CI, 1.96–3.01), whereas their counterparts with BMI ≥ 25 kg/m^2 had an RR of 1.32 (95% CI, 1.09–1.61) ($P = 0.004$). BMI had no apparent association with the risk of developing breast carcinoma with mixed or negative ER/PR status. Waist-to-hip ratio also was investigated but was not included in the final analysis because it was not as strongly predictive of breast carcinoma risk as was BMI/weight change since age 18 years and because data regarding this ratio were less complete. Adjustment for current BMI quintile also did not significantly alter our findings. Additional stratification according to HRT type and BMI resulted in case numbers that were too small to allow meaningful comparisons.

To address the issue of whether the different assay types introduced bias into our findings, analyses were performed separately for tumors assayed using biochemical methods and tumors assayed using IHC methods. Results were similar, with differences among the three hormone receptor status categories being slightly weaker in the group of tumors that were assayed biochemically. The most likely explanation is that biochemical assays, which are less sensitive than IHC assays, led to the misclassification of some ER-positve/PR-positive or mixed-status tumors as ER-negative/PR-negative tumors, thereby attenuating any differences observed among the three categories. To assess the extent to which cases with unavailable data on ER/PR status biased our results, sensitivity analyses were conducted under a range of assumptions regard-

ing ER/PR status in such cases. Even in the most extreme scenarios (e.g., all tumors with unavailable data had positive ER/PR status or all tumors with unavailable data had negative ER/PR status), our findings were not substantially different. Finally, to address the potential association between HRT use and early-stage tumors, which may be more likely to have positive ER/PR status, we performed an analysis that was limited to Stage I breast carcinoma; this analysis also yielded similar findings.

DISCUSSION

Our findings indicate that current HRT use is more likely to be associated with the development of ER-positive/PR-positive tumors than with the development of ER-negative/PR-negative tumors. Furthermore, for HRT users, the risk of developing tumors with mixed ER/PR status was intermediate between the risk of developing hormone receptor–negative disease and the risk of developing hormone receptor–positive disease. This association between HRT use and hormone receptor expression was evident in patients who used ERT as well as in patients who used CHRT, although it was stronger in the latter group. Breast carcinoma risk (and particularly ER-positive/PR-positive breast carcinoma risk) also was elevated among HRT users with BMI < 25 kg/m^2 relative to users with BMI ≥ 25 kg/m^2.

In breast carcinoma prevention trials conducted in the United States and Europe,[25] tamoxifen has exhibited efficacy in decreasing the risk of ER-positive tumor development, but not in reducing the risk of ER-negative tumor development. Therefore, the hypothesis that HRT is associated only with the development of ER-positive disease is biologically plausible. Previous studies have also suggested that other hormonal risk factors, such as early age at menarche,[26,27] nulliparity,[26,28] late age at first birth,[28] and BMI,[26,28] have a more pronounced effect on hormone receptor–positive tumors than on hormone receptor–negative tumors. Finally, according to the SEER database, the incidence of hormone receptor–positive breast carcinoma continues to increase, whereas the incidence of hormone receptor–negative breast carcinoma has remained constant over time.[29] Although much of the observed increase in breast carcinoma incidence has been attributed to increased screening use, HRT may also be partially responsible for the differential increase in hormone receptor–positive breast carcinoma incidence over time, as this differential increase appears to mirror reported increases in HRT use (as of last year's Women's Health Initiative report on HRT[30]).

Several studies have failed to demonstrate a correlation between HRT use and tumor hormone receptor status,[8–11] possibly due to insufficient analytic power resulting from the limited numbers of cases available. In the current study, detailed data on HRT type and duration of HRT use were available, and past and current HRT users were analyzed separately from each other. Some studies have separated ER-positive/PR-negative tumors from ER-negative/PR-positive ones to evaluate the individual contributions of ER status and PR status to hormonal sensitivity; however, due to the limited number of ER-negative/PR-positive tumors ($n = 83$) in the current study, we were unable to perform such an analysis with stratification according to HRT use, and therefore, ER-negative/PR-positive tumors were grouped together with ER-positive/PR-negative tumors. The majority of tumors with mixed ER/PR status were positive for ER expression and negative for PR expression (84.6%); thus, findings made in the mixed-status group primarily reflect the association between HRT and ER-positive/PR-negative breast carcinoma. Similar results were obtained when ER-negative/PR-positive tumors were excluded from this group.

Central pathologic review was not performed, and approximately half of all breast tumors analyzed had their ER/PR status assessed by biochemical methods, which can be less sensitive than IHC methods. As was confirmed in a subgroup analysis, the use of a less sensitive assay would attenuate, rather than exaggerate, any detected differences. Therefore, if all tumors had been assessed by IHC methods, which now are commonly used, it is likely that the observed differences according to ER/PR status would have been greater than those that were actually documented. Questionnaire cycle also was controlled for in the current study to account for secular time trends.

The Women's Health Initiative, which has published data on breast tumor characteristics in relation to HRT, reported no difference in ER status or PR status between patients receiving placebo and patients receiving CHRT.[21] Nonetheless, this conclusion was based on findings from 182 cases in the CHRT arm and 127 cases in the placebo arm for which data on ER status were available, and tumors were not classified according to joint ER/PR status. Another point to be considered is that the median follow-up duration in the Women's Health Initiative study was only slightly greater than 5 years; in the current analysis, differences between hormone receptor status categories grew stronger as the duration of HRT use increased. The Women's Health Initiative also recently reported that the use of estrogen alone did not significantly increase breast carcinoma risk.[31] The results of the current study may still be consistent with those

reported by the Women's Health Initiative, as we observed an increase in risk only with respect to ER-positive/PR-positive tumors and only after longer durations of current HRT use (i.e., $>$ 5 years).

Like several other investigators, we found that HRT use elevated breast carcinoma risk to a greater extent in leaner women.[1,22,32] Women with lower BMIs may also have lower circulating endogenous hormone levels; if this were the case, then exogenous estrogens would have a greater impact on the underlying hormonal milieu in these women compared with women with higher BMIs. This scenario provides an example of the way in which endogenous and exogenous hormonal factors can interact to influence breast tumor characteristics.

Conjugated estrogens were the most commonly used ERT agents in the current study population, and conjugated estrogens and medroxyprogesterone were the most commonly used CHRT agents. As is evident from the case numbers reported in Table 5, we did not have sufficient analytic power to further stratify patients according to type of progesterone administration (i.e., sequential or concurrent). As in the Women's Health Initiative study, CHRT (compared with ERT) appeared to be associated with a more pronounced increase in breast carcinoma risk after $<$ 5 years of current use and also with a greater breast carcinoma risk overall.

Because of the observational nature of the Nurses' Health Study, HRT use was not randomly assigned; therefore, breast carcinoma risk factors were not evenly distributed between women who had never used HRT and women who were current users. The principal risk factors that were consistently associated with the preferential development of ER-positive/PR-positive tumors rather than ER-negative/PR-negative tumors in the current cohort were older age and surgically induced menopause. HRT users tended to be younger than nonusers and thus might have been expected to develop ER-negative/PR-negative tumors more frequently; however, we found the exact opposite to be true. Ovarian suppression has been used to treat premenopausal women with hormone receptor–positive tumors.[33] HRT users were more likely to have experienced surgically induced menopause than were women who had never attempted postmenopausal hormone use, and again, a bias against hormone receptor–positive tumors in HRT users would be expected to result; nonetheless, again, the actual findings of the current study ran counter to the effects of this bias. When the analysis was limited to women who experienced natural menopause, the observed associations between HRT use and hormone receptor status were similar if not slightly stronger.

HRT users also were more likely to undergo mammographic screening and to receive clinical breast examinations. Although frequent screening may lead to the diagnosis of smaller, earlier-stage tumors,[30,34,35] it is not clear as to whether screening use would influence the likelihood of detecting tumors with positive ER/PR status after tumor size was controlled for.[36] Overall mammographic screening rates were high within the current cohort, and consequently, screening was less likely to be a source of bias. Regardless, when the current analysis was restricted to women who had recently received a screening mammogram or a clinical breast examination, our findings were essentially unchanged. Most participants in the Nurses' Health Study are Caucasian, and thus it is not known whether the findings of the current study also apply to members of other racial/ethnic groups. The distribution of ER/PR status in breast tumors is known to vary according to ethnicity.[37]

It is not known how the preferential development of ER-positive/PR-positive breast tumors in HRT users would affect disease-related mortality, as hormone receptor–positive tumors may be associated with a more favorable prognosis compared with hormone receptor–negative tumors.[38] The effects of HRT on other tumor characteristics, such as histology, size, grade, and lymph node status, will be investigated in future analyses of the Nurses' Health Study cohort. Because of the observational nature of the Nurses' Health Study, no conclusions regarding causality can be made, and the hypothesis that HRT stimulates the growth of occult ER-positive/PR-positive tumors or causes such tumors to develop de novo cannot be assessed.

A growing body of evidence suggests that hormone receptor–positive breast carcinoma and hormone receptor–negative breast carcinoma are distinct entities, rather than variations of a single disease type. Aside from being less sensitive to endocrine therapy, hormone receptor–negative tumors may exhibit increased sensitivity to adjuvant chemotherapy compared with hormone receptor–positive tumors,[39,40] and patterns of recurrence may also vary according to hormone receptor status.[41] cDNA microarray analysis of gene expression patterns has demonstrated that hormone receptor–positive and hormone receptor–negative tumors are also phenotypically distinct from each other, with complex differences involving genes in the ER signaling pathway, among others.[42,43] Our finding that HRT was associated only with the development of ER-positive/PR-positive breast tumors supports the view that hormone receptor–positive tumors and hormone receptor–negative tumors are distinct entities.

Hormone receptor status should be considered in all analyses of hormonal risk factors, as important hormonal influences may be underestimated if ER-negative/PR-negative and ER-positive/PR-positive breast tumors are grouped together. The results of the current study highlight the importance of differentiating between tumor types when analyzing the effects of both endogenous and exogenous hormonal factors, as well as the importance of identifying alternative chemotherapeutic targets (i.e., targets outside the hormonal pathway) in hormone receptor–negative breast tumors.

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