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INCREASED RISK OF BREAST CANCER FOLLOWING DIFFERENT REGIMENS OF HORMONE REPLACEMENT THERAPY FREQUENTLY USED IN EUROPE

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Epidemiologic studies have shown an increased risk of breast cancer following hormone replacement therapy (HRT). The aim of this study was to investigate whether different treatment regimens or the androgenecity of progestins influence the risk of breast cancer differently. The Danish Nurse Cohort was established in 1993, where all female nurses aged 45 years and above received a mailed questionnaire (*n* = 23,178). A total of 19,898 women returned the questionnaire (86%). The questionnaire included information on HRT types and regimens, reproductive history and lifestyle-related factors. Breast cancer cases were ascertained using nationwide registries. The follow-up ended on 31 December 1999. Women with former cancer diagnoses, women with missing information on HRT, surgical menopause, premenopausal, as well as hysterectomized women were excluded, leaving 10,874 for analyses. Statistical analyses were performed using Cox proportional hazards model. A total of 244 women developed breast cancer during follow-up. After adjustment for confounding factors, an increased risk of breast cancer was found for the current use of estrogen only (RR = 1.96; 95% CI = 1.16–3.35), for the combined use of estrogen and progestin (RR = 2.70; 95% CI = 1.96–3.73) and for current users of tibolone (RR = 4.27; 95% CI = 1.74–10.51) compared to the never use of HRT. In current users of combined HRT with testosterone-like progestins, the continuous combined regimens were associated with a statistically significant higher risk of breast cancer than the cyclical combined regimens (RR = 4.16, 95% CI = 2.56–6.75, and RR = 1.94, 95% CI = 1.26–3.00, respectively). An increased risk of breast cancer was noted with longer durations of use for the continuous combined regimens (*p* for trend = 0.048). The European traditional HRT regimens were associated with an increased risk of breast cancer. The highest risk was found for the use of continuous combined estrogen and progestin.

Key words: *hormone replacement therapy; breast cancer; breast neoplasm; epidemiology; estradiol; estrogens; progestin; sex steroids; menopause*

Epidemiologic studies have found a modestly increased risk of breast cancer following the ever or current use of hormone replacement therapy (HRT), but data on the risk of breast cancer following the use of different HRT regimens are limited. The latest pooled analysis including mainly studies from the United States was published in 1997.[1] It was found that the increased risk of breast cancer is confined to the current or recent use of HRT for 5 years or more, and the magnitude of this increase in risk per year resembles the increased risk of breast cancer associated with a delayed natural menopause. Women in the United States have traditionally been treated with equine conjugated estrogens (CE), although the prescription of regimens opposed by progestins has increased during the last 2 decades. The progestin preferred in the United States is medroxyprogesterone acetate (MPA), which resembles natural progesterone and is administered either cyclically or continuously. In Scandinavia and some other European countries, the predominant regimens prescribed is 17-β-estradiol together with the more androgenic testosterone-derived progestins, mainly norethistosterone acetate (NETA) or levonorgestrel (LNG), which has increasingly been administered in the continuous treatment mode.[1]

Recently, one arm of the large randomized trial of the Women Health Initiative (WHI), testing the continuous treatment regimens of CE and MPA against placebo, was stopped earlier than intended due to adverse effects in the treated group, including an increased risk of breast cancer (HR = 1.26; 95% CI = 1.00–1.56). Another arm of the study randomizing hysterectomized women to CE or placebo is continuing as planned until the year 2005.[2] At the same time, another randomized trial with subsequent open-label observation of HRT treatment and follow-up, the Heart and Estrogen/Progestin Replacement Study 2 (HERS2), reported on noncardiovascular outcomes, which for breast cancer represented an increased relative risk of 27%, although not statistically significant.[3] In the following discussions worldwide, it has been pointed out that the results from these 2 randomized studies may not apply to European women, as the HRT compounds preferred in the United States and Europe are different. The latest evidence comes from the Million Women's Study in the United Kingdom, and results confirm an increased risk of breast cancer following the current use of HRT, with the highest risk following the use of combined estrogen/progesterone regimens.[4]

Only a few recent studies originating from both Europe and the United States have estimated the risk of breast cancer following the use of cyclical or continuous combined HRT.[5–9] These studies reported risk estimates in different directions for the 2 regimens, but the most recent study found no significant difference between the cyclical and the continuous regimens.[4]

A recent review emphasizes that the European HRT types with the combined cyclical or continuous addition of testosterone-like progestins may confer a higher risk of breast cancer than other treatment regimens.[10] The key concern is whether the different

Abbreviations: BBD, benign breast disease; BSO, bilateral salpingooophorectomy; CE, conjugated estrogen; CIS, carcinoma *in situ*; DBCG, Danish Breast Cancer Group Cooperation; E, estrogen; HR, hazard ratio; HRT, hormone replacement therapy; LNG, levonorgestrel; LPR, Lands Patient Register; MPA, medroxyprogestrerone acetate; NETA, norethistosterone acetate; OC, oral contraceptive; P, progestin; RR, relative risk.

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treatment modes, *i.e.*, cyclical or continuous addition of progestins, or the androgenecity of progestins, influence the risk of breast cancer to a different degree.

The aims of the present study were to evaluate the magnitude of the breast cancer risk conferred by the different regimens of HRT used in Scandinavia and some European countries in natural postmenopausal women with an intact uterus, and to investigate whether the type of progestin or the treatment mode, *i.e.*, the cyclical or continuous combined regimens, influences the risk of breast cancer differently. The study was based on a nationwide cohort of Danish nurses. The Scientific and Ethical Committee of Frederiksberg and Copenhagen has approved the Danish Nurse Cohort, including analyses in the present study (KF 11-035/00). The Danish Data Protection Agency has been notified and had no objections including the access to the Danish Cancer Registry and National Registry of Hospital Discharges (2001-54-0860). Data are kept by the National Institute of Public Health, Copenhagen.

MATERIAL AND METHODS

Cohort

The Danish Nurse Cohort was established in 1993, when Danish nurses above the age of 44 years, identified through membership of the Danish Nurses Organization, received a mailed questionnaire (n = 23,178). Two reminders were sent out and the response rate was 86%.[11]

Ascertainment of exposure

The mailed questionnaire of 1993 served as baseline information. The questionnaire included detailed questions on the past or current use of HRT as well as duration of use. The type of HRT was available for current users only. Brand names were listed to facilitate the identification of HRT type and regimens. Furthermore, the questionnaire included details on known risk factors for breast cancer and confounders such as menarche, parity, age at first birth, alcohol use, physical activity, body mass index (BMI), benign breast disease, intake of oral contraceptives, hysterectomies, menopausal status and type of menopause.

Using a pharmacoepidemiologic register covering 2 Danish counties, the information on the use of HRT was validated on a subset of women living in these counties comparing the self-reported use with data on HRT prescriptions in the register. Each woman was identified through the personal identification number in Denmark and linked to the register. The sensitivity of the questionnaire on current HRT use was 78.4%, as mainly very short-term users with 1–5 prescriptions in the databases had not categorized themselves as HRT users in the questionnaire. The specificity was 98.4%.[12]

Ascertainment of outcome

Breast Cancer cases were identified by linkage to the Danish Cancer Registry (a registry with information on all cancer diagnoses in Denmark since 1943), the Danish Breast Cancer Group Cooperation (DBCG) registry (a clinical database on all breast cancers in protocoled treatment in Denmark containing information on diagnosis, surgical and adjuvant treatment, survival and histopathologic details since 1977) and the Lands Patient Register (LPR) registry (the National Registry of Patients containing information on all hospital admissions, diagnoses and operations performed in Denmark since 1977). The Danish Cancer Registry was available in a complete form up to 31 December 1997, the LPR was available up to 31 December 1999 and the DBCG was available in a complete form up to May 2001. Self-reported data on breast cancer were available from the questionnaire. The Danish Cancer Registry is complete with respect to prevalent cases, although some excess number of breast cancers exists due to carcinoma *in situ* (CIS) sometimes being reported as invasive cancer.[13]

Identified cases from all data sources were listed and manually checked. To ensure completeness of endpoint registration, follow-up ended at 31 December 1999. An extended follow-up until 30 April 2001 was conducted, but the information in the latest time period was available only from one source, the DBCG registry, and this extended follow-up therefore is not considered to be complete with respect to incident cases.[14]

Analysis

The cohort consists of 19,898 responders. At baseline in 1993, we excluded all identified breast cancer cases, CIS of the breast and other invasive cancers, except for nonmelanoma skin cancer (n = 1,086). Furthermore, women with missing information on HRT (n = 267), premenopausal women (n = 5,084) and women with a surgical menopause (n = 571), *i.e.*, bilateral oophorectomy, were excluded. Finally, hysterectomized women (n = 2,016) were excluded, leaving a total of 10,874 women for follow-up and analysis. More than one exclusion criterion was fulfilled in several women.

Women were considered postmenopausal if the menstrual bleeding had ceased, or they were bleeding while currently taking HRT. Menopausal age was defined as the age at cessation of menstrual bleedings or start of HRT use in women who did not stop menstruating prior to initiating use of HRT.

For current users of HRT in 1993, the HRT brands reported in the questionnaire were translated into type and regimens of HRT use, *i.e.*, the systemic use of estrogen only and combined regimens. Furthermore, the combined users were categorized as cyclical or continuous users and the type of progestin was coded as either progesterone-like progestins or testosterone-like progestins. The use of estrogen comprises the use of estradiol, as only 16 women in the entire cohort reported the use of conjugated estrogens. Women reporting use of tibolone were placed in a separate group. Women with missing information on type of HRT or women reporting several brands were coded as users of other HRT. Vaginally applied estrogen users were considered never users.

The Cox proportional hazards model for left-truncated and right-censored data was used in the modeling of time to breast cancer outcome. The nurse's age was used as an underlying time where the age at study entry is considered as the delayed entry time in the analysis. The first step in the analysis was modeling the outcome of interest univariate, with the HRT exposure variable unadjusted for confounders (except age, which is the delayed entry variable) and estimating the relative risks (RRs) and their 95% confidence intervals (CIs). The second step in analysis was multivariate modeling of the breast cancer outcome, estimating the RRs of HRT exposure with 95% CIs adjusted for the confounders: age at menopause, age at menarche, parity, age at first childbirth, use of oral contraceptives (OCs), former benign breast disease (BBD), smoking, night work, BMI, height, physical activity, alcohol intake. Stepwise selection was made to identify the significant confounders, which were kept in the model together with the HRT variable. The third step of the analysis consisted of effect modification analysis, where each of the significant covariates in the models was tested for interaction with the HRT variable. Additionally, we tested for interaction with BMI and alcohol. For every Cox model, the proportional hazard assumption was checked. Missing values were excluded from analysis. The analysis was performed in Stata version 7.0.

RESULTS

In this population of 10,874 natural postmenopausal women, a total of 2,726 women (25.1%) were current users of HRT, 1,582 women (14.5%) were past users and 6,566 (60.4%) women had never used HRT at baseline in 1993. Information on type of HRT was available for current users. A total of 543 women used estrogen only (20%), 1,958 women used combined estrogen and progestin (72%), 79 women used tibolone (3%) and 146 (5%) women used other HRT. Furthermore, there was information on the type of progestin in 1,844 women using combined regimens, with 23% using progesterone-like progestins and 77% using testosterone-like progestins. Information on treatment mode was

available for 1,938 women using combined HRT regimens, with 82% using cyclical regimen and 18% using continuous regimens. Information on duration was available for 2,592 users of HRT. Women used HRT for 0–42 years, with mean duration of 7.2 ± 6.3 years.

A total of 244 women developed breast cancer during follow-up. The time from baseline to breast cancer diagnosis was 3.36 ± 1.28 and the mean follow-up time for the whole cohort was 6.34 ± 0.98 years. The potential and established risk factors for breast cancer showed expected distributions and trends in univariate analysis (Table I). There was a significantly higher risk for breast cancer with late menopause, the ever use of oral contraceptives and in women with previous BBD. Adjustment for age is underlying. The multivariate analysis adjusted for significant variables in the final model, which were benign breast disease and age at menopause. An increased risk of breast cancer was found following the ever use of HRT (RR = 1.91; 95% CI = 1.45–2.50) and the current use of HRT (RR = 2.42; 95% CI = 1.81–3.26). No significantly increased risk was seen for past users of HRT. We found no increased risk of breast cancer with longer duration of use in the overall analysis (Table II). No significant effect modification was found. The unopposed estrogen-only treatment increased breast cancer risk nearly 2-fold (RR = 1.96; 95% CI = 1.16–3.35), while the combined therapy of estrogen and progestin increased breast cancer risk nearly 3-fold (RR = 2.70; 95% CI = 1.96–3.73). Comparing these 2 estimates, with estrogen-only therapy as the reference, the risk estimates were not significantly different from each other ($p = 0.26$).

The differential risk of breast cancer according to type of hormone compound was analyzed on women who provided complete information on compound type and regimens of interest. The exposure to HRT was categorized into the current use of estrogen, the current use of estrogen combined with either progesterone-like progestins (MPA) or testosterone-like progestins (NETA/LNG) in a cyclical or continuous treatment mode and the use of tibolone. Women who had specified more than one HRT compound, used other HRT or combined estrogen/progestin HRT regimens with unknown type or regimen, were categorized into other HRT regimens. Progesterone-like progestins were administered in a cyclical regimen only (Table III).

Risk estimates for breast cancer were increased for both types of progestins in the cyclical treatment mode for the progesterone-like progestins (RR = 3.02; 95% CI = 1.80–5.05) and for the testosterone-like progestins (RR = 1.94; 95% CI = 1.26–3.00). There was no significant difference between the 2 types of progestins ($p = 0.144$; Table III).

The effect of treatment regimens on the risk of breast cancer, *i.e.*, the cyclical or continuous combined administration of estrogen and progestin, was analyzed in the same model. To avoid any possible confounding by type of progesterone, we compared the users of the combined HRT regimens with the testosterone-like progestins in the cyclical or the continuous treatment mode. The continuous combined regimens were associated with a 4-fold increased risk of breast cancer (RR = 4.16; 95% CI = 2.56–6.75), while the cyclical combined regimens with testosterone-like progestins increased risk of breast cancer 2-fold (RR = 1.94; 95% CI = 1.26–3.00). The difference in breast cancer risk for the 2 regimens of testosterone-like progestins was significant ($p = 0.01$).

The current use of tibolone increased the risk of breast cancer significantly (RR = 4.27; 95% CI = 1.74–10.51). Other HRT regimen increased the risk of breast cancer, although not statistically significant (RR = 1.53; 95% CI = 0.67–3.50).

Longer duration of HRT use in general was not associated with increasing risk of breast cancer. However, when dividing the

TABLE I – POTENTIAL AND ESTABLISHED RISK FACTORS FOR BREAST CANCER: DESCRIPTIVE STATISTICS AND UNIVARIATE ANALYSIS

Covariate	Definition	Frequency, *n* (%)	Number of cases ($n = 244$)	RR (95% CI; $n = 10{,}874$)[a]
Menopausal age	1, < 44 years	1,334 (13.2)	25	1.00
	2, 45–49 years	3,886 (38.5)	76	1.02 (0.65–1.61)
	3, 50–54 years	4,368 (43.2)	102	1.16 (0.74–1.82)
	4, ≥ 55 years	514 (5.1)	19	1.70 (0.92–3.14)
Menarche	0, ≤ 12 years	2,039 (19.2)	51	1.00
	1, > 12 years	8,555 (80.8)	188	0.85 (0.62–1.16)
Parity	0, No children	2,086 (19.4)	52	1.00
	1, one child or more	8,681 (80.6)	189	0.87 (0.63–1.20)
Age at first childbirth	1, 15–24 years	2,694 (31.0)	53	1.00
	2, 25–29 years	4,179 (48.0)	86	0.99 (0.70–1.40)
	3, 30–34 years	1,371 (15.7)	40	1.33 (0.86–2.06)
	4, ≥ 35 years	459 (5.3)	9	0.90 (0.43–1.85)
BBD	0, No	8,771	175	1.00
	1, Yes	1,913 (17.9)	65	1.73 (1.30–2.30)
OC	0, No	6,687 (62.1)	138	1.00
	1, Yes	4,083 (37.9)	105	1.37 (1.04–1.80)
Night work	0, No	5,654 (90.5)	119	1.00
	1, Yes	592 (9.5)	18	1.41 (0.86–2.33)
Smoking	1, Never	3,012 (29.4)	63	1.00
	2, Past	3,097 (30.3)	73	1.12 (0.80–1.58)
	3, Current	4,128 (40.3)	91	1.07 (0.78–1.48)
BMI	1, < 18.5 kg/m^2	367 (3.4)	7	1.00
	2, 18.5–25 kg/m^2	7,297 (67.7)	161	1.07 (0.50–2.29)
	3, 25–30 kg/m^2	2,536 (23.5)	55	1.02 (0.46–2.24)
	4, > 30 kg/m^2	580 (5.4)	19	1.56 (0.66–3.72)
Physical activity	1, Low	2,565 (24.0)	66	1.00
	2, Medium	7,253 (67.8)	156	0.82 (0.61–1.10)
	3, High	877 (8.2)	19	0.97 (0.58–1.60)
Alcohol	0, No	2,204 (21.2)	53	1.00
	1, 1 unit or more	8,205 (78.8)	182	0.92 (0.67–1.25)
Age	1, < 50 years	852 (7.8)	13	1.00
	2, 50–60 years	5,403 (49.7)	121	1.32 (0.51–3.41)
	3, 60–70 years	3,301 (30.4)	79	1.22 (0.41–3.63)
	4, > 70 years	1,318 (12.1)	31	1.35 (0.34–5.44)

[a]Age-adjusted.

TABLE II – HRT USE AND THE RISK OF BREAST CANCER

Use of HRT (person-years, 68,912)	Frequency (%; n = 10,874)	Cases (n = 244)	Univariate,[a] RR (95% CI)	Multivariate,[b] RR (95% CI)
Never	6,566 (60.4)	110	1.00	1.00
Ever	4,308 (39.6)	134	1.94 (1.50–2.51)	1.91 (1.45–2.50)
Never	6,566 (60.4)	110	1.00	1.00
Past	1,582 (14.5)	31	1.14 (0.76–1.69)	1.16 (0.76–1.77)
Current	2,726 (25.1)	103	2.55 (1.93–3.37)	2.42 (1.81–3.26)
Never	6,566 (61.1)	110	1.00	1.00
Past	1,582 (14.7)	31	1.14 (0.77–1.71)	1.17 (0.76–1.78)
Current ≤ 1 year	487 (4.5)	14	2.24 (1.24–4.07)	2.28 (1.26–3.15)
Current 2–4 years	648 (6.0)	17	1.88 (1.10–3.20)	1.84 (1.07–3.15)
Current 5–9 years	659 (6.1)	27	2.74 (1.77–4.24)	2.58 (1.64–4.05)
Current 10–14 years	418 (3.9)	20	2.95 (1.82–4.78)	3.08 (1.87–5.06)
Current 15+ years	380 (3.6)	17	2.49 (1.48–4.17)	2.56 (1.49–4.39)
Duration unknown[c]	134			

[a]Age-adjusted.–[b]Significant covariates in the multivariate model were benign breast disease (0, yes; 1, no) and menopausal age (0, < 55 years; 1, 55+ years).–[c]Current users with missing information on duration were excluded (n = 134).

TABLE III – HRT TYPE AND THE RISK OF BREAST CANCER

Use of HRT (person-years, 68,912)	Frequency (%;n = 10,874)	Cases (n = 244)	Univariate,[a] RR (95% CI)	Multivariate,[b] RR (95% CI)
Never HRT	6,566 (60.4)	110	1.00	1.00
Past HRT	1,582 (14.6)	31	1.14 (0.76–1.69)	1.16 (0.76–1.77)
Estrogen	543 (5.0)	16	1.86 (1.10–3.14)	1.95 (1.15–3.32)
Estrogen + progesterone-like progestin cyclical	433 (4.0)	20	3.30 (2.02–5.38)	3.02 (1.80–5.05)
Estrogen + testosterone-like progestin cyclical	1,054 (9.7)	32	2.16 (1.43–3.24)	1.94 (1.26–3.00)
Estrogen + testosterone-like progestin continuous	341 (3.1)	23	4.36 (2.77–6.88)	4.16 (2.56–6.75)
Current tibolone	79 (0.7)	5	3.95 (1.61–9.69)	4.27 (1.74–10.51)
Current other HRT	276 (2.5)	7	1.63 (0.76–3.51)	1.53 (0.67–3.50)

[a]Age-adjusted.–[b]Significant covariates in the multivariate model were benign breast disease (0, yes; 1, no) and menopausal age (0, < 55 years; 1, 55+ years).

TABLE IV – HRT REGIMEN AND THE RISK OF BREAST CANCER IN USERS OF COMBINED HRT WITH TESTOSTERONE-LIKE PROGESTINS

Use of HRT (person-years, 68,912)		Frequencies (%; n = 10,874)	Number of cases (n = 196)	Univariate,[a] RR (95% CI)	Multivariate,[b] RR (95% CI)	Trend test, p value
Never HRT		6,566 (68.8)	110	1.00	1.00	
Past HRT		1,582 (16.6)	31	1.14 (0.76–1.70)	1.18 (0.77–1.80)	
Estrogen plus testosterone-like progestin cyclical	< 5 years	490 (5.2)	10	1.62 (0.81–3.25)	1.58 (0.79–3.17)	
Estrogen plus testosterone-like progestin cyclical	5–9 years	246 (2.6)	9	2.49 (1.24–4.96)	2.47 (1.23–4.95)	
Estrogen plus testosterone-like progestin cyclical	10+ years	270 (2.8)	10	2.25 (1.17–4.33)	2.18 (1.09–4.33)	0.555
Estrogen plus testosterone-like progestin continuous	< 5 years	139 (1.5)	4	1.96 (0.72–5.36)	1.96 (0.72–5.36)	
Estrogen plus testosterone-like progestin continuous	5–9 years	88 (0.9)	6	4.54 (1.98–10.41)	4.96 (2.16–11.39)	
Estrogen plus testosterone-like progestin continuous	10+ years	96 (1.0)	10	6.20 (3.23–11.89)	6.78 (3.41–13.48)	0.048
Other HRT/missing duration[c]		1,397				

[a]Age-adjusted.–[b]Significant covariates in the multivariate model were benign breast disease (0, yes; 1, no) and menopausal age (0, < 55 years; 1, 55+ years).–[c]Women with other/unknown HRT type, estrogen users, missing information on type of progesterone or treatment mode and duration (n = 1397) have been excluded from analysis.

current users of testosterone-like progestins at baseline into the cyclical and continuous treatment mode, an increased risk of breast cancer with longer duration was found for the continuous treatment mode (p for trend = 0.048; Table IV). The use of continuous combined HRT for 10 years or more increased the risk of breast cancer 6-fold (RR = 6.78; 95% CI = 3.41–13.48) compared to never users.

A separate analysis was conducted assessing the risk of breast cancer according to HRT use in 1,070 hysterectomized women above the age of 55 years (natural menopause occurred at the age of 55 years in 90% of women in this cohort). The risk estimates for breast cancer following the current use of estrogen only at baseline compared to never users were close to the estimate for women with an intact uterus (RR = 1.84; 95% CI = 0.79–4.28) adjusted for age. No significant covariates were identified.

During the extended follow-up through 30 April 2001, a total of 306 women could be identified as breast cancer cases, but the identification of cases cannot be considered to be complete for this period due to some late updating by the registries. The same analysis was performed based on the larger number of breast cancer cases, and the estimates were close to main analyses, showing consistency and confirming results from the shorter follow-up period.

DISCUSSION

Our study comprises one of relatively few studies estimating the risk of breast cancer following the use of different types and regimens of HRT. In this Danish nationwide cohort of nurses, we found a significantly increased risk of breast cancer following the

use of both estrogen-only and the combined estrogen/progestin therapy. Most studies have found a very modest increase in breast cancer risk or a neutral effect of the treatment with estrogen only.[8,9,15–20] A single-population-based case-control study from Sweden found an increased risk of breast cancer following the use of estradiol (OR = 1.94; 95% CI = 1.47–2.55),[6] which is close to our estimate for the current use of estrogen only (RR = 1.96; 95% CI = 1.16–3.35). This is confirmed by the recent findings from the Million Women's Study in the United Kingdom, where estrogen-only therapy is associated with an increased risk of breast cancer (RR = 1.30; 95% CI = 1.22–1.38).[4]

Estradiol and conjugated estrogens have generally been considered to be medium potency estrogens with the same adverse effects with respect to breast cancer; thus, they have usually been analyzed together as one exposure group. In our study, only 16 women in the cohort had used conjugated estrogens, and this is why our risk estimates represent the risk of breast cancer in women exposed to estradiol. A recent study on human epithelial breast cells showed a potential effect of estradiol on neoplastic transformation.[21] This raises the concern whether exposure to estradiol might confer a higher risk of breast cancer than conjugated estrogens and may suggest that in the future these 2 types of estrogen should be analyzed separately as different exposure groups. However, the recent study from the United Kingdom found both types of estrogens to increase the risk of breast cancer equally, but there was the possibility of women having switched preparations in about 1/3 of the total population, which might have introduced some bias.[4]

In the present study, the combined estrogen/progestin treatment was associated with higher-risk estimates for breast cancer than the estrogen-only treatment, although the difference in breast cancer risk was not statistically significant. When trying to assess the different effects of estrogen-only *vs*. combined therapy regimens, it must be borne in mind that the combined estrogen/progestin replacement therapy is usually prescribed to women with an intact uterus. The treatment with estrogen only is generally prescribed for older women or for women who have had a hysterectomy. In accordance with this knowledge, women using HRT regimens as estrogen-only therapy in our study were a median of 2 years older than women using the combined treatment regimens, with a median age of 57 years (range, 45–82) compared to 55 years (range, 44–79). However, this age difference is considered to be small and age is entered in the statistical model, enabling a comparison across the HRT regimens. With respect to duration of HRT use, a total of 22% of the women using estrogen-only therapy at baseline were very long-term users (15 years or more) compared to 11% using the combined regimens. However, women on estrogen only might have used other combined HRT types previously, and our data do not provide information on HRT type in past users. Another important issue is the possible introduction of a bias due to hysterectomized women. Women who have had a hysterectomy may differ with respect to various characteristics and may be at a lower baseline risk than women with an intact uterus.[22] Furthermore, age at menopause is uncertain in hysterectomized women, and including them in observational studies might underestimate the true effect of HRT.[23] We therefore excluded women with a hysterectomy for the main analysis.

The key question concerning the risk of breast cancer according to the androgenecity of the progestin is difficult to answer. Previous studies from the United States lack sufficient numbers of women using testosterone-like progestins, and previous studies from Europe comprise mainly women on testosterone-like progestins. However, risk estimates for breast cancer from European studies have generally been high.[4,6,10] We were only able to investigate the effect of the 2 types of progestins both combined with estradiol in the cyclical treatment regimens, and in our study the current use of progestins with differing androgenecity does not seem to confer a different risk of breast cancer. However, the progesterone-like progestins had only been on the Danish market since 1988, *i.e.*, 5 years prior to baseline in 1993, and the questionnaire does not provide information on HRT type for past users. A total of 54% of women using progesterone-like progestins have used HRT for 5 years or more, suggesting that the type of progestin used earlier must have been the testosterone-like progestin. Even though women could have switched preparation at any time during the 5 years prior to baseline, we reanalyzed data restricting analysis to women with HRT use for 5 years or less, but the estimates were remaining equally increased. However, our data on this issue are thus limited and further research is needed.

In our study, the continuous combined regimens were found to be more harmful with respect to breast cancer than the cyclical regimens. The duration for testosterone-like progestin users on cyclical HRT was median 5 years (0–33 years) and on the continuous regimens was median 5 years (0–32 years). As the current long-term use is distributed equally throughout both the cyclical and continuous HRT regimens, the effect does not seem to be influenced by difference in duration. The impact on breast cancer risk by treatment mode has been investigated by others, but with different conclusions. Ross *et al.*[7] found that the cyclical HRT regimens were associated with an increased risk of breast cancer. By contrast, 2 recent studies from the United States reported an increased risk of breast cancer following the use of the continuous combined regimens, while the cyclical regimens showed no significant increased risk of breast cancer.[8,9] Finally, the latest study from the United Kingdom finds no difference in breast cancer risk due to treatment mode, stratified into durations of less than and 5 years or more.[4] In agreement with our results, a Swedish case-control study found the risk of breast cancer to be increased following the use of combined estradiol and testosterone-like progestins in the continuous treatment mode for 5 years or more compared to the cyclical regimens, and risk estimates were higher than those from U.S. studies.[6] It is possible that progestins of a different androgenecity have different biologic effects on breast tissue when administered in the cyclical or continuous treatment mode, and the effect may vary when combined with conjugated estrogens or estradiol, respectively.

The higher risk of breast cancer found for the continuous combined rather than the cyclical treatment of estradiol and testosterone-like progestins might reflect a dose-response relationship of the progestin. The most frequently used cyclical regimen provides a monthly progesterone dose of 10 mg NETA, whereas the most frequent continuous combined regimen provides a monthly load of 28 mg NETA. This is emphasized by the increasing risk of breast cancer with duration of use for testosterone-like progestins in the continuous treatment mode but not for the cyclical treatment mode. In our study, the use of progesterone-like progestins comprised only cyclical users; we therefore have no data on a possible different effect of duration for cyclical or continuous use of progesterone-like progestins. A recent follow-up study based on the Women's Health Study found an increased risk of breast cancer with increasing doses of estrogen, but no dose-response relationship for progesterone-like progestins.[8]

The increased risk of breast cancer following the use of tibolone is in agreement with recent findings.[4] However, in our set of data, we are not able to control sufficiently for family history of breast cancer, as we do not have information on first-degree relatives with breast cancer. A total of 31% of the whole population as opposed to 35% of tibolone users report any female relatives with former breast cancer. As tibolone has been considered a safe alternative regarding breast cancer, this preparation might have been prescribed primarily to women with an increased risk of breast cancer due to family history.

Supportive of the epidemiologic findings are studies on cell proliferation and mammographic densities in women receiving HRT. A recent study on proliferation of breast epithelial cells found an increased epithelial cell proliferation following use of the combined estrogen/progestin treatment, together with increased epithelial density, localized to the terminal duct lobular unit, which is the site of development for most breast cancers.[24] Furthermore,

the discontinuation of progestins seems to reduce the estradiol-induced proliferation of breast epithelial cells and trigger the apoptotic mechanism.[25] This could explain the lower risk of breast cancer following the cyclical regimens.

Studies on mammographic density have shown an increased density for women on the combined estrogen-progestin treatment compared to estrogen only. A Swedish study found that the continuous combined regimens were associated with an increased mammographic density of 52%, compared to 13% for the cyclical regimens.[26] Another recent study found significant higher rates of breast density following continuous combined treatment with either type of progestin. Comparing the 2 types of progestins administered in a continuous form, the breast density was increased, especially following the use of testosterone-like progestins, although this difference did not reach statistical significance.[27]

Addressing the issue of breast cancer risk following HRT, our study has several strengths. This cohort consists of a homogeneous occupational group, which eliminates the potential confounding by educational standard or occupation. Nurses have good qualifications regarding answering health-related questions and providing information on the use of different medications. Our exposure data are reliable and endpoint registration is considered to be complete.

However, the study has some inherent weakness as well. It is a prospective study with exposure information available at baseline only. Some misclassification could therefore occur over time. The women reporting short duration of use at baseline might well have used HRT for some years before developing breast cancer. This might explain why this study could not show an increasing risk of breast cancer with longer duration of use and the risk of developing breast cancer in women using HRT for < 1 year at baseline is as high as with longer durations of use. Furthermore, the number of cases is not as large as a case-control design could have given, but the problem of recall bias is minimized. Our study did not provide information on mammographic screening. A formal screening program was set up in only 2 out of 15 Danish counties in the study period. Women on HRT may have had more mammograms and contact with their physicians for purposes of preventive medicine, introducing a surveillance bias. However, if this were to overestimate the true risk of breast cancer, it would have been expected to occur equally for all types of HRT.

From the European point of view, the findings of different risk estimates for the development of breast cancer following the continuous combined and cyclical therapy are certainly important and provide a new perspective when counseling women in their choice of HRT. Whether the risk of breast cancer varies for the different types of progestins remains to be further elucidated.

In this nationwide cohort of Danish nurses, the risk of breast cancer is increased following both estrogen-only and the combined estrogen/progestin treatment in naturally postmenopausal women. The continuous combined treatment regimens of estradiol plus progestins was associated with the highest risk estimates: a 4-fold increased risk of breast cancer. The difference in breast cancer risk between the cyclical and continuous combined therapy regimens is highly statistically significant. The androgenecity of the progestin does not seem to influence the risk of breast cancer differently. These findings are important in the perspective of counseling of European women in their choice of HRT.

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