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EPIDEMIOLOGY

Pre-diagnostic sex hormone levels and survival among breast cancer patients

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Abstract

Purpose Higher levels of circulating sex steroid hormones are associated with increased breast cancer risk, though their association with prognosis remains unclear. We evaluated the association between circulating sex hormone levels and breast cancer survival in two large cohorts.

Methods We evaluated this association among 2073 breast cancer cases from the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII) cohorts. Women in this analysis provided a blood sample in 1989–1990 (NHS) or in 1996–1999 (NHSII) and were subsequently diagnosed with breast cancer. Levels of estradiol (postmenopausal women only), testosterone, dehydroepiandrosterone-sulfate (DHEAS), and sex hormone-binding globulin (SHBG) were measured in plasma. Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI) for survival, adjusting for patient and tumor characteristics.

Results A total of 639 deaths and 160 breast cancer deaths occurred over follow-up through 2015. Compared to women in the lowest quartile, postmenopausal women in the highest quartile of estradiol experienced a 1.43-fold overall mortality rate (HR 1.43, 95% CI 1.03–1.97, *P*-trend = 0.04) and a nonsignificantly higher breast cancer mortality rate (HR 1.50, 95% CI 0.75–2.98, *P*-trend = 0.12). Higher DHEAS levels were nonsignificantly associated with better overall survival (HR_{Q4vsQ1}=0.79, 95% CI 0.57–1.10, *P*-trend = 0.05), though not with breast cancer survival. No associations were observed between testosterone or SHBG and survival.

Conclusions Pre-diagnostic postmenopausal circulating estradiol levels were modestly associated with worse survival among breast cancer patients. Further studies should evaluate whether circulating hormone levels at diagnosis predict cancer prognosis or treatment response.

Keywords Breast cancer · Prognosis · Estradiol · Testosterone · DHEAS · SHBG

Introduction

Sex steroid hormones, particularly estrogens, play a vital role in the development and progression of breast cancers [1]. Estrogens increase the cellular proliferation rate in the

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breast, and as such, the use of aromatase inhibitors and selective estrogen receptor modulators in breast cancer treatment directly targets estrogen receptor signaling pathways in an attempt to mitigate these effects [2, 3]. Androgens are the biologic precursors of estrogens, though they may exert independent effects on carcinogenesis and tumor progression in the breast. Studies of the effects of androgens on cellular proliferation in the breast have yielded conflicting results, suggesting that the effects of androgens may differ by estrogen receptor expression in the model system [4, 5].

Sex steroid hormone levels are consistently positively associated with breast cancer risk among postmenopausal women, with relative risks comparing highest to lowest quintiles of 2.0 (95% CI 1.5–2.7) for estradiol, 2.2 (95% CI 1.3–2.4) for testosterone, and 1.8 (95% CI 1.3–2.4) for dehydroepiandrosterone sulfate (DHEAS) in a pooled analysis of nine prospective studies [6]. Additionally, sex hormone-binding globulin (SHBG), which binds to androgens and estrogens in circulation, likely making them less bioavailable, was associated with 40% lower breast cancer risk (OR 0.6, 95% CI 0.4–1.0) (highest vs. lowest quintiles) [6]. These findings have been replicated in subsequent studies [7–10]. In studies of postmenopausal women, relative risks of androgen and breast cancer risk are attenuated after adjustment for estradiol, suggesting that the androgen association is driven in part by conversion to estrogens [11, 12]. The evidence to date suggests that increased post-diagnostic hormone levels are also associated with worse breast cancer survival outcomes; however, prior studies are limited by modest sample sizes, inability to control for important potential confounders, and predominantly used measures of hormone levels collected post-diagnosis [13–18]. There is likewise little evidence for how circulating sex hormones may correlate with tumor characteristics. Given the long-term reliability of circulating sex steroid hormones [12], we utilized pre-diagnostic circulating sex hormones levels as a proxy for concentrations at diagnosis and assessed their associations with breast cancer prognosis in two large prospective cohorts of US women.

Methods

Nurses' Health Studies

The Nurses' Health Study (NHS) is composed of 121,700 US registered nurses who completed a baseline questionnaire at ages 30–55 years in 1976. The Nurses' Health Study II (NHSII) includes 116,429 US registered nurses who returned a baseline questionnaire in 1989 at ages 25–42. Members of both cohorts complete biennial questionnaire that provide detailed information about demographic and lifestyle variables, and their medical history. Details of the cohort procedures have been described previously [19], and all study protocols have been approved by the institutional review board of Brigham and Women's Hospital (Boston, MA).

New diagnoses of breast cancer are self-reported by cohort members in the biennial questionnaires. With participant consent, cohort investigators attempt to obtain medical records pertinent to the cancer to confirm the diagnosis, and extract information regarding cancer histopathology and treatment. Over 99% of new breast cancer diagnoses are confirmed following medical record review.

Endpoints

Deaths in the cohorts are reported by next of kin or by postal authorities or determined through targeted searches of the National Death Index [20]. Causes of death are then determined through review of the death certificate or medical records. Overall survival was defined as the time (in months) between diagnosis of breast cancer and death from any cause, censoring other individuals at their last follow-up time. Breast cancer-specific survival was defined as the time from breast cancer diagnosis to death from breast cancer, censoring other individuals at date of death from non-breast cancer causes, or at the end of follow-up. Other cause-specific mortality endpoints were defined in a similar manner. All endpoints were ascertained through December 2015.

Sex hormone assays

Blood samples were collected from subsets of the NHS and NHSII cohorts, and details of these collections have been described extensively elsewhere [7, 21, 22]. Briefly, blood samples were obtained from 32,826 members of the NHS cohort in 1989–1990 (ages 43–69 at the time of collection). Of this group, 18,717 women provided a second sample in 2000–2001. In the NHSII cohort, 29,611 women provided blood samples in 1996–1999 (ages 31–48). Of these women, 18,521 pre-menopausal women provided two timed samples in the early follicular and mid-luteal phases of their menstrual cycle, while the remaining 11,090 women provided a single untimed sample. In all collections, the women were provided collection kits to have their blood drawn and then shipped the samples with an ice pack back to our laboratory for processing. Samples were then aliquoted and stored at − 130 °C.

Estradiol, testosterone, DHEAS, and SHBG were assessed as prognostic markers in this study. Though estrone and androstenedione have the highest concentrations in circulation in postmenopausal women, these hormones are very modestly associated with breast cancer risk and were therefore not evaluated in our study [6]. The laboratory assays used to measure estradiol, testosterone, DHEAS, and SHBG

have been described previously [7, 12, 21–23]. Concisely, estradiol, testosterone, and DHEAS were measured by radioimmunoassay at Quest Diagnostics (San Juan Capistrano, CA) for NHS cases diagnosed between 1990 and 1998 and NHSII cases diagnosed between 1995 and 2003. For NHS cases diagnosed from 2000 to 2010 and NHSII cases from 2005 to 2009, estradiol and testosterone were measured by liquid chromatography-tandem mass spectrometry at the Mayo Clinic (Rochester, MN). Correlations between assays were > 0.9 [12]. DHEAS was measured by solid-phase, competitive chemiluminescent enzyme immunoassay (Siemens Healthcare Diagnostics, Deerfield, IL) for cases diagnosed in 2000 or later. SHBG was measured by solid-phase two-site chemiluminescent enzyme immunometric assay (Immulite; DPC, Inc.) at two sites (Longcope Laboratory and Massachusetts General Hospital). Within-batch coefficients of variation ranged from 8 to 12% [12].

Pre- and postmenopausal women at the time of blood draw from NHS and NHSII were included for analyses examining testosterone, DHEAS, and SHBG, while analyses examining estradiol were restricted to only women from NHS who were postmenopausal at the time of blood draw. Women with hormone values below the limit of detection were assigned a value at half of the limit of detection. One outlying value for estradiol and eight for testosterone (none for DHEAS or SHBG) were identified using the generalized extreme studentized deviate test of outliers and were excluded [24]. For the members of NHS that provided two blood samples, the mean was calculated between the two samples, if the woman reported the same menopausal status and menopausal hormone therapy (MHT) use at the second blood collection as she reported in the first collection. Otherwise, only the first sample was included. For the members of NHSII that provided timed blood samples, the mean of the levels in the follicular and luteal phases was used. For testosterone, DHEAS, and SHBG, average batch recalibration was used to adjust for batch effects, accounting for cohort, age, menopausal status, and MHT use [25]. For estradiol, average batch recalibration was performed separately for women using MHT and not using MHT, and accounted for age only as only postmenopausal cases from NHS were included.

Statistical analysis

The associations between patient, tumor, and treatment variables and mean hormone levels were evaluated using analysis of covariance (ANCOVA), adjusting for age at diagnosis. Hormone levels were log2-transformed to better approximate a normal distribution in the ANCOVA. Global F tests of heterogeneity (or tests for trend for ordinal variables) were used to test the significance of hormone-covariate associations.

The association between pre-diagnostic circulating sex hormone levels and survival following breast cancer diagnosis was evaluated using Cox proportional hazards models to estimate hazard ratios (HR) and 95% confidence intervals (95% CI) with time since diagnosis (months) as the metameter. Hormone levels were modeled in quartiles and as log2-transformed continuous variables. Models were stratified by cohort, and adjusted for age at diagnosis, time since blood draw, menopausal status/MHT use at blood draw, date of diagnosis, tumor stage, tumor grade, HER2 expression, ER expression, smoking status at diagnosis, BMI at diagnosis, and physical activity at diagnosis. Treatment variables were considered as potential covariates, but were not strongly correlated with hormone levels, and were not included in final models. Missing indicators were used for categorical variables, and individuals with missing BMI or physical activity was excluded from multivariable-adjusted analyses. Tests for trend were conducted by using a Wald test for a variable taking the median concentration of each quartile of the hormone. The proportional hazards assumption was evaluated by a likelihood ratio test for the hormone-by-follow-up time interaction [26]. Hormone-by-ER interactions were also evaluated using likelihood ratio tests.

In a sensitivity analysis, the associations between hormones and cause of death were evaluated using a Lunn-McNeil competing risks analysis, wherein the dataset was augmented to create observations for each individuals for each cause of death (breast cancer, cardiovascular disease, other cancer, other cause) [27]. Hazard ratios for each cause of death were estimated using Cox proportional hazards models stratified by cause of death. All other covariates were constrained to yield a single hazard ratio for all causes of death. A P value for heterogeneity across causes of death was calculated using a likelihood ratio test comparing the model with cause-specific mortality endpoints to the model with all-cause mortality only.

As applicable, this study adhered to the REMARK guidelines for prognostic markers [28]. All statistical tests were two-sided and used a 0.05 level of significance, and no corrections were performed for multiple testing. All analyses were conducted in SAS version 9.4.

Study population

A total of 2156 participants had received a diagnosis of stage 0–III breast cancer after providing a blood sample and had at least one measured analyte of interest. Fifty-nine women were diagnosed with breast cancer within 6 months of providing the blood sample and were excluded to reduce the potential influence of reverse causation. One woman had an implausible survival time and was excluded. Finally, 23 women died of breast cancer within the first year of follow-up after diagnosis and were assumed to have had metastatic

disease at the time of diagnosis and were subsequently excluded, leaving a final analytic population of 2073 women.

Results

The 2073 breast cancer patients with at least one measured hormone were followed for a median of 12.9 years post-diagnosis (interquartile range 8.7–16.7). Six hundred thirty-nine total deaths occurred over the follow-up period, including 160 from breast cancer, 100 from other cancers, 71 from cardiovascular disease, and 308 from other causes.

Age-adjusted mean levels of estradiol and testosterone in relation to patient, tumor, and treatment variables are shown in Table 1. Age at diagnosis was inversely associated with mean levels of estradiol ($P < 0.001$) and testosterone ($P < 0.001$). Mean postmenopausal estradiol levels were considerably higher among women using MHT at blood draw (mean 17.2 pg/mL, 95% CI 16.0–18.5) compared to women not using MHT (mean 7.6 pg/mL, 95% CI 7.2–8.1) ($P < 0.001$). In contrast, testosterone levels did not differ by menopausal status or MHT use ($P = 0.19$). Women with ER+ breast cancers had higher mean estradiol ($P = 0.005$) and testosterone ($P = 0.01$) levels, while pre-diagnostic testosterone levels were lower among women with HER2+ breast cancers ($P = 0.002$). There were no clear correlations between hormone levels and tumor grade or treatment received; however, testosterone was inversely associated with stage ($P = 0.04$). Mean estradiol levels were positively associated with BMI ($P = 0.002$) and inversely associated with physical activity ($P = 0.01$). Testosterone was only suggestively associated with physical activity ($P = 0.08$). Smoking was associated with both mean estradiol and testosterone concentrations, though the directions of the associations differed, with an inverse association observed for estradiol ($P = 0.03$) and a suggestive positive association with testosterone ($P = 0.06$).

In the fully adjusted Cox model accounting for patient and tumor characteristics (Table 2), postmenopausal women in the highest quartile of pre-diagnostic estradiol level experienced 1.43-fold higher overall mortality (HR 1.43, 95% CI 1.03–1.97, P-trend = 0.04) compared to women in the lowest quartile. A doubling in postmenopausal estradiol concentration was associated with a nonsignificant 10% increase in overall mortality (HR 1.10, 95% CI 1.00–1.22). These associations were similar in magnitude, but did not achieve statistical significance, when restricting to women who were not using MHT at the time of blood draw. Among postmenopausal MHT users, there was no apparent association between estradiol concentrations and overall survival, suggesting that this association is driven by endogenous estradiol levels. In contrast, a doubling in DHEAS level was associated with a 12% reduction in overall mortality (HR 0.88, 95% CI 0.80–0.98, P-trend across quartiles = 0.05) among pre- and postmenopausal women combined. No associations were observed between testosterone (HR per doubling = 0.95, 95% 0.84–1.07) or SHBG (HR per doubling = 0.93, 95% 0.83–1.03) and overall survival. The association with postmenopausal estradiol was similar when restricting the analysis to women who were diagnosed with breast cancer within 5 years of their blood draw, while there was no association with DHEAS (Supplemental Fig. 1). In a sensitivity analysis, there was no significant heterogeneity between hormone concentrations and cause of death in a competing risks model (Supplemental Table 1).

Estradiol was nonsignificantly associated with breast cancer-specific survival (HR per doubling = 1.17, 95% CI 0.95–1.44, P-trend across quartiles = 0.12) (Table 3). When restricting to non-MHT users, this association appeared stronger (HR per doubling 1.40, 95% CI 0.96–2.05), though again was nonsignificant. In contrast with overall survival, DHEAS level was not associated with breast cancer survival (HR per doubling = 0.95, 95% CI 0.78–1.15, P-trend across quartiles = 0.59). Testosterone and SHBG levels were not associated with breast cancer survival.

No significant interactions were observed between pre-diagnostic circulating hormone levels and tumor ER expression with respect to overall or breast cancer survival (Table 4). Nonsignificant positive relative risks between estradiol level and mortality were observed for both ER + and ER − tumors, though the associations were nonsignificantly higher among ER − breast cancers. There were suggestive inverse associations between the androgens and breast cancer mortality among ER − breast cancers. Similarly, no significant hormone-by-ER interactions were observed when restricting to women diagnosed with breast cancer within 5 years of their blood draw (Supplemental Fig. 1). The association for estradiol among ER − cancers in this restricted subset was inverse (HR 0.87, 95% CI 0.63–1.21), in contrast with what was observed for breast cancer survival for ER − cancers in the total population (HR 1.17, 95% CI 0.85–1.60).

Discussion

In a prospective analysis of breast cancer patients from two large cohorts of US registered nurses, we found that higher pre-diagnostic postmenopausal concentrations of estradiol in plasma were modestly associated with worse survival. Higher DHEAS levels were associated with modestly better overall survival, but not with breast cancer-specific survival among pre- and postmenopausal women combined. Testosterone and SHBG were not associated with survival among breast cancer patients.

Table 1 Patient, tumor, and treatment characteristics in relation to age-adjusted mean pre-diagnostic estradiol (pg/mL) and testosterone (ng/dL) levels among women with stage 0–III breast cancer

Characteristic	Estradiol ($n=1077$)			Testosterone ($n=2041$)		
	n	Mean (95% CI)	*P* value*	*n*	Mean (95% CI)	*P* value*
Age at cancer diagnosis (years)[a]			< 0.001			< 0.001
< 50	1	26.0 (N/A)		274	26.0 (24.6–27.5)	
50–59	97	14.3 (12.2–16.8)		492	24.9 (23.9–26.0)	
60–69	540	10.9 (10.2–11.6)		656	22.7 (21.9–23.5)	
70 +	439	9.3 (8.6–10.0)		619	22.4 (21.5–23.2)	
Year of diagnosis			0.02			0.52
1990–1994	312	10.7 (9.7–11.8)		319	23.2 (22.0–24.4)	
1995–1999	446	11.5 (10.7–12.4)		537	23.5 (22.6–24.5)	
2000–2004	209	9.1 (8.1–10.2)		643	23.5 (22.7–24.4)	
2005 and later	110	8.7 (7.4–10.3)		542	23.7 (22.8–24.7)	
Time from blood draw to breast cancer (years)			0.30			0.29
< 5	392	10.4 (9.6–11.3)		660	23.9 (23.1–24.8)	
5–9	467	11.1 (10.3–11.9)		808	23.4 (22.6–24.2)	
10 +	218	9.3 (8.3–10.4)		573	23.2 (22.3–24.2)	
Menopausal status at blood draw			< 0.001			0.19
Pre-menopausal	–	–		576	24.5 (23.1–25.9)	
Postmenopausal not using MHT	661	7.6 (7.2–8.1)		668	23.4 (22.4–24.4)	
Postmenopausal using MHT	416	17.2 (16.0–18.5)		797	22.9 (22.2–23.8)	
Tumor ER expression			0.005			0.01
Positive	788	10.8 (10.2–11.4)		1493	23.7 (23.1–24.3)	
Negative	154	8.9 (7.8–10.1)		280	21.9 (20.7–23.2)	
Tumor HER2 expression			0.81			0.002
Positive	188	10.3 (9.2–11.5)		346	21.8 (20.8–22.9)	
Negative	451	10.1 (9.4–11.0)		960	23.9 (23.2–24.7)	
Tumor grade			0.87			0.58
Grade 1 well differentiated	232	10.3 (9.3–11.4)		413	22.7 (21.6–23.8)	
Grade 2 moderately differentiated	429	10.5 (9.7–11.3)		725	23.7 (22.9–24.6)	
Grade 3 poorly differentiated	152	10.1 (8.9–11.4)		327	22.1 (20.9–23.3)	
Tumor stage			0.89			0.04
Stage 0	159	10.9 (9.6–12.3)		417	24.3 (23.2–25.4)	
Stage I	621	10.2 (9.6–10.9)		1048	23.6 (23.0–24.3)	
Stage II	240	10.7 (9.7–11.8)		459	22.8 (21.9–23.8)	
Stage III	57	10.8 (8.8–13.3)		117	22.7 (20.8–24.7)	
Type of surgery received			0.36			0.87
Lumpectomy	495	10.7 (9.9–11.5)		820	23.5 (22.7–24.3)	
Mastectomy	430	11.2 (10.4–12.1)		663	23.4 (22.6–24.3)	
Receipt of chemotherapy			0.80			0.09
Yes	297	10.9 (9.9–12.0)		581	22.8 (21.9–23.7)	
No	587	10.7 (10.0–11.5)		873	23.8 (23.1–24.6)	
Receipt of radiation therapy			0.75			0.58
Yes	602	10.5 (9.8–11.2)		1019	23.3 (22.6–24.0)	
No	367	10.6 (9.8–11.6)		566	23.6 (22.7–24.6)	
Receipt of hormone therapy			0.21			0.56
Yes	765	10.8 (10.2–11.5)		1266	23.4 (22.8–24.0)	
No	218	10.0 (9.0–11.1)		344	23.0 (21.9–24.2)	
Body mass index (kg/m^2) at diagnosis			0.002			0.79
< 25	472	9.8 (9.1–10.5)		965	23.4 (22.7–24.1)	

Table 1 (continued)

Characteristic	Estradiol ($n=1077$)			Testosterone ($n=2041$)		
	n	Mean (95% CI)	*P* value*	*n*	Mean (95% CI)	*P* value*
25–29	341	10.8 (10.0–11.8)		623	24.0 (23.2–24.9)	
30+	220	12.0 (10.8–13.3)		388	23.4 (22.3–24.5)	
Physical activity (MET hours/day) at diagnosis			0.01			0.08
< 1.0	362	11.3 (10.4–12.2)		663	24.1 (23.3–25.0)	
1.0–1.9	230	10.5 (9.5–11.7)		411	23.5 (22.5–24.6)	
2.0–3.9	248	10.4 (9.4–11.4)		493	23.3 (22.3–24.3)	
4.0+	225	9.5 (8.6–10.5)		452	22.9 (22.0–24.0)	
Smoking status at diagnosis			0.03			0.06
Never	452	11.1 (10.4–12.0)		1040	23.1 (22.4–23.8)	
Former	527	10.2 (9.6–11.0)		833	23.8 (23.0–24.6)	
Current	88	8.9 (7.5–10.5)		151	25.3 (23.5–27.3)	

Analyses are restricted to women without missing data for variable of interest. Hormones were log2-transformed for analysis of covariance

**P* values are from global test of heterogeneity or test for trend (age, year of diagnosis, time since blood draw, stage, grade, body mass index, physical activity)

[a]Values are not age-adjusted

Table 2 Multivariable analysis of overall survival by pre-diagnostic circulating estradiol, testosterone, DHEAS, SHBG level

Hormone	Cancers	Deaths	HR (95% CI)					HR (95% CI)
			Q1	Q2	Q3	Q4	*P*-trend*	Log2 (hormone)
Estradiol								
Model 1	1077	483	1.00	1.18 (0.90–1.54)	1.43 (1.10–1.85)	1.48 (1.10–1.99)	0.02	1.11 (1.01–1.22)
Model 2	1032	457	1.00	1.13 (0.85–1.50)	1.42 (1.07–1.89)	1.43 (1.03–1.97)	0.04	1.10 (1.00–1.22)
Estradiol (non-MHT users)								
Model 2	628	276	1.00	1.13 (0.79–1.62)	1.12 (0.77–1.63)	1.46 (1.00–2.13)	0.04	1.14 (0.97–1.34)
Estradiol (MHT users)								
Model 2	404	181	1.00	1.45 (0.92–2.27)	1.52 (0.98–2.37)	1.15 (0.73–1.82)	0.95	1.04 (0.92–1.18)
Testosterone								
Model 1	2041	622	1.00	0.82 (0.66–1.02)	0.81 (0.65–1.01)	0.92 (0.74–1.14)	0.51	0.97 (0.87–1.09)
Model 2	1970	590	1.00	0.83 (0.66–1.05)	0.81 (0.64–1.01)	0.91 (0.73–1.14)	0.45	0.95 (0.84–1.07)
DHEAS								
Model 1	1630	447	1.00	1.03 (0.82–1.30)	0.79 (0.60–1.03)	0.72 (0.52–0.99)	0.01	0.87 (0.79–0.96)
Model 2	1573	420	1.00	1.10 (0.87–1.41)	0.79 (0.60–1.05)	0.79 (0.57–1.10)	0.05	0.88 (0.80–0.98)
SHBG								
Model 1	2050	630	1.00	0.82 (0.65–1.02)	0.80 (0.63–1.01)	0.85 (0.66–1.09)	0.34	0.92 (0.84–1.02)
Model 2	1981	598	1.00	0.79 (0.62–0.99)	0.76 (0.59–0.97)	0.87 (0.66–1.14)	0.62	0.93 (0.83–1.03)

Model 1: stratified by cohort, and adjusted for age at diagnosis (continuous), time since blood draw (continuous), and menopausal status/MHT use at blood draw (pre-menopausal, postmenopausal no MHT, postmenopausal using MHT)

Model 2: covariates in Model 1 + date of diagnosis (continuous), tumor stage (0, I, II, III), tumor grade (1, 2, 3, missing), HER2 expression (positive, negative, missing), ER expression (positive, negative, missing), smoking status at diagnosis (never, current, former), BMI at diagnosis (continuous), and physical activity at diagnosis (continuous)

*Tests for trend were conducted by using a Wald test for a variable taking the median concentration of each quartile of the hormone

A role of sex steroid hormones in the development and progression of breast cancer was initially hypothesized more than a century ago [29]. Estrogens exert a proliferative effect on normal and neoplastic breast epithelium via ER signaling pathways, and estrogen metabolites may also inflict genotoxic insult [30]. As biologic precursors to estrogens, androgens may contribute to the development and progression of breast cancers through their conversion to estrogens.

Table 3 Multivariable analysis of breast cancer-specific survival by pre-diagnostic circulating estradiol, testosterone, DHEAS, SHBG level

	Cancers	Deaths	HR (95% CI)					HR (95% CI)
			Q1	Q2	Q3	Q4	*P*-trend*	Log2 (hormone)
Estradiol								
Model 1	1077	101	1.00	0.73 (0.40–1.35)	1.00 (0.57–1.77)	1.21 (0.66–2.21)	0.27	1.08 (0.89–1.30)
Model 2	1032	94	1.00	0.68 (0.34–1.37)	1.33 (0.70–2.51)	1.50 (0.75–2.98)	0.12	1.17 (0.95–1.44)
Estradiol (non-MHT users)								
Model 2	628	47	1.00	1.43 (0.60–3.40)	1.09 (0.43–2.76)	2.24 (0.87–5.76)	0.11	1.40 (0.96–2.05)
Estradiol (MHT users)								
Model 2	404	47	1.00	1.07 (0.40–2.82)	1.29 (0.53–3.16)	0.93 (0.37–2.34)	0.80	1.03 (0.80–1.33)
Testosterone								
Model 1	2041	157	1.00	1.04 (0.69–1.57)	0.65 (0.41–1.05)	0.88 (0.57–1.35)	0.31	0.88 (0.70–1.10)
Model 2	1970	148	1.00	1.04 (0.68–1.61)	0.66 (0.40–1.07)	0.97 (0.61–1.52)	0.56	0.89 (0.70–1.13)
DHEAS								
Model 1	1630	119	1.00	1.30 (0.80–2.11)	0.88 (0.50–1.54)	0.93 (0.52–1.67)	0.48	0.95 (0.78–1.14)
Model 2	1573	112	1.00	1.46 (0.87–2.45)	0.88 (0.49–1.59)	1.00 (0.54–1.88)	0.59	0.95 (0.78–1.15)
SHBG								
Model 1	2050	160	1.00	0.80 (0.51–1.25)	0.79 (0.50–1.26)	1.03 (0.63–1.68)	0.68	0.99 (0.82–1.20)
Model 2	1981	151	1.00	0.67 (0.42–1.07)	0.59 (0.36–0.99)	0.92 (0.54–1.56)	0.83	0.94 (0.76–1.17)

Model 1: stratified by cohort, and adjusted for age at diagnosis (continuous), time since blood draw (continuous), and menopausal status/MHT use at blood draw (pre-menopausal, postmenopausal no MHT, postmenopausal using MHT)

Model 2: covariates in Model 1 + date of diagnosis (continuous), tumor stage (0, I, II, III), tumor grade (1, 2, 3, missing), HER2 expression (positive, negative, missing), ER expression (positive, negative, missing), smoking status at diagnosis (never, current, former), BMI at diagnosis (continuous), and physical activity at diagnosis (continuous)

*Tests for trend were conducted by using a Wald test for a variable taking the median concentration of each quartile of the hormone

Table 4 Multivariable analysis of overall and breast cancer-specific mortality per doubling of pre-diagnostic circulating estradiol, testosterone, DHEAS, SHBG level stratified by tumor ER expression

Hormone	Overall survival			Breast cancer survival		
	ER+	ER−	*P*-interaction	ER+	ER−	*P*-interaction
Estradiol						
Cases/deaths	763/341	146/70		763/59	146/27	
HR (95% CI)	1.06 (0.95–1.19)	1.12 (0.93–1.35)	0.63	1.10 (0.86–1.41)	1.17 (0.85–1.60)	0.74
Testosterone						
Cases/deaths	1452/435	268/90		1452/100	268/35	
HR (95% CI)	0.95 (0.83–1.09)	1.01 (0.74–1.37)	0.75	0.95 (0.72–1.25)	0.86 (0.51–1.44)	0.73
DHEAS						
Cases/deaths	1190/321	209/60		1190/80	209/25	
HR (95% CI)	0.88 (0.79–0.99)	0.92 (0.72–1.17)	0.75	1.01 (0.80–1.28)	0.83 (0.59–1.18)	0.34
SHBG						
Cases/deaths	1461/442	273/92		1461/102	273/36	
HR (95% CI)	0.95 (0.84–1.07)	0.83 (0.66–1.04)	0.26	0.95 (0.74–1.23)	0.96 (0.64–1.44)	0.98

HR is stratified by cohort and adjusted for age at diagnosis (continuous), time since blood draw (continuous), menopausal status and MHT use at blood draw (pre-menopausal, postmenopausal no MHT, postmenopausal using MHT), date of diagnosis (continuous), tumor stage (0, 1, 2, 3), tumor grade (1, 2, 3, missing), HER2 expression (positive, negative, missing), smoking status at diagnosis (never, current, former), BMI at diagnosis (continuous), and physical activity at diagnosis (continuous)

However, androgens have demonstrated both independent proliferative and anti-proliferative effects in the breast, with the direction dependent both on the androgen and the model system evaluated [4, 5, 31–33]. Androgens may act as estrogen agonists in estrogen-replete environments, but estrogen antagonists in estrogen-rich environments [5].

While the positive association between circulating hormones and breast cancer risk has been well established [6, 34], the handful of epidemiologic studies to date that have evaluated circulating hormones as potential prognostic markers for breast cancer has yielded inconsistent findings. These studies have been conducted predominantly in dietary or lifestyle modification trials among breast cancer survivors or hospital-based cohorts and have had relatively low sample sizes (range 31–153 events). Correspondingly, these studies have had limited capacity to adjust for potential confounding by patient and tumor characteristics. With one exception in which serum samples were collected at diagnosis [18], circulating hormone concentrations in prior studies have been measured following breast cancer diagnosis, with sample collections ranging from a median 3 months post-surgery to a mean 4.6 years post-diagnosis. While our pre-diagnostic design has its limitations, post-diagnostic designs have potential complications of treatment influencing hormone levels, as well as potential inherent selection biases in that women must survive to provide a sample. The heterogeneity in design and analytic approach has likely contributed to the inconsistency in findings, but it remains unclear which factors drive this heterogeneity. Our finding that higher postmenopausal estradiol levels are associated with moderately worse breast cancer survival mirrors prior studies [16, 17]; however, others have not observed an association [15]. We and others did not find that testosterone levels were associated with worse survival outcomes [15, 16], though several prior studies have reported twofold to sevenfold relative risks [13, 14, 18]. While one study reported a significant inverse association for circulating SHBG [15], others, including ourselves, did not observe an association [13, 16]. Finally, to our knowledge, we are the first to evaluate circulating DHEAS as a prognostic marker for breast cancer. With these inconsistencies, further large studies evaluating circulating hormone concentrations measured at diagnosis would be invaluable in clarifying the potential prognostic utility of these biomarkers.

Given the effects of estrogens and androgens on the breast, it might follow that the associations between circulating hormones and breast cancer progression would be stronger for hormone receptor-positive cancers. We found no significant heterogeneity of the hormone-survival associations by tumor ER status. The HEAL study investigators likewise found no effect heterogeneity by tumor ER expression for estradiol, testosterone, or SHBG [15]. However, our analysis to assess heterogeneity was underpowered and it may be that this potential interaction is more apparent in hormone levels measured at diagnosis, rather than years prior. It remains unclear how well circulating hormones correlate with hormone receptor signaling in the breast. The limited evidence suggests that, although positively correlated, estrogen levels are significantly higher in the breast tissue than in circulation [35–37]. Circulating estradiol has been shown to be associated with the expression of severable established estrogen response genes in tumor tissue, including *PGR, GREB1, TFF1*, and *PDZK1* [38]. The few studies that have evaluated the correlation between androgen levels in the breast and in circulation have found coefficients ranging from −0.81 to 0.23 [39–41].

A major strength of this study relative to several prior studies is the ability to better control for potential confounding factors. In particular, BMI, physical activity, and smoking are correlated with sex hormone concentrations and have been shown to be prognostic factors for breast cancer [42, 43]. Smoking was a particularly strong confounder for analyses evaluating all-cause mortality. Though a disadvantage in other respects, the use of pre-diagnostic circulating hormone levels ensures that concentrations are not affected by breast cancer therapies and may better reflect the baseline hormonal milieu in the breast that led to carcinogenesis. Finally, this study also benefits from its large sample size and extended follow-up on its participants.

There are several notable limitations to our study, chief among which is the use of pre-diagnostic blood samples to represent circulating hormone levels at the time of diagnosis. Our group has demonstrated previously that circulating hormone levels are stable over a 10-year period, observing within-individual intra-class correlations of 0.69 for estradiol, 0.71 for testosterone, 0.54 for DHEAS, and 0.74 for SHBG [12]. Though the median time between blood collection and breast cancer diagnosis was 7 years in this study, we may be concerned that the breast cancer may influence hormone levels, such that the pre-diagnostic concentrations are not representative of the concentrations that would have been observed at the time of diagnosis. A sensitivity analysis restricting to individuals who were diagnosed with breast cancer within 5 years of providing a blood sample yielded similar results to the primary analysis. The congruence of these results suggests that that pre-diagnostic hormone levels can be interpreted as representative of the levels that would be observed at diagnosis. An additional potential limitation is that this study was restricted to breast cancer patients who were members of the blood collection sub-cohorts in NHS and NHSII. However, the included breast cancer cases are similar to those diagnosed in the total NHS and NHSII cohorts, with respect to patient and tumor characteristics. Finally, information regarding tumor grade and tumor ER and HER2 expression was not available for a subset of the study population (10–35%), which could result in residual confounding from these factors.

In summary, we observed that pre-diagnostic circulating estradiol levels were associated with moderately worse survival among postmenopausal breast cancer patients, while circulating androgen and SHBG levels were not prognostic in pre- and postmenopausal women combined. Future

studies should attempt to measure circulating hormone levels at the time of diagnosis prior to treatment initiation, to better assess sex hormones as potential markers of breast cancer prognosis or predictors of treatment response.

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Compliance with ethical standards

Conflict of interest MB receives sponsored research support from and has been a consultant to Novartis. MB has served on the scientific advisory board of Gtx, Inc. and currently serves on the scientific advisory board of Kronos Bio. The remaining authors declare they have no conflict of interest.

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