


# Joint relative risks for estrogen receptor-positive breast cancer from a clinical model, polygenic risk score, and sex hormones

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## Abstract

**Background** Models that predict the risk of estrogen receptor (ER)-positive breast cancers may improve our ability to target chemoprevention. We investigated the contributions of sex hormones to the discrimination of the Breast Cancer Surveillance Consortium (BCSC) risk model and a polygenic risk score comprised of 83 single nucleotide polymorphisms. **Methods** We conducted a nested case-control study of 110 women with ER-positive breast cancers and 214 matched

controls within a mammography screening cohort. Participants were postmenopausal and not on hormonal therapy. The associations of estradiol, estrone, testosterone, and sex hormone binding globulin with ER-positive breast cancer were evaluated using conditional logistic regression. We assessed the individual and combined discrimination of estradiol, the BCSC risk score, and polygenic risk score using the area under the receiver operating characteristic curve (AUROC).

**Results** Of the sex hormones assessed, estradiol (OR 3.64, 95% CI 1.64–8.06 for top vs bottom quartile), and to a lesser degree estrone, was most strongly associated with ER-positive breast cancer in unadjusted analysis. The BCSC risk score (OR 1.32, 95% CI 1.00–1.75 per 1% increase) and polygenic risk score (OR 1.58, 95% CI 1.06–2.36 per standard deviation) were also associated with ER-positive cancers. A model containing the BCSC risk score, polygenic risk score, and estradiol levels showed good discrimination for ER-positive cancers (AUROC 0.72, 95% CI 0.65–0.79), representing a significant improvement over the BCSC risk score (AUROC 0.58, 95% CI 0.50–0.65).

**Conclusion** Adding estradiol and a polygenic risk score to a clinical risk model improves discrimination for postmenopausal ER-positive breast cancers.

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**Keywords** Breast cancer · Single nucleotide polymorphisms · Sex hormones · Risk assessment · Cancer surveillance and screening · Chemoprevention

## Abbreviations

AUROC Area under the receiver operating characteristic curve  
BCSC Breast Cancer Surveillance Consortium  
BI-RADS Breast Imaging Reporting and Data System

BMI	Body mass index
CCR	California Cancer Registry
CPMC	California Pacific Medical Center
GWAS	Genome-wide association study
LR	Likelihood ratio
OR	Odds ratio
PRS	Polygenic risk score
SFMR	San Francisco Mammography Registry
SHBG	Sex hormone binding globulin
SNPs	Single nucleotide polymorphisms
USPSTF	United States Preventive Services Task Force

## Introduction

ER-positive cancers represent at least 80% of breast cancers diagnosed in the United States [1]. Preventive medications such as tamoxifen and raloxifene can reduce the risk of estrogen receptor (ER)-positive breast cancers in high-risk women but have potential adverse effects [2]. Uptake is partially limited by the ability to identify high-risk women with the most favorable benefit-harm tradeoff [3, 4].

The United States Preventive Services Task Force (USPSTF) recommends using risk prediction models to identify candidates for chemoprevention but acknowledges that current models have modest discrimination [3]. Validated models such as the Breast Cancer Risk Assessment Tool and Tyrer-Cuzick model incorporate clinical risk factors such as age, race/ethnicity, family history, history of prior breast biopsy, and history of benign breast disease [3, 5–7]. The Breast Cancer Surveillance Consortium (BCSC) risk model also includes mammographic breast density, a strong risk factor, and tends to have the highest discrimination of available models [8, 9].

Single nucleotide polymorphisms (SNPs) can improve the performance of clinical risk models. Individual SNPs have modest impacts on risk, but polygenic risk scores (PRS) representing the cumulative effects of multiple SNPs exhibit strong associations with risk that are largely independent from clinical risk factors [10–12]. PRS have been shown to improve the discrimination of the BCSC [11, 12] and other [13] risk models. The magnitude of improvement in the area under the receiver operating characteristic curve (AUROC) of these models is modest [11, 12].

Circulating sex hormone levels may improve prediction beyond SNPs and clinical models [7, 14, 15]. Estrone and estradiol have been most robustly associated with breast cancer, particularly ER-positive cancers. Elevated estradiol levels are positively associated with breast cancer risk [14–20], while undetectable levels are strongly protective against ER-positive cancers [21]. Sex hormone binding globulin (SHBG) binds estradiol, affecting bioavailable

levels, and has been inversely associated with risk of invasive breast cancer [14]. The reported effects of testosterone on breast cancer risk have been heterogeneous [14, 18, 19, 22–24].

Many prior studies have examined the relationships between individual hormones and breast cancer risk [14–16, 18–20, 22, 24]. Fewer studies have assessed the combined effects of hormones in risk prediction, particularly in conjunction with risk prediction models. In one study, sex hormones (with the most parsimonious combination being estrone sulfate, testosterone, and prolactin) improved the performance of the Gail and Rosner–Colditz risk models by AUROC of 0.06 and 0.03 (relative improvements of 11 and 6%), respectively [25]. This analysis did not account for breast density or SNPs.

A prediction model for ER-positive breast cancer, which includes sex hormones, SNPs, and clinical risk factors could potentially improve on the discrimination of more restrictive models and help identify women most likely to benefit from chemoprevention by virtue of their risk of ER-positive breast cancer. To investigate this hypothesis, we studied the associations of four sex hormones—estrone, estradiol, testosterone, and SHBG—with ER-positive breast cancer in postmenopausal women. We assessed whether the addition of one or a combination of sex hormones improved the discrimination of the BCSC risk score alone, and the BCSC risk score modified by an 83-SNP PRS.

## Methods

### Study population

We conducted a nested case-control study [12] within the California Pacific Medical Center (CPMC) Research Institute cohort, comprised of women undergoing screening mammography at the Breast Health Center at CPMC. Between 2004 and 2011, women were asked to provide blood samples for research. Women who provided informed consent for blood collection completed a questionnaire with demographic information and risk factor data, which were collected and pooled by the San Francisco Mammography Registry (SFMR). The protocol was approved by the institutional review boards at CPMC and the University of California, San Francisco.

Blood samples were collected from 19,276 women without a diagnosis of invasive or pre-invasive breast cancer at the time of blood draw. A nested case-control study was performed on 324 participants with blood collected between 9/3/2004 and 11/30/2011. We excluded women self-identified as premenopausal, perimenopausal, or postmenopausal as a direct result of surgery or medical

treatments, such as chemotherapy. Women on hormonal therapy or selective estrogen receptor modulators at the time of blood draw were also excluded. Cases were ascertained by linkage to the California Cancer Registry (CCR) and defined as pathologically-confirmed diagnoses of invasive breast cancers with positive/elevated ER expression on immunohistochemical staining. ER-negative cases and those with unavailable ER status were excluded. Women without breast cancer as of last linkage to the CCR on 10/31/2013 were matched as controls in a 2:1 ratio to cases based on age, race/ethnicity, and date of index mammogram. Six cases were matched 1:1 with controls due to missing data, resulting in 110 cases and 214 controls.

### Sex hormone measurement

Blood samples for sex hormone measurements were collected at the time of consent. Whole blood was centrifuged within 15 min of venipuncture and serum was aliquoted into 1 mL tubes stored at  $-80^{\circ}\text{C}$ . Samples were shipped on dry ice by overnight courier to Mayo Medical Laboratory (Rochester, MN) for hormone measurement. Liquid chromatography–mass spectrometry (LC–MS, Agilent Technologies, Santa Clara, CA) was used to measure estrone, estradiol, and testosterone levels. Prior to LC–MS, estradiol and estrone were extracted with methylene chloride and underwent derivatization with dansyl chloride followed by high-pressure liquid chromatography. SHBG was measured using a Siemens chemiluminescent assay (Siemens Healthcare Diagnostics, Deerfield, IL). The inter- and intra-assay coefficients of variation for each assay are shown in Supplementary Table S1. Women with estradiol levels over 25 pg/ml were excluded from the analysis given the possibility that extreme values were due to unreported or incorrectly ascertained exogenous hormone usage or menopausal status. The free estradiol index was calculated by dividing the total estradiol level by the SHBG level and multiplying by 100.

### SNP genotyping and calculation of polygenic risk score

Whole blood was sent to the Genomics Core at the University of Minnesota for DNA extraction. A total of 113 cases and 113 matched controls randomly selected from the overall dataset were genotyped using an OncoArray platform (Illumina, San Diego, CA), resulting in 1:1 matching within the genotyped subgroup. We included 83 SNPs (Supplementary Table S2) selected based on review of the genome-wide association study (GWAS) catalog and published associations with invasive breast cancer in Caucasian, Asian, or Hispanic populations, as previously

described [12]. The PRS was calculated using a previously described method using published odds ratios and allele frequencies [12]. In brief, it is the composite likelihood ratio (LR) of breast cancer representing the individual effects of each SNP assuming that the SNPs are inherited independently (in linkage equilibrium) and that there are no interactions between them.

### BCSC model

The BCSC risk model provides estimates of 5-year absolute risk using age, race/ethnicity, presence of a first-degree relative with breast cancer, history of breast biopsy, and breast density [8, 9]. Community radiologists participating in the SFMR assessed breast density on the index mammogram (acquired 1997–2011) according to the Breast imaging reporting and data system (BI-RADS) fourth edition or earlier categories: almost entirely fat (a), scattered fibroglandular densities (b), heterogeneously dense (c), and extremely dense (d) [26]. Although an updated version of the BCSC model has been published [9], we used version 1.0 [8] to allow the calculation of risk estimates for women older than 74 years.

### Statistical analysis

Demographic data and risk factors were compared between cases and controls using the chi-squared test for categorical measures and the unpaired *t* test for body mass index. The median hormone levels across cases and controls were compared using the non-parametric *k*-sample equality of medians test. Statistical tests were two-sided, with  $\alpha = 0.05$ .

To examine the effects of individual hormones on ER-positive breast cancer risk, we performed univariate conditional logistic regression. Hormone levels were categorized into quartiles based on their respective distributions in controls. We compared point estimates across quartiles using tests of linear trend.

To examine the relative contributions of sex hormone levels, the BCSC risk score, and PRS to ER-positive risk, we performed conditional logistic regression using univariate and multivariable models within the subset of women ( $n = 218$ , i.e., 109 matched case-control pairs) with complete genotype, hormone, density, and clinical data. Hormone levels were  $\log_2$ -transformed so that a one-unit increase represented a doubling of levels. The PRS was standardized according to the mean and standard deviation of the PRS in controls. The 5-year risk estimate generated by BCSC version 1.0 was used as a continuous variable in logistic regression models.

Area under the receiver operating characteristic curve (AUROC) was used to compare discrimination. To avoid

the bias introduced when the same data are used to both fit and evaluate the model, we performed tenfold cross-validation to confirm the internal validity of the model. Briefly, we split the dataset into ten equally-sized groups containing randomly-selected case-control pairs, used 9/10 as a training dataset to fit a regression model, then used the fitted model to generate predicted probabilities in the remaining 1/10. We repeated this process ten times so that each 1/10 of the dataset was used once as a validation set, and then used the aggregate predicted probabilities to calculate the final cross-validated AUC and 95% CI using a stratified bootstrap approach ( $n = 1000$  replications) accounting for matched case-control clusters. Reported AUROCs represent cross-validated estimates. We compared AUROCs using a Wald test with bootstrap variance and covariance estimates.

Primary statistical analysis was performed using STATA 14.1 (StataCorp, College Station, TX, USA). The PRS was generated using R (R Foundation, Vienna, Austria), and the BCSC risk estimate was generated using SAS Version 9.3 (SAS Institute, Cary, NC, USA).

## Results

We identified 110 postmenopausal women with ER-positive, invasive breast cancers and 214 matched controls. Approximately 80% of cases and controls were Caucasian/White by self-report (Table 1). Cases were more likely to have a positive family history of breast cancer, prior breast biopsy, and higher body mass index, although the absolute difference was  $1 \text{ kg/m}^2$ . The distribution of breast density was generally similar between cases and controls, with most women having scattered fibroglandular densities (BI-RADS b) or heterogeneously dense (BI-RADS c) breasts. Fatty breasts (BI-RADS a) were more common in cases, while extremely dense breasts (BI-RADS d) were more common in controls. However, the overall difference in density did not reach statistical significance ( $p = 0.12$ ).

Cases had higher median estrone and estradiol levels while controls had higher SHBG (Table 1). Cases and controls had similar testosterone levels. The distributions of all hormone levels were right-skewed, while log-transformed levels approximated a normal distribution (Supplementary Figure S1).

We examined the associations between individual sex hormones and ER-positive breast cancer using univariate logistic regression (Table 2). Estradiol had the strongest association with ER-positive breast cancer, with increasing levels corresponding to higher risk. The associations reached statistical significance in the highest (OR 3.64, 95% CI 1.64–8.06) and second-highest (OR 3.12, 95% CI 1.41–6.90) quartiles relative to the bottom quartile, and

followed a linear trend. Free estradiol index showed a similar trend. Estrone levels above 14 pg/ml were associated with an approximately twofold increase in risk. SHBG was inversely associated with risk, though no quartile associations reached statistical significance. The second-highest quartile of testosterone was associated with elevated risk (OR 2.55, 95% CI 1.23–5.26) but a null effect could not be excluded for the other quartiles.

The individual and combined contributions of the BCSC risk score, PRS, and estradiol levels to risk prediction were evaluated in a subset of 218 women representing 109 cases and 109 matched controls who were genotyped in addition to having hormone levels and breast density measured. We selected estradiol because it was most robustly associated with ER-positive breast cancer in univariate logistic regression.

In univariate analysis, estradiol was associated with ER-positive cancer, as were the PRS and BCSC risk score (Table 3, column 1). In a model containing the BCSC risk score and estradiol levels, the OR per doubling of estradiol levels slightly increased from 1.57 (95% CI 1.13–2.19) to 1.79 (95% CI 1.24–2.58). The ORs and confidence intervals per 1% increase in the BCSC risk score and per doubling of estradiol modestly increased when combined in a joint model (Table 3, column 2), but remained similar when the PRS was added to the model (Table 3, column 3). Adjustment for BMI slightly attenuated the associations between estradiol and ER-positive cancer but did not have a substantial effect on the BCSC risk score or the PRS (Supplementary Table S3).

We compared the discrimination of the above models using ROC curve analysis generated by tenfold cross-validation (Table 4). AUROCs for estradiol and the PRS were higher than that of the BCSC risk score, though differences did not reach statistical significance. Adding estradiol to the BCSC risk score resulted in modestly improved discrimination from AUROC 0.58 (95% CI 0.50–0.65) to 0.67 (95% CI 0.57–0.71),  $p = 0.02$ , while the AUROC increase with adding PRS to the BCSC risk score was of borderline statistical significance. The combination of the BCSC risk score, PRS, and estradiol levels had the highest discrimination out of all models, with AUROC 0.72 (95% CI 0.65–0.79), representing an AUROC increase of 0.14 (24%) relative to the BCSC risk score alone,  $p < 0.001$ . Furthermore, the model including BCSC risk score, PRS, and estradiol had improved discrimination relative to the BCSC-PRS ( $p = 0.02$ ) and BCSC-estradiol models ( $p = 0.01$ ). The ROC curves for the BCSC risk score alone and with the addition of the PRS and/or estradiol levels are shown in Fig. 1. We confirmed that inclusion of estrone, testosterone, or SHBG to models containing the BCSC risk score and estradiol levels did not further improve model performance (Supplementary Table S4).

**Table 1** Study demographics, all patients

Characteristic	Controls ( <i>n</i> = 214)	Cases ( <i>n</i> = 110)	<i>p</i> value
Matched variables			
Age, years—median (I.Q.R.)	62 (57–67)	61.5 (57–67)	
Race—no. (%)			
White	172 (80.4)	89 (80.9)	
Hispanic	9 (4.2)	5 (4.6)	
Asian	30 (14.0)	15 (13.6)	
Mixed	2 (0.9)	1 (0.9)	
Other (Non-Asian)	1 (0.4)	0 (0)	
Unmatched variables			
First-degree relative with breast cancer—no. (%)	32 (15.0)	36 (32.7)	<0.001
History of breast biopsy—no. (%)	49 (22.9)	36 (32.7)	0.06
Mean Body Mass Index—median (I.Q.R.)	24.3 (21.6–27.3)	25.8 (23.0–28.3)	0.05
Breast density, BI-RADS category—no. (%)			
a, almost entirely fat	26 (12.2)	18 (16.4)	0.12
b, scattered fibroglandular densities	99 (46.3)	49 (44.5)	
c, heterogeneously dense	69 (32.2)	40 (36.4)	
d, extremely dense	20 (9.4)	3 (2.7)	
Sex hormone levels—median (I.Q.R.)			
Estrone (pg/ml)	14 (10–19)	16 (12–22)	0.002
Estradiol (pg/ml)	2.8 (1.9–4.3)	3.5 (2.3–4.8)	0.005
Free estradiol index	5.5 (3.2–9.9)	7.2 (4.3–12)	0.014
Testosterone (pg/ml)	10 (7.5–16)	12 (8.4–16)	0.32
Sex hormone binding globulin (nmol/L)	51.4 (37.5–70.8)	44.7 (36.1–65.2)	0.20

*I.Q.R* Interquartile Range, *BI-RADS* breast imaging reporting and data system

We further examined the physiologic relationship between estradiol and ER-positive breast cancer using a cubic splines model (Figure S2). The risk of ER-positive breast cancers increased linearly with rising estradiol levels until a threshold of approximately 5 pg/ml, beyond which there was no further risk elevation associated with higher estradiol levels. The precision of our OR estimate was limited in the upper range of estradiol levels due to scarcity of data.

## Discussion

The addition of estradiol levels and the PRS to the BCSC model improved its performance, with the combined model having an AUROC of 0.72 (95% CI 0.65–0.79) for ER-positive cancers, representing a statistically significant improvement from the BCSC risk score, both alone and in combination with estradiol or an 83-SNP polygenic risk score. The sample size and retrospective nature of the analysis limits our ability to definitively assess discrimination and calibration, particularly the precision of our AUROC point estimates. Nevertheless, our results contribute to the growing body of literature showing that the

addition of circulating measures such as SNPs and hormone levels can improve the performance of clinical risk models.

While the PRS has been shown to improve the discrimination of the BCSC model in several reports [11, 12], these analyses focused on invasive breast cancer overall. Differential effects of clinical risk factors [27], breast density [28, 29], and SNPs [30, 31] with ER subtype have been reported. Neither the BCSC model nor PRS are fitted to receptor subtype, but these models are likely well-fitted to ER-positive cancers given that at least 80% of breast cancers diagnosed in the United States are ER-positive [27], with the proportion rising with older age [1]. In our study, it is possible that both the PRS and estradiol levels contributed subtype-specific prediction, suggesting that measurement of both biomarkers may be of clinical value.

Our study is the first to investigate the combined contributions of clinical risk factors, breast density, SNPs (as represented by a PRS), and sex hormones to breast cancer risk prediction. The most comparable study examined the contributions of seven sex hormones to the Gail and Rosner–Colditz risk models [25]. The authors used stepwise regression to identify a subset of hormones (estrone, testosterone, and prolactin) that maximized predictive

**Table 2** Unadjusted associations between hormone levels and estrogen receptor-positive breast cancer

Hormone level, by quartiles	Controls <i>n</i> (%)	Cases <i>n</i> (%)	O.R. (95% CI)	<i>p</i> <sub>trend</sub>
Estrone (pg/ml)				0.002
<10	57 (26.2)	17 (15.5)	1.0 (ref)	
10–14	55 (25.7)	19 (17.3)	1.23 (0.58–2.61)	0.59
14–19	58 (27.1)	41 (37.3)	<b>2.49 (1.23–5.03)</b>	<b>0.011</b>
≥19	45 (21.0)	33 (30.0)	<b>2.61 (1.27–5.39)</b>	<b>0.009</b>
Estradiol, total (pg/ml)				<0.001
<1.9	53 (24.8)	12 (10.9)	1.0 (ref)	
1.9–2.8	53 (24.8)	23 (20.9)	2.20 (0.95–5.11)	0.066
2.8–4.3	50 (23.4)	33 (30.0)	<b>3.12 (1.41–6.90)</b>	<b>0.005</b>
≥4.3	58 (27.1)	42 (38.2)	<b>3.64 (1.64–8.06)</b>	<b>0.001</b>
Free estradiol index				0.004
<3.2	62 (29.0)	19 (17.3)	1.0 (ref)	
3.2–5.5	56 (26.2)	25 (22.7)	1.52 (0.76–3.05)	0.24
5.5–9.9	52 (24.3)	29 (26.4)	1.85 (0.91–3.76)	0.089
≥9.9	44 (20.6)	37 (33.6)	<b>2.64 (1.36–5.13)</b>	<b>0.004</b>
Testosterone (pg/ml)				0.12
<7.5	51 (23.8)	17 (15.5)	1.0 (ref)	
7.5–10	60 (28.0)	27 (24.6)	1.41 (0.66–3.00)	0.37
10–16	54 (25.2)	42 (38.2)	<b>2.55 (1.23–5.26)</b>	<b>0.012</b>
≥16	49 (22.9)	24 (21.8)	1.57 (0.71–3.43)	0.26
Sex hormone binding globulin (nmol/L)				0.093
<37.5	54 (25.2)	31 (28.2)	1.0 (ref)	
37.5–51.4	53 (24.8)	35 (31.8)	1.16 (0.62–2.18)	0.63
51.4–70.8	54 (25.2)	27 (24.6)	0.86 (0.44–1.66)	0.65
≥70.8	53 (24.8)	17 (15.5)	0.60 (0.30–1.18)	0.14

Bold values denote statistically significant associations ( $p < 0.05$ )

**Table 3** Univariate and multivariate logistic regression on hormones in combination with the BCSC model and polygenic risk score

Risk factor	Univariate O.R. (95% CI)	<i>p</i> value	BCSC and estradiol O.R. (95% CI)	<i>p</i> value	BCSC, estradiol and PRS O.R. (95% CI)	<i>p</i> value
Estradiol, per doubling	1.57 (1.13–2.19)	0.01	1.79 (1.24–2.58)	0.002	1.82 (1.26–2.64)	0.001
Polygenic risk score, per standard deviation	1.58 (1.06–2.36)	0.02	–	–	1.63 (1.07–2.50)	0.02
BCSC risk score, per 1% increase	1.32 (1.00–1.75)	0.05	1.51 (1.11–2.06)	0.01	1.52 (1.10–2.10)	0.01

power of the respective models. Direct comparison of the AUROCs in our study to those reported by Twroger et al. [25] is limited by differences in study design, statistical analysis, and performance of risk models evaluated in each study. Neither the Gail nor Rosner–Colditz models incorporate breast density, and the study [25] did not evaluate the effects of genetic variants.

Our findings around the association of the individual sex hormones with breast cancer risk are qualitatively consistent with previous studies. Estradiol has been robustly associated with risk. In our study, estradiol had the strongest association with ER-positive breast cancer in univariate analyses, and the results of our cubic splines analysis suggested a threshold effect. Estrone has also been associated with breast cancer risk in numerous studies

[14, 19, 20], consistent with our results. Our results suggest that SHBG (which modulates estradiol's physiologic effects) provides a small improvement in prediction, but our analysis was not powered to definitively assess this observation. Testosterone did not meaningfully contribute to risk prediction in our dataset, which is unsurprising given the heterogeneous results in the literature [14, 18, 19, 22–24].

Our findings should be interpreted in light of several considerations. First, the discrimination of the BCSC risk score in our dataset was lower than in the previous studies, where the AUROCs ranged from 0.65 to 0.66 [8, 9]. The lower AUROC we observed was likely due to matching based on age and race/ethnicity (both strong predictors of invasive breast cancer in the BCSC model). Additionally,

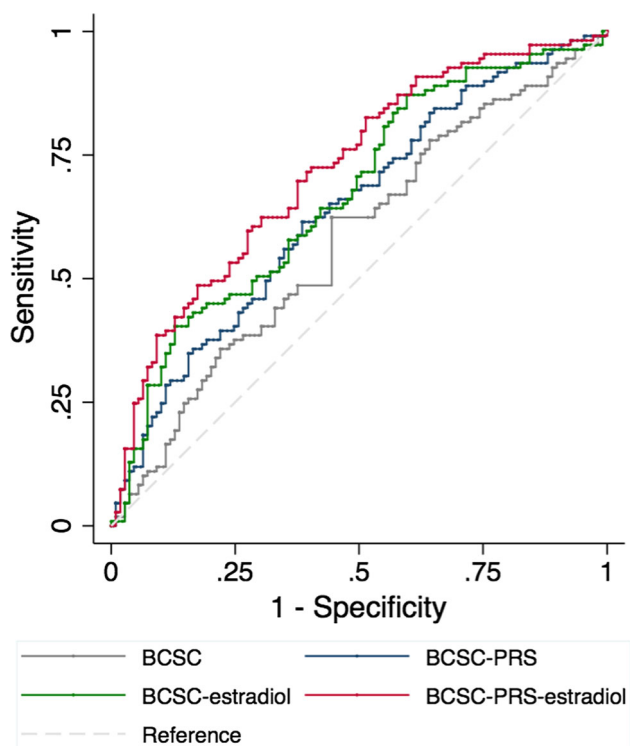
**Table 4** Areas under the receiver operating characteristic curve for models containing the BCSC model, polygenic risk score, and sex hormones

Model	Area under the curve	95% CI	<i>p</i> value <sup>a</sup>
BCSC	0.58	0.50–0.65	–
PRS	0.68	0.61–0.75	0.07
Estradiol only	0.65	0.58–0.72	0.21
BCSC-PRS	0.64	0.57–0.71	0.07
BCSC-estradiol	0.67	0.60–0.74	0.02
BCSC-PRS-estradiol	0.72	0.65–0.79	<0.001 <sup>b,c</sup>

<sup>a</sup> Compared with BCSC model alone

<sup>b</sup> *p* = 0.01 versus BCSC-estradiol model

<sup>c</sup> *p* = 0.02 versus BCSC-PRS model



**Fig. 1** Receiver operating characteristic curves for estrogen receptor-positive breast cancers are shown for the Breast Cancer Surveillance Consortium (BCSC) model alone and in combination with estradiol level, polygenic risk score (PRS), or both. The *dashed reference line* corresponds to an area under the curve of 0.5

the distribution of breast density did not differ significantly between cases and controls, possibly due to a combination of chance and the restriction of our analysis to postmenopausal women with ER-positive breast cancers, a group where the association with breast density is attenuated with increasing age [27]. The discrimination of the BCSC risk score would likely be higher in larger, unmatched studies.

Importantly, the sample size of our study limited the precision of our point estimates and comparisons. In particular, the AUROC for the PRS was higher than previously reported, though the latter studies' AUROCs ranged from 0.60 to 0.62, which overlaps with our confidence intervals [10, 11]. The AUROC for the PRS in combination with the BCSC risk score was slightly lower than that of the PRS alone possibly due to sample splitting for cross-validation, and relative insensitivity of the AUROC for improvements in discrimination with the addition of risk factors [32]. Our AUROC comparisons must also be interpreted in light of an underlying assumption of the Wald test, that the differences in AUROC estimators follows a normal distribution. Due to our small sample size, this assumption may not have been satisfied [33].

Additionally, the case-control design limited our ability to assess calibration. Careful evaluation of model calibration in independent, preferably unmatched, datasets is essential prior to adoption of such a tool into clinical practice. Lastly, our population was predominantly white, creating the need for further assessment of discrimination and calibration in a multiracial, multiethnic population.

Our statistical approach differed from those described by others. We did not adjust our main analysis for additional covariates beyond those included in the BCSC model. Previously published studies have conditioned on such variables as parity, number of live births, age at menarche, and time since menopause [14]. These analytical approaches tend to isolate the causal effect of the hormonal pathway by controlling for potential mediators, likely leading to a conservative estimate of the hormone's effect. In contrast, our goal was to investigate the net predictive power of sex hormones, which encompasses the effects of mediating and confounding pathways. We examined hormones without adjustment for covariates to determine whether they improved the prediction of our models. For example, we did not adjust for BMI because the relationship between obesity and breast cancer risk is primarily mediated through its effect on circulating estrogen levels [34–36]. Supporting this assumption, adjusting for BMI in secondary analyses minimally attenuated the association between estradiol and ER-positive breast cancer.

We chose ER-positive cancers as our endpoint given the direct causal relationship between estradiol (and other sex hormones) with these cancers. In the clinical setting, our results are therefore most applicable to chemoprevention. Guidelines empirically recommend using risk prediction models to identify high-risk women eligible for chemoprevention [3, 37], although such models are not specific to particular subtypes of breast cancer. Selective estrogen receptor modulators (SERMs) and aromatase inhibitors have established efficacy in preventing the development of ER-positive cancers in high-risk women. Improving risk

prediction for ER-positive cancers may improve our ability to identify women who may specifically benefit from chemoprevention, although this approach merits further evaluation in specimens stored from randomized trials.

The threshold effect of estradiol is consistent with prior studies showing that high-risk women with undetectable estradiol levels were not at elevated risk of developing breast cancers, and did not benefit from chemoprevention with raloxifene, a selective estrogen receptor modulator [21]. Although our splines analysis is purely exploratory and limited by sample size, future studies could further investigate whether a threshold relationship exists and attempt to identify an estradiol level cutoff that could be used alone, or in conjunction with risk models, to risk-stratify women for ER-positive cancer.

Our results suggest that the addition of estradiol to clinical risk factors, breast density, and a PRS may improve the prediction of ER-positive breast cancer in postmenopausal women. This combination of predictors may improve the identification of postmenopausal women who are most likely to benefit from chemoprevention.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Specifically, each registry in the Breast Cancer Surveillance Consortium and the Statistical Coordinating Center (SCC) have received institutional review board approval for either active or passive consenting processes or a waiver of consent to enroll participants, link data, and perform analytic studies. All procedures are Health Insurance Portability and Accountability Act (HIPAA) compliant and

all registries and the SCC have received a Federal Certificate of Confidentiality and other protection for the identities of women, physicians, and facilities who are subjects of this research.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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