

Added value of serum hormone measurements in risk prediction models for breast cancer for women not using exogenous hormones: Results from the EPIC cohort

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Abbreviations:

AIC:	Akaike information criterion
AUROC:	Area under the receiver operating curve
BCRAT:	Breast Cancer Risk Assessment Tool
BMI:	Body mass index
CDC:	United States Centers for Disease Control and Prevention
DCIS:	Ductal carcinoma <i>in situ</i>
DHEAS:	Dehydroepiandrosterone sulfate
DKFZ:	German Cancer Research Center
EPIC:	European Prospective Investigation into Cancer and Nutrition
ER:	Estrogen receptor
IDI:	Integrated discrimination improvement
IGF-I:	Insulin-like growth factor-I
IGFBP3:	Insulin-like growth factor binding protein 3
NRI:	Net reclassification improvement
OC:	Oral contraceptive
PMH:	Postmenopausal hormones
SHBG:	Sex hormone binding globulin

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Translational Relevance: Breast cancer risk prediction models are increasingly being considered in the context of mammography screening, given the need to balance potential benefits of screening against possible negative side effects such as over-diagnosis or false positive findings, and financial costs. Our model demonstrates opportunities for higher discrimination in models incorporating blood-based biomarkers, including estradiol and testosterone, hormones with established cross-laboratory standardization protocols.

Abstract

Purpose: Circulating hormone concentrations are associated with breast cancer risk, with well-established associations for postmenopausal women. Biomarkers may represent minimally invasive measures to improve risk prediction models.

Experimental Design: We evaluated improvements in discrimination gained by adding serum biomarker concentrations to risk estimates derived from risk prediction models developed by Gail et al. and Pfeiffer et al. using a nested case-control study within the EPIC cohort including 1217 breast cancer cases and 1976 matched controls. Participants were pre- or postmenopausal at blood collection. Circulating sex steroids, prolactin, insulin-like growth factor I, IGF binding protein 3 and sex hormone binding globulin (SHBG) were evaluated using backward elimination separately in women pre- and postmenopausal at blood collection. Improvement in discrimination was evaluated as the change in C-statistic from a modified Gail or Pfeiffer risk score alone vs. models including the biomarkers and risk score. Internal validation with bootstrapping (1000-fold) was used to adjust for over-fitting.

Results: Among women postmenopausal at blood collection, estradiol, testosterone and SHBG were selected into the prediction models. For breast cancer overall, discrimination was 5.3 percentage points higher than the modified Gail model alone, and 3.4 percentage points higher than the Pfeiffer model alone, after accounting for over-fitting. Discrimination was more markedly improved for estrogen receptor (ER)+ disease (percentage point change in C-statistic: 7.2, Gail; 4.8 Pfeiffer). We observed no improvement in discrimination among women premenopausal at blood collection.

Conclusions: Integration of hormone measurements in clinical risk prediction models may represent a strategy to improve breast cancer risk stratification.

Introduction

Risk prediction models aim to identify women at increased risk of breast cancer who may benefit from targeted screening or prophylactic chemoprevention. For women in the general population who have no indication of carrying a highly predisposing genetic mutation (e.g., BRCA1/2), current prediction models include menstrual and reproductive history, body mass index (BMI), past and current use of oral contraceptives (OC) or postmenopausal hormones (PMH), and basic information on family history and/or previous diagnosis of benign breast disease as predictors (1, 2). In addition, some first models have been developed integrating polygenetic risk scores based on common polymorphisms, and, for women participating in breast cancer screening, models may also include mammographic density (3-8).

Serum hormone concentrations represent potential additional predictors for these models. Endogenous hormones, including androgens, estrogens, insulin-like growth factor I (IGF-I) and prolactin, have been associated with risk in both pre- and postmenopausal breast cancer (9-14), suggesting that selected hormone measurements could be used for improving risk models for the identification of higher-risk women. However, only one prior investigation, limited to postmenopausal women, has evaluated whether the addition of circulating hormones to risk prediction models improves discrimination (15).

Here, we present a study from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, in which we assessed whether the inclusion of dehydroepiandrosterone sulfate (DHEAS), testosterone, estradiol, estrone, sex hormone binding globulin (SHBG), IGF-I, IGF binding protein 3 (IGFBP3) or prolactin could improve risk prediction of invasive breast cancer for pre- and postmenopausal women, as compared to established and validated risk scores from the Breast Cancer Risk Assessment Tool (BCRAT, based on the Gail model; (1)) and, on a more restricted subset of data and as a secondary analysis, from the more recently developed model by Pfeiffer and colleagues (2).

Methods

Study population

The EPIC cohort has been described in detail previously (16, 17). Briefly, more than 500,000 study participants (367,903 women) were recruited from 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) between 1992 and 2002. Participants from Sweden and Norway did not contribute data to this analysis. In addition to questionnaire-based data and anthropometric measures, serum samples were collected at baseline using a standardized protocol and were stored at $\leq -150^{\circ}\text{C}$.

Incident cancer cases are identified via record linkage with regional cancer registries (Denmark, Italy, the Netherlands, Spain, and the United Kingdom), health insurance records, cancer and pathology registries, and active follow-up of study subjects (France, Germany, Greece, and Naples, Italy). Data on vital status are obtained from mortality registries, in combination with data collected by active follow-up.

Case and Control Selection

Between 1993 and 2010 a total of 10,713 incident cases of invasive breast cancer were identified. Of these, up to 1590 cases were included in a series of nested case-control studies on the relationship of breast cancer risk with endogenous hormone levels, as reported in detail previously (9, 10, 18-21).

Women with known menopausal status, not using exogenous hormones (i.e., OC or PMH), and with no reported history of cancer (except non-melanoma skin cancer) were eligible for this study. Women who were 42 years or younger or reported having had regular menses in the last 12 months were classified as premenopausal. Women were classified as postmenopausal when they reported not having any menses over the past 12 months, when older than 55 years of age, or when reporting bilateral oophorectomy. Women older than 42 years and with incomplete information on menopausal status were classified as peri-

menopausal/unknown menopausal status and were generally excluded from EPIC studies on endogenous hormones. Cases were selected in two study phases. In phase 1 (through 2004), all eligible cases were included; in phase 2, all ER- cases, plus an equal number of ER+ cases were randomly selected among cases matching each ER- case for recruitment center (after 2004).

Cases were restricted to incident invasive breast cancers diagnosed during follow-up; cases of ductal carcinoma *in situ* (DCIS) were excluded. Up to two control subjects were matched to each case, using incidence density sampling. Matching factors were: study recruitment center, menopausal status (premenopausal, postmenopausal), age at enrollment (± 6 months), time of day of blood collection (± 1 hour), fasting status (< 3 hours; 3–6 hours, > 6 hours) and, for premenopausal women, menstrual cycle phase. We restricted the current study to women with available data on at least estradiol, testosterone, SHBG, and IGF-I, resulting in a total study population of 1217 cases and 1976 matched controls.

Laboratory Analyses

This study includes concentrations of circulating (serum) testosterone, DHEAS, estrone, estradiol, SHBG, androstenedione, progesterone, IGF-I, IGFBP3, and prolactin. Measurement of the biomarkers has been described in detail previously (9, 10, 18-21). For all hormone measurements, blood samples from cases and matched controls were analyzed within the same analytical batch and laboratory technicians were blinded to the case-control status of the study subjects. The assays were performed at the International Agency for Research into Cancer (IARC; Lyon, France) and at the German Cancer Research Center (DKFZ; Heidelberg, Germany), using commercially available immunoassays. Serum progesterone concentrations were measured only in premenopausal women, as ovarian progesterone synthesis ceases after menopause.

Statistical Analyses

For all cases and controls, we calculated 5-year and 10-year risk scores for breast cancer, using established models by Gail (1) and by Pfeiffer (2), and using available data on

menopausal status, ages at menarche and at menopause, duration of PMH use, parity, number of children and age at first full term pregnancy, family history of breast cancer, alcohol consumption at recruitment and BMI (kg/m^2). Information on personal history of breast biopsies and benign breast disease (i.e., hyperplasia) was not available and was set to missing in the estimation of Gail-model risk estimates. Given missing data on these two risk factors, we henceforth refer to our Gail-model risk estimates as results from a modified Gail model. When information on family history was missing ($n=1693$, 53%), it was set to zero. The Pfeiffer model is designed for women older than 50 years of age, thus estimates using this model are available for only 60% of our study sample. Given the small number of premenopausal women older than age 50 in our study ($n=45$ cases) improvement to this model was evaluated only among women postmenopausal at blood collection. Further, sporadic missing values in the risk factor variables in the Pfeiffer risk model ranging from 0% (BMI) to 6% (age at menopause) led to the exclusion of 92 case-sets from the analyses using the Pfeiffer risk score.

All biomarker measurements were log2-transformed. Estrogens and androgens were measured in two study phases (9, 18, 20, 21). To harmonize the measurements from the two study phases, the log2-transformed values from phase 2 were standardized to the distribution in phase 1 using mean and standard deviation, by menopausal status. Among women premenopausal at blood collection, we used menstrual cycle phase-specific residuals for estrone, estradiol and progesterone, calculated as a woman's individual hormone value minus menstrual cycle phase-specific mean value from a local linear regression model. This accounts for within-person variability in these hormones across the menstrual cycle. Relative risk estimates were derived using conditional logistic regression, which was calibrated towards the absolute risk estimates from the pre-specified modified Gail and Pfeiffer epidemiological risk models as an offset-variable, thus including the pre-defined absolute risk score for each participant in the model with a fixed beta of 1. In a single-step backwards elimination process, biomarkers with a p-value below 0.157, indicating improvement in the Akaike information criterion (AIC; (22)) were retained in the model. Biomarker concentration

availability did not overlap among all cases and controls (i.e., different subsets of biomarkers were available for subsets of the study population). Therefore, we evaluated one biomarker from each pathway in these analyses (i.e., androgens: testosterone; estrogens: estradiol; growth factors: IGF-I; SHBG), to achieve the maximum sample size with overlapping biomarker values. Testosterone and estradiol are both part of the United States Centers for Disease Control and Prevention (CDC) Hormone Standardization Program, designed to ensure comparability of results across laboratories (www.cdc.gov/labstandards/hs.html). Thus, these markers in particular are attractive candidates as circulating markers to be included in clinical risk prediction models.

Improvement in risk estimation was assessed with C-statistic (equivalent to the area under the receiver operating curve (AUROC)) for the effect of the biomarkers alone, and in terms of change in C-statistic from addition of biomarkers to the calculated absolute risk estimates. We assessed the increase in average risk difference between cases and non-cases with the integrated discrimination improvement (IDI) and evaluated the frequency of improvement of prediction with the net reclassification improvement (NRI; continuous).

Internal validation with bootstrapping (1000-fold) was applied to adjust for over-fitting from model development and estimation. The mean “optimism” estimate for the C-statistics, IDI, and NRI was subtracted from the observed estimates, and 2.5 and 97.5 percentiles of the bootstrap estimates are presented as 95% confidence limits. Analyses were performed separately for women pre- and postmenopausal at blood collection. In addition, we also conducted analyses considering only estrogen receptor positive (ER+) breast cancer cases. All analyses were conducted in SAS v9.3 (Cary, NC, USA).

Results

This study included a total of 1217 breast cancer cases (430 pre- and 787 postmenopausal at time of blood donation) with calculated 5-year (and 10-year) risk scores from the modified Gail model and measurements of testosterone, estradiol, SHBG and IGF-I.

A total of 661 cases contributed to analyses using the Pfeiffer model (restricted to postmenopausal women >50 years of age at blood collection).

Women were median age 56 years (range: 26-77) at blood collection, and the majority had at least one full-term pregnancy (cases: 83%; controls: 86%), with median two full-term pregnancies (range: 1-14) among parous women (**Table 1**). The median predicted 5- and 10-year risks as calculated by the modified Gail and Pfeiffer models were only slightly higher among the breast cancer cases as compared to the controls, and the modified Gail and Pfeiffer risk scores provided only weak discrimination between cases and controls (C statistics, postmenopausal women, modified Gail: 52.7%; Pfeiffer: 53.8%). Median concentrations for each of the evaluated biomarkers are presented in **Table 2**.

Testosterone, androstenedione, and DHEAS were significantly associated with risk among premenopausal women in models adding the serum hormones individually to the modified Gail-risk score as offset. Among this subgroup, significant ORs for a one-unit change in log2-transformed hormone concentration ranged from 1.24 (95% confidence interval (CI): 1.01-1.53) for DHEAS to 1.33 (1.08-1.64) for testosterone (**Table 3**). In premenopausal women, the addition of single hormone concentrations to the calculated modified Gail model epidemiologic risk score improved discrimination (quantified by change in C-statistic) up to 2.3 percentage points (androstenedione). Among postmenopausal women, testosterone, androstenedione, DHEAS, SHBG, estrone, estradiol, and IGF-I were significantly associated with breast cancer risk. Statistically significant OR estimates ranged from 1.24 for both androstenedione (95% CI: 1.09-1.42) and DHEAS (95% CI: 1.11-1.40) to 1.56 (1.32-1.84) for estradiol and 1.66 (1.33-2.06) for estrone. SHBG was significantly inversely associated with postmenopausal breast cancer risk (OR: 0.86 (0.77-0.96)). The associations for ER+ disease were somewhat stronger than those observed for total breast cancer. In this sample of women not using exogenous hormones at time of blood donation, prolactin was not associated with breast cancer risk. In postmenopausal women adding single serum hormone measurements improved discrimination up to 4.5 percentage points

for breast cancer overall (estradiol). Results were similar in models using the Pfeiffer risk score as offset (restricted to postmenopausal women; **Supplemental Table 1**).

We next carried out a backward elimination step with testosterone, estradiol, IGF-I and SHBG as candidates for a multi-biomarker risk prediction model. Testosterone was selected by backward elimination for prediction of premenopausal risk; estradiol, testosterone and SHBG were selected for postmenopausal women in models using the modified Gail score (**Table 4**) and Pfeiffer score (**Supplemental Table 2**) as offset.

Among women premenopausal at blood collection, we observed no improvement in discrimination, relative to the modified Gail model alone, in the full multi-biomarker model or the selected model including testosterone, for overall breast cancer or ER+ disease (**Table 5**). Discrimination for ER+ disease in premenopausal women was not improved when the model was refit among cases with ER+ tumors and their matched controls (four candidate hormones, change in discrimination: 1.7 percentage points, IDI: 0.0%, NRI: 11%). In contrast, among postmenopausal women and for overall breast cancer, adding these four hormone measurements to prediction models improved the C-statistic by 5.6 percentage points relative to the modified Gail score (correcting for optimism due to overfitting). The IDI indicates an average increase in risk difference of 0.18% and the NRI shows improved prediction in 16% of the women. Results were similar in the model including the three selected biomarkers (relative to modified Gail score: 5.3 percentage point improvement in discrimination, IDI of 0.17% and NRI of 16%). Applying the model to ER+ disease, discrimination was improved by 7.5 percentage points in the full model, and 7.2 percentage points in the selected model, compared to the modified Gail score alone. When the model was refit among cases with ER+ tumors and their matched controls, testosterone and estradiol were selected into the prediction model with OR effects of 1.20 and 1.17, respectively, in postmenopausal women. Model discrimination improved by 7.0 percentage points (0.18% IDI, 18% NRI) for the four hormones together, and by 6.7 percentage points (0.17% IDI, 19% NRI) for the selected model, after adjustment for over-optimism.

Improvement in discrimination was similar in models using the Pfeiffer score as offset, though somewhat weaker (all cases: improvement in discrimination, percentage points, all markers: 3.9; selected markers: 3.4; ER+ cases, all markers: 4.9; selected markers: 4.8; **Supplemental Table 3**).

For postmenopausal women the inclusion of BMI (continuous) as an additional predictor to the modified Gail model did not improve model prediction and was not estimated as significant effect (C: 58.9, p-value for BMI estimate: 0.19); however, when BMI was added as a predictor, SHBG was no longer selected into the model (p=0.26).

We included prolactin concentrations on 465 postmenopausal and 234 premenopausal cases and their matched controls in a secondary analysis. Among postmenopausal women, results from the risk prediction models were similar in models including and excluding prolactin as a candidate biomarker. However, amongst premenopausal women no biomarker was selected into the risk prediction model when prolactin was included as a candidate biomarker (data not shown). We evaluated improvements in discrimination based on 10-year risk estimates; results were similar to those presented for 5-year risk estimates.

Discussion

Measurements of endogenous hormones significantly improved risk discrimination among postmenopausal women not using PMH. Relative to the modified Gail model alone, the selected model including estradiol, testosterone, and SHBG concentrations improved discrimination in terms of C-statistic by 5.3 percentage points for breast cancer overall and 7.2 percentage points for ER+ disease, after correcting for overfitting. Improvement in discrimination was similar when hormone concentrations were added to the Pfeiffer model (improvement in discrimination, percentage points, all cases: 3.4; ER+ cases: 4.8). The improvement in discrimination observed among postmenopausal women in the current study is similar in magnitude to that reported for polygenic risk scores, or mammographic density

patterns (3-7). We observed no improvement in discrimination for women premenopausal at blood collection.

We included three measures of prediction model performance in this study: (1) the C statistic, which provides a summary measure of the discriminatory capacity of a risk prediction model; (2) the IDI, describing the change in the difference in absolute risk estimates between subsequent cases and controls from the modified Gail (or Pfeiffer) model alone and absolute risk estimates from models additionally including hormones; and, (3) the continuous NRI, showing the proportion of the population in which the model more accurately predicts absolute risk estimates in subsequent cases and controls, before as compared to after including hormones. While all three measures are clearly related, they each provide a different perspective on the extent to which the models including hormones improve discrimination in the study population, relative to the risk factor models alone. Comparing absolute risk estimates from the modified Gail model alone to those from the model including hormones, the IDI indicates the mean difference in absolute risk estimates of women subsequently diagnosed as cases vs. controls significantly increased by 0.17-0.19 percentage points in postmenopausal women (e.g., modified Gail model alone, 0.04 percentage point difference; additionally including hormones, 0.04 + 0.18 improvement = 0.22 percentage point difference). According to the NRI, after inclusion of hormones in the model, improved prediction discrimination was observed in 16-20% of the study population, with correctly increased risk estimates for future cases and decreased estimates for controls (i.e., improvement over and above potential changes in the wrong direction, regardless of magnitude of change).

Our findings confirm results from a recent study by Tworoger et al. in the Nurses' Health Study (15). Tworoger et al. observed a 5.9 percentage point improvement in discrimination for invasive breast cancer, relative to the modified Gail model, in the selected model with estrone sulfate, testosterone, and prolactin. Further, as was observed in our study, Tworoger et al. observed somewhat stronger discrimination for ER+ disease (change in AUC 8.8 percentage points, relative to modified Gail model) in the selected models. In

contrast to the previous study, prolactin was not selected into our model. However, these data were only available on a subset of our study population. Further, circulating estrone sulfate concentrations were not available for cases and controls in our study. However, estrogens estrone and estradiol were considered.

Addition of hormones resulted in stronger improvement of risk prediction estimates from the modified Gail model vs. the Pfeiffer model. At baseline, the differences in 5-year absolute risk estimates between subsequent cases and controls were larger in the Pfeiffer model (0.07 percentage points) than in the modified Gail model (0.04 percentage points). This is due, in part, to the standard inclusion of BMI in the Pfeiffer model. BMI is known to be a significant determinant of serum SHBG and sex hormones (particularly estrogens), in postmenopausal women (23-27). However, consistent with the previous study by Tworoger et al. (15), improvements in risk prediction using the modified Gail model were robust to adjustment for BMI.

Present recommendations for identifying women at sufficiently high risk to benefit from chemoprevention (28) include reference to the BCRAT originally developed by Gail et al. (1), with the aim to reduce costs not only in terms of financial expense, but also to optimize expected medical benefits against possible negative side effects (e.g., increased risk of endometrial cancer). Breast cancer risk prediction models are increasingly being considered in the context of mammography screening (29), given recent data suggesting screening may have limited benefit for some women (30), and the need to balance potential benefits of screening against possible negative side effects such as over-diagnosis or false positive findings and financial costs. Our model demonstrates opportunities for higher discrimination in models incorporating blood-based biomarkers, including estradiol and testosterone, hormones with established cross-laboratory standardization protocols (31). However, a cost-benefit analysis is required to determine the potential net value of implementing blood-based biomarkers in the screening context.

The median predicted 5- and 10-year risks as calculated by the modified Gail and Pfeiffer models were only slightly higher among the breast cancer cases as compared to the

controls, and the modified Gail and Pfeiffer risk scores provided only weak discrimination between cases and controls. This weak discrimination, as compared to reports from full cohort analyses, can be explained by the matching of breast cancer case and control subjects by a number of key predictors in the risk scores, including age and menopausal status, and by the exclusive focus on women not using exogenous hormones at the time of blood donation, since PMH use is another factor adding to risk stratification. A limitation of this study, and to some extent the prior investigation by Tworoger et al. (15), is that we used a modified Gail model because of a lack of data on selected risk factors. Data for family history of breast cancer and history of benign breast disease are lacking in the EPIC cohort. Further, we used data from a single biomarker measurement in these analyses; blood samples were collected up to 14 years prior to diagnosis (median 4 years). However, the within-person stability of these markers over time has been demonstrated previously (32-34). Further, the investigation by Tworoger et al. included the average of two hormone measurements from samples taken 10 years apart for select hormones, and for women diagnosed after the second blood draw. We observed similar improvements in discrimination in our study using one blood sample, suggesting that one measurement may be sufficient to predict longer term risk. Future studies should evaluate whether discrimination may be further improved by adding mammographic density, among women already part of screening programs, as well as genetic markers. These data were not available in sufficient numbers for the cases and controls included in this investigation. Tumor ER status was missing for 34% of cases. However, cases with and without ER status data were similar with respect to baseline absolute risk estimates, and therefore we do not expect this to have introduced bias into our results.

In summary, we confirm the improvement in the discriminatory capacity of risk prediction models with the addition of hormone concentrations, among postmenopausal women not using PMH at blood collection. Integration of hormone measurements in clinical risk prediction models may represent a strategy to improve risk stratification for breast cancer.

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For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at <http://epic.iarc.fr/access/index.php>

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Table 1. Description of epidemiological risk factors and risk score estimates in cases and matched controls, presented as n (%) or median (min-max): EPIC breast cancer nested case-control study

	Cases (n=1217)	Controls (n=1976)
Age at recruitment, years	56.2 (26.6 - 76.4)	56.3 (26.4 - 76.8)
Body mass index (BMI), kg/m²	25.3 (16.6 - 46.1)	25.2 (16.0 - 45.3)
Age at menarche, years	13 (≤8; ≥20)	13 (≤8 - ≥20)
Ever full-term-pregnancy	1016 (83%)	1699 (86%)
Age at first full-term-pregnancy, years^a	25.0 (16.0 - 44.0)	25.0 (16.0 - 43.0)
Number of full-term pregnancies^a	2.0 (1.0 - 9.0)	2.0 (1.0 - 14.0)
Menopausal status at blood collection		
Premenopausal	430 (35%)	684 (35%)
Postmenopausal	755 (62%)	1233 (62%)
Surgical postmenopausal	32 (3%)	59 (3%)
Age at menopause (among postmenopausal), years	50 (15 - 59)	50 (24 - 62)
Family history of breast cancer		
Yes	87 (7%)	87 (4%)
No	466 (38%)	860 (44%)
Unknown	664 (55%)	1029 (52%)
Alcohol consumption, baseline		
non-drinker	246 (20%)	443 (22%)
0-6 g/d	489 (40%)	831 (42%)
> 6 g/d	482 (40%)	701 (35%)
5-year breast cancer risk estimates		
Modified Gail-model	1.68% (0.07 - 4.59)	1.64% (0.07 - 4.54)
Pfeiffer's model ^b	1.53% (0.72 - 3.55)	1.47% (0.66 - 3.86)
10-year breast cancer risk estimates		
Modified Gail-model	3.52% (0.26 - 8.43)	3.46% (0.25 - 8.43)
Pfeiffer's model	3.13% (1.59 - 6.96)	3.02% (1.46 - 7.10)
Tumor estrogen receptor status^c		
Positive	560 (46%)	
Negative	242 (20%)	

^a Among parous women

^b Data available for 1041 controls (53%) and 661 cases (54%)

^c Missing for 34% of cases; percentages reflect distribution among women with known estrogen receptor status. Through 2004 all cases included; after 2004, all ER-negative cases plus an equal number of ER-positive cases

Table 2: Distribution of circulating biomarker concentrations in cases and controls, by menopausal status at blood collection: EPIC breast cancer nested case-control study

Hormone	Premenopausal at blood collection ^a						Postmenopausal at blood collection					
	Cases			Controls			Cases			Controls		
	n	Median (95%-range)		n	Median (95%-range)		N	Median (95%-range)		n	Median (95%-range)	
Testosterone (ng/ml)^b	430	0.46	(0.16 - 1.11)	684	0.45	(0.16 - 0.96)	787	0.39	(0.11 - 1.01)	1292	0.35	(0.09 - 0.93)
Androstenedione (ng/ml)	270	1.52	(0.55 - 4.08)	516	1.44	(0.45 - 3.38)	561	0.96	(0.29 - 2.84)	1057	0.87	(0.25 - 2.53)
DHEAS (µg/dl)	270	127.0	(37.1 - 352.8)	516	123.8	(32.4 - 298)	559	84.5	(19.0 - 281.8)	1061	73.3	(17.19 - 243.6)
SHBG (nmol/l)^b	430	48.01	(15.33 - 124.32)	684	45.82	(14.9 - 115)	787	32.6	(10.31 - 94.2)	1292	34.6	(9.9 - 95.6)
Estrone (E1) (pg/ml)	269	96.34	(28.77- 297.53)	508	98.22	(28.6 -294.9)	535	42.4	(16.4 - 108.1)	1036	39.7	(15.1 - 83.2)
Estradiol (E2) (pg/ml)^b	430	102.9	(27.1- 404.0)	678	97.89	(17.5 - 390.5)	787	25.5	(12.42 - 84.2)	1292	23.3	(10.7 - 65.2)
Progesterone(ng/ml) ^b	405	5.08	(0.3 - 37.8)	635	5.38	(0.28 - 49.7)						
IGF-I (ng/ml)^b	430	273.5	(142.6 - 430.1)	684	272.2	(140 - 425)	787	218.0	(102.7 - 395.7)	1292	211.9	(107.4 - 375.1)
IGF-BP3 (ng/ml)	258	3229	(1814 - 6462)	490	3227	(1738 - 6281)	550	3323	(1532 - 6692)	1024	3245	(1690 - 6525)
Prolactin (ng/ml)	268	8.74	(3.77 - 27.3)	257	9.35	(3.92 - 25.0)	500	5.88	(2.87 - 18.88)	483	5.69	(2.93 - 14.49)

Bold: Markers used in multi-biomarker risk prediction model ^afor premenopausal women, estradiol, estrone, and progesterone calibrated to day 21 of menstrual cycle phase, study phase 1; ^bHormones measured in two study phases. Phase 2 values calibrated to mean and standard deviation observed in measurements from phase 1

Table 3: Associations between candidate biomarkers and breast cancer risk by menopausal status at blood collection, using 5-year risk from modified Gail-model as a regression offset: EPIC breast cancer nested case-control study

	Premenopausal at blood collection					Postmenopausal at blood collection				
	Case sets	p	OR*	95% CI	ΔC ^a	Case sets	p	OR*	95% CI	ΔC ^a
All breast cancer cases										
Testosterone	430	0.0073	1.33	(1.08 - 1.64)	1.0	787	1.88E-07	1.40	(1.24 - 1.59)	3.7
Androstenedione	270	0.0209	1.31	(1.04 - 1.65)	2.3	561	0.0014	1.24	(1.09 - 1.42)	1.9
DHEAS	270	0.0370	1.24	(1.01 - 1.53)	1.3	559	0.0003	1.24	(1.11 - 1.40)	2.8
SHBG	430	0.7617	0.97	(0.81 - 1.17)	0	787	0.0074	0.86	(0.77 - 0.96)	1.3
Estrone (E1)	269	0.4308	1.09	(0.89 - 1.33)	0	535	4.80E-06	1.66	(1.33 - 2.06)	3.1
Estradiol (E2)	430	0.2473	1.08	(0.95 - 1.22)	0.1	787	2.32E-07	1.56	(1.32 - 1.84)	4.5
Progesterone	405	0.3740	0.97	(0.89 - 1.04)	0					
IGF-I	430	0.8359	1.04	(0.74 - 1.46)	0.1	787	0.0454	1.25	(1.00 - 1.55)	0.9
IGF-BP3	258	0.4826	1.24	(0.68 - 2.27)	0.7	550	0.2517	1.19	(0.88 - 1.60)	0.5
Prolactin	268	0.9497	1.01	(0.76 - 1.34)	0	500	0.2327	1.13	(0.92 - 1.40)	0.2
ER+ breast cancer cases										
Testosterone	197	0.0583	1.35	(0.99 - 1.85)	1.7	363	<.0001	1.49	(1.23 - 1.80)	5.0
Androstenedione	113	0.0651	1.39	(0.98 - 1.96)	4.1	251	0.0022	1.37	(1.12 - 1.68)	4.2
DHEAS	113	0.0378	1.41	(1.02 - 1.95)	4.3	251	0.0014	1.36	(1.13 - 1.64)	5.3
SHBG	197	0.5623	0.92	(0.70 - 1.22)	0.2	363	0.1154	0.87	(0.74 - 1.03)	1.4
Estrone (E1)	112	0.2411	1.21	(0.88 - 1.65)	-0.0	239	0.0065	1.58	(1.14 - 2.20)	2.4
Estradiol (E2)	197	0.0719	1.19	(0.98 - 1.44)	1.7	363	<.0001	1.71	(1.31 - 2.22)	6.3
Progesterone	186	0.2943	0.94	(0.84 - 1.06)	0.6					
IGF-I	197	0.2674	1.35	(0.79 - 2.29)	1.8	363	0.0906	1.31	(0.96 - 1.80)	1.8
IGF-BP3	110	0.2230	1.77	(0.71 - 4.46)	3.8	244	0.3986	1.22	(0.77 - 1.94)	0.8
Prolactin	191	0.7907	0.96	(0.68 - 1.34)	0.4	335	0.1010	1.25	(0.96 - 1.63)	1.0

Bold: Markers used in multi-biomarker risk prediction model

* Effects presented as per doubling of concentration (from log2-transformed measurements).

^aΔC represents change in C-statistic comparing model with offset for breast cancer risk score alone to one with risk score plus individual biomarker; expressed here as percentage point difference

Table 4: Estimated biomarker effects on risk of breast cancer, per doubling of hormone concentration, by menopausal status at blood collection. Estimates adjusted for modified Gail-risk score from common model (i.e. mutually adjusted) and in selected model: EPIC breast cancer nested case-control study

	Premenopausal at blood collection				Postmenopausal at blood collection			
	Full model		Selected		Full model		Selected	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Testosterone	1.31	(1.06 - 1.62)	1.33	(1.08 - 1.64)	1.25	(1.08 - 1.45)	1.26	(1.09 - 1.45)
Estradiol (E2)	1.06	(0.91 - 1.23)			1.31	(1.08 - 1.58)	1.31	(1.08 - 1.58)
SHBG	0.98	(0.81 - 1.19)			0.91	(0.81 - 1.01)	0.90	(0.81 - 1.00)
IGF-I	0.99	(0.70 - 1.41)			1.12	(0.89 - 1.40)		

Table 5: Performance of full and selected models by menopausal status at blood collection in terms of concordance-statistic ("C"), integrated discrimination improvement index (IDI), and net-reclassification improvement (NRI continuous), each with 95%-Confidence interval and optimism-correction from 1000 bootstrap-samples: EPIC breast cancer nested case-control study

	%C (95% CI)		IDI (95% CI)		NRI (95% CI)	
Premenopausal at Blood Collection						
Modified Gail Model	52.4	(48.9 - 55.8)				
Hormones only	52.6	(49.1 - 56.1)				
Full model, all cases	53.4	(49.9 - 56.9)				
Improvement	1.0		0.0002	(-0.0001 - 0.0004)	0.05	(-0.07 - 0.17)
Optimism ^a	1.1	(-0.6 - 3.8)	0.0002	(-0.0001- 0.0008)	0.04	(-0.06 - 0.16)
Corrected Improvement ^{a,b}	-0.1	(-0.3 - 2.0)	0.0000	(0.0000 - 0.0006)	0.01	(-0.04 - 0.16)
Full model, ER+ cases	53.3	(48.1 - 58.8)				
Improvement	2.0		0.0002	(-0.0002 - 0.0006)	0.03	(-0.15 - 0.21)
Optimism ^a	1.5	(-1.5 - 5.6)	0.0003	(-0.0002 - 0.0011)	0.07	(-0.09 - 0.22)
Corrected Improvement ^{a,b}	0.5	(-0.8 - 3.7)	0.0000	(-0.0001 - 0.0007)	-0.04	(-0.13 - 0.18)
Selected model, all cases	53.3	(49.9 - 56.8)				
Improvement	1.0		0.0002	(-0.0001- 0.0004)	0.04	(-0.08 - 0.16)
Optimism ^a	0.9	(-0.8 - 3.7)	0.0002	(-0.0001- 0.0008)	0.04	(-0.08 - 0.15)
Corrected Improvement ^{a,b}	0.0	(-0.6 - 1.9)	0.0000	(0.0000- 0.0006)	0.00	(-0.03 - 0.14)
Selected model, ER+ cases	53.0	(47.8 - 58.1)				
Improvement	1.6		0.0002	(-0.0002 - 0.0006)	0.06	(-0.11 - 0.24)
Optimism ^a	1.3	(-1.8 - 5.4)	0.0002	(-0.0002 - 0.0010)	0.06	(-0.11 - 0.22)
Corrected Improvement ^{a,b}	0.3	(-0.7 - 3.7)	0.0000	(-0.0001 - 0.0006)	0.00	(-0.10 - 0.13)
Postmenopausal at Blood Collection						
Modified Gail Model	52.7	(50.1 - 55.2)				
Hormones only	57.7	(55.2 - 60.2)				
Full model, all cases	58.8	(56.3 - 61.3)				
Improvement	6.1		0.0021	(0.0013 - 0.0028)	0.17	(0.09 - 0.26)
Optimism ^a	0.5	(-1.6 - 2.9)	0.0003	(-0.0005 - 0.0017)	0.01	(-0.06 - 0.09)
Corrected Improvement ^{a,b}	5.6	(4.7 - 6.2)	0.0018	(0.0017 - 0.0039)	0.16	(0.13 - 0.21)
Full model, ER+ cases	60.1	(56.4 - 63.7)				
Improvement	7.7		0.0020	(0.0010 - 0.0031)	0.19	(0.06 - 0.33)
Optimism ^a	0.2	(-2.9 - 3.7)	0.0002	(-0.0010 - 0.0019)	0.00	(-0.12 - 0.12)
Corrected Improvement ^{a,b}	7.5	(6.3 - 8.1)	0.0019	(0.0017 - 0.0037)	0.19	(0.13 - 0.26)
Selected model, all cases	58.5	(56.0 - 61.0)				
Improvement	5.8		0.0021	(0.0013 - 0.0028)	0.17	(0.08 - 0.26)
Optimism ^a	0.5	(-1.5 - 2.9)	0.0003	(-0.0005 - 0.0017)	0.02	(-0.06 - 0.09)
Corrected Improvement ^{a,b}	5.3	(4.2 - 6.2)	0.0017	(0.0017 - 0.0038)	0.16	(0.12 - 0.21)
Selected model, ER+ cases	59.7	(56.1 - 63.4)				
Improvement	7.4		0.002	(0.0010 - 0.0030)	0.21	(0.08 - 0.34)
Optimism ^a	0.3	(-3.0 - 3.8)	0.0002	(-0.0010 - 0.0020)	0.00	(-0.12 - 0.13)
Corrected Improvement ^{a,b}	7.2	(5.3 - 8.1)	0.0018	(0.0016 - 0.0037)	0.21	(0.13 - 0.26)

^a 95%-confidence intervals from bootstrapping ^b statistics from improvement minus optimism

Clinical Cancer Research

Added value of serum hormone measurements in risk prediction models for breast cancer for women not using exogenous hormones: Results from the EPIC cohort

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