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Short Report

Endometrial cancer risk prediction including serum-based biomarkers: results from the EPIC cohort

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Endometrial cancer risk prediction models including lifestyle, anthropometric and reproductive factors have limited discrimination. Adding biomarker data to these models may improve predictive capacity; to our knowledge, this has not been investigated for endometrial cancer. Using a nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, we investigated the improvement in discrimination gained by adding serum biomarker concentrations to risk estimates derived from an existing risk prediction model based on epidemiologic factors. Serum concentrations of sex steroid hormones, metabolic markers, growth factors, adipokines and cytokines were evaluated in a step-wise backward selection process; biomarkers were retained at $p < 0.157$ indicating improvement in the Akaike information criterion (AIC). Improvement in discrimination was assessed using the *C*-statistic for all biomarkers alone, and change in *C*-statistic from addition of biomarkers to preexisting absolute risk estimates. We used internal validation with bootstrapping (1000-fold) to adjust for over-fitting. Adiponectin, estrone, interleukin-1 receptor antagonist, tumor necrosis factor-alpha and triglycerides were selected into the model. After accounting for over-fitting, discrimination was improved by 2.0 percentage points when all evaluated biomarkers were included and 1.7 percentage points in the model including the selected biomarkers. Models including etiologic markers on independent pathways and genetic markers may further improve discrimination.

What's new?

Predicting cancer requires lots of different information. Risk prediction models of endometrial cancer risk include questionnaire data on lifestyle, body measurements and reproductive factors. Could biomarker data improve the predictive value of these models? Using data from the EPIC cohort, these authors looked at serum concentrations of a variety of biomarkers, including sex steroid hormones, growth factors and others. They achieved a modest improvement in discrimination after incorporating biomarker data into endometrial cancer risk prediction models.

Introduction

Endometrial cancer (EC) risk prediction models identify women who would most likely benefit from targeted screening or prevention. Incidence of EC is relatively low, estimated at 13.6 cases per year per 100,000 women in Europe.[1] Therefore, risk prediction models designed to identify 'high' vs. lower risk populations of women for targeted intervention or screening programs need high specificity to avoid invasive follow-up on false positives, while ensuring a high proportion of true high risk women are identified.

Risk models based on questionnaire data alone provide moderate predictive capacity for endometrial cancer.[2,3] We recently reported discrimination capacity of 77% (*C*-statistic) in a model fit in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort;[3] this model included body mass index (BMI), menopausal status, ages at menarche, first full-term pregnancy and menopause, oral contraceptive (OC) use and duration, parity, duration of hormone therapy (HT) and smoking status. Pfeiffer *et al.* developed an endometrial cancer risk prediction model in US-based cohorts, reporting an area under the curve (AUC) of 0.68 for a model including a somewhat smaller set of variables than those selected into the model in the EPIC cohort (BMI, menopausal status, age at menopause, BMI, OC use, parity, HT use and smoking).[2] These models provide important insights for future population based approaches to predict endometrial cancer risk, although additional predictors are needed to improve discrimination. To our knowledge, the extent to which circulating biomarkers improve endometrial cancer risk prediction has not been addressed.

A factor analysis within an EPIC nested case–control study identified three hormonal and metabolic axes associated with endometrial cancer risk: (*i*) steroid hormones; (*ii*) insulin resistance/metabolic syndrome and (*iii*) inflammation.[4] Biomarkers along these axes have been independently associated with disease risk in previous analyses.[5–14] We investigate here whether the addition of biomarkers to a risk score based on epidemiological questionnaire data improves predictive capacity for endometrial cancer.

Methods

The EPIC cohort has been described in detail previously.[15,16] Briefly, >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. In addition to questionnaire-based data and anthropometric measures, serum samples were collected at baseline using a standardized protocol; samples and have been in long-term storage at ≤ -150°C, with the exception of Sweden, where samples are stored at -70°C.

Cohort follow-up

Incident cancer cases were identified via record linkage with regional cancer registries (Denmark, Sweden, Italy, the Netherlands, Norway, Spain and the United Kingdom), health insurance records, cancer and pathology registries and active follow-up of study subjects (France, Germany, Greece, Naples and Italy). Data on vital status were obtained from mortality registries, in combination with data collected by active follow-up. End of follow-up corresponds to latest dates of complete follow-up for both cancer incidence and vital status (June 1999–December 2003) at the time when the nested case–control analyses were performed.

Case and control selection

Case and control selection has been described in detail previously.[5–10] Women with known menopausal status, not using exogenous hormones at blood draw (e.g., OC and HT), and with no reported hysterectomy or history of cancer (except nonmelanoma skin cancer) were eligible for this study. Cases were restricted to incident epithelial endometrial cancers diagnosed during follow-up; nonepithelial cases 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 and perimenopausal), age at enrollment ($\pm$6 months), time of day of blood collection ($\pm$1 hr), fasting status (<3, 3–6 and >6 hr) and for premenopausal women, phase of menstrual cycle (follicular, periovulatory and luteal). This analysis included 247 cases and 469 matched controls.

Biomarker measurements

The biomarkers investigated here include C-reactive protein (CRP), interleukin 6 (IL6), IL1 receptor antagonist (IL1Ra), tumor necrosis factor-alpha (TNFα), TNF receptor 1 (TNFR1), TNFR2, testosterone, dehydroepiandrosterone-sulfate (DHEAS), estrone, estradiol, sex hormone-binding globulin (SHBG), androstenedione, C-peptide, insulin-like growth factor-binding protein 1 (IGFBP1), IGFBP2, high-density lipoprotein (HDL) cholesterol, triglycerides, total cholesterol, glucose and adiponectin. Measurement of the biomarkers has been described in detail.[4–10] In brief, 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 majority of the assays were performed at the International Agency for Research on Cancer (Lyon, France) using commercially available immunoassays. Interleukin-1 receptor antagonist and soluble tumor necrosis factor (TNF) receptors were measured at the German Cancer Research Center (DKFZ; Heidelberg, Germany). Blood lipids were measured at the Hôpital Edouard Herriot (Lyon, France) using an enzymatic colorimetric test. Serum estradiol concentrations were measured only in postmenopausal women because of the variation in estradiol levels during the menstrual cycle among premenopausal women. Glucose was not measured in samples from women recruited at the Oxford, United Kingdom, study center because samples were kept at room

Table 1. Baseline characteristics of the endometrial cancer nested case–control study: EPIC cohort [median (min; max), or *n* (%)]

	Cases, *n* = 247	Controls, *n* = 469
Age at blood collection, years	56.9 (40.5; 69.6)	57.2 (39.9; 69.7)
Body mass index (BMI), kg/m^2	27.4 (18.2; 48.8)	25.7 (15.7; 43.6)
Age at first period, years	13.0 (9.0; 18.0)	13.0 (9.0; 20.0)
OC pill use, ever	81 (33%)	194 (41%)
Cumulative duration of pill use, years[1]	3.0 (1.0; 25.0)	4.0 (1.0; 25.0)
Completed full-term pregnancy, ever	200 (81%)	424 (90%)
Age at first full-term pregnancy, years[2]	24.0 (16.0; 40.0)	25.0 (15.0; 41.0)
Menopausal status		
Premenopausal	37 (15%)	61 (13%)
Postmenopausal	175 (71%)	339 (72%)
Perimenopausal	35 (14%)	69 (15%)
Age at menopause, years[3]	51.0 (33.0; 62.0)	50.0 (33.0; 59.0)
HT use, ever[3]	48 (19%)	69 (15%)
Duration of HT use, years[4]	1.0 (0.08; 20.0)	1.0 (0.08; 10.0)
Smoking status		
Never	168 (68%)	292 (62%)
Former	43 (17%)	88 (19%)
Smoker	36 (15%)	87 (19%)
5-year risk estimate, EC model	0.3% (0.03; 3.12)	0.2% (0.01; 2.19)

[1]Among ever users.
[2]Among women with completed full term pregnancy.
[3]Among postmenopausal women.
[4]Among postmenopausal ever HT users.

temperature for >24 hr before processing, and glucose concentrations are not stable with delayed processing.

Statistical analyses

All biomarker measurements were log2-transformed. A considerable fraction of IL1Ra and TNFα measurements were below the detection limit (LOD) (52 and 18% of values below LOD). Indicator variables for IL1Ra and TNFα below LOD were included as interaction terms, given the high proportion of values below LOD. Sporadic missing analyte values for the remaining biomarkers, for reasons such as insufficient volume or technical failure, varied between 0% for C-peptide and IGFBP1 to 5% for estrone. Missing values for the remaining biomarkers were imputed using the center- and menopausal status-specific mean biomarker value. Estrone was measured in both pre- and postmenopausal women. Given within-person variability in estrone across the menstrual cycle in premenopausal women, we used menstrual cycle phase-specific residuals from a local linear regression model. We included an interaction term with an indicator variable for post-menopausal status for both estrone and estradiol (measured only in postmenopausal women). Biomarker values were adjusted for center, age, menopausal status and fasting status (matching factors) and regression residuals were used for all further analyses. Absolute risk estimates for all subjects were calculated according to the previously defined EC risk model based on the full EPIC cohort[3] including the following exposures: BMI (kg/m^2), menopausal status, age at menarche and at menopause, OC use (overall and by BMI categories) and duration of use, parity, age at first full-term pregnancy, duration of HT and smoking status (by menopausal status). Relative risk estimates of the biomarkers were derived with conditional logistic regression, which was calibrated towards the absolute risk estimates as an offset-variable. In a step-wise backwards selection process biomarkers with a *p* values below 0.157, indicating improvement in the Akaike information criterion (AIC; i.e., balancing model fit with number of included parameters),[17] were retained in the model.

Improvement in risk estimation was assessed with *C*-statistic (equivalent to the area under the receiver operating curve (AUROC)) for all biomarkers alone, and change in C from addition of biomarkers to preexisting absolute risk estimates. In addition we assessed the integrated discrimination improvement (IDI) and net reclassification improvement (NRI; continuous).[18]

Internal validation with bootstrapping (1000-fold) was applied to adjust the performance outcomes for over-fitting from model development and estimation.[17] The median 'optimism' estimate for the *C*-statistics, IDI and NRI was subtracted from the observed estimates; optimism was calculated on the full study population.

Table 2. Odds ratios (OR) and 95% confidence intervals (95% CI) for individual biomarker concentrations and endometrial cancer risk, in full model and in selected model: EPIC cohort

Biomarker	Effect in full model		Effect as selected	
	OR[1]	95% CI	OR[1]	95% CI
Adiponectin	0.74	0.53–1.04	0.77	0.58–1.02
Total cholesterol	0.98	0.41–2.39		–
HDL cholesterol	1.08	0.55–2.12		–
C-peptide	0.95	0.66–1.38		–
C-reactive protein	1.09	0.92–1.28		–
Androstenedione	0.83	0.53–1.30		–
DHEAS	0.84	0.63–1.11		–
Estrone	1.68	1.16–2.44	1.54	1.16–2.04
Estrone × postmenopausal	1.15	0.81–1.64		–
Estradiol × postmenopausal	0.99	0.55–1.77		–
Glucose	0.89	0.43–1.84		–
IGFBP1	1.04	0.85–1.27		–
IGFBP2	1.04	0.78–1.39		–
IL1Ra (< LOD vs. ≥ LOD)	1.00	0.81–1.23	0.98	0.80–1.20
IL1Ra (among ≥LOD)	1.28	1.06–1.54	1.26	1.06–1.51
IL6	0.99	0.75–1.31		–
SHBG	0.99	0.68–1.44		–
Testosterone	1.25	0.82–1.91		–
TNF Receptor 1	1.50	0.52–4.35		–
TNF Receptor 2	0.74	0.31–1.75		–
TNFα (< LOD vs. ≥ LOD)	1.08	0.84–1.40	1.05	0.82–1.34
TNFα (among ≥ LOD)	1.15	0.92–1.44	1.18	0.95–1.45
Triglycerides	0.82	0.55–1.25	0.78	0.57–1.06

[1]For a 1 unit increase in log2 regression residual; regression residuals from models adjusting biomarker values for center, age, menopausal status and fasting status (matching factors).
Abbreviation: LOD, limit of detection.

Results

Cases and controls were median age 57 years at recruitment, and the majority of study participants were parous, never users of OCs and never smokers and postmenopausal at recruitment (Table 1). Cases had higher BMI at recruitment than controls (27.4 vs. 25.7 kg/m^2). The 5-year risk of endometrial cancer was estimated to be between 0.01 and 3%. Distributions of the investigated biomarkers are provided in Table S1.

Among the evaluated biomarkers, only estrone and IL1Ra were statistically significantly positively associated with endometrial cancer risk (estrone, OR_{log2}: 1.54 [95% CI: 1.16–2.04]; IL1Ra among women with values >LOD, OR_{log2}: 1.26 [1.06–1.51]) in a multivariate model including EC-risk estimates as offset (i.e., adjusted for the variables in the risk prediction model; Table 2). Adiponectin, estrone, IL1Ra, TNFα and triglycerides were selected into the final model using the threshold $p < 0.157$.

The *C*-statistic of the EC risk prediction model was 62.7% in this sample. After accounting for optimism (i.e., over-fitting), the discrimination of a model including biomarkers alone was 62.3% (Table 3). The EC risk model was improved by 2.0 percentage points in the model considering all evaluated biomarkers (*C*-statistic, EC risk model: 62.7%, optimism adjusted, all biomarkers: 64.7%) and by 1.7 percentage points in the model including the selected biomarkers (*C*-statistic: 64.4%). The EC risk models had somewhat higher discrimination in models restricted to postmenopausal women (*C*-statistic, EC risk model alone: 65.5%; optimism adjusted *C*-statistic, including all biomarkers: 66.4%; including selected biomarkers: 65.8%). The difference between risk estimates for cases and controls increased by an average of 0.1% (IDI). The NRI indicates that the model including the selected hormones provided a more accurate risk prediction score for 19.0% of cases and controls.

Discussion

Inclusion of biomarkers in an endometrial cancer risk prediction model resulted in modest improvements in discrimination. The biomarkers included in this study were assessed to investigate biological pathways in the development of EC. The selected markers represent intermediates on etiologic pathways (e.g., between adiposity and EC risk), but don't necessarily contribute independent information to other, questionnaire based markers related to the same pathways, such as BMI to the metabolic syndrome, or reproductive history and hormone-use and the balance of sex steroid hormones. This questionnaire information is included in the predefined risk score. Thus, after including extensive questionnaire information into the risk model, these biomarkers contribute only enough additional, independent information to slightly improve prediction. The full predictive potential of the biomarkers alone (62.3%) was similar to the discriminative capacity of the comprehensive epidemiological risk score (62.7%).

The aim of this investigation was to investigate the extent to which biomarkers improved discrimination of an existing risk prediction model. The performance of the endometrial cancer risk model presented here (nested case–control study) is lower than the model previously reported (cohort study[3]) due to the matched case–control study design. Endometrial cancer risk is strongly impacted by both age and menopausal status, and cases and controls in the present study were matched on these factors. Furthermore, duration of menopausal hormone therapy use was an important predictor in our questionnaire-based risk prediction model, and women using exogenous hormones at blood draw were excluded from the nested case–control study.

Our study has several limitations. First, our analysis of model performance is based on the case–control data alone, among women not using exogenous hormones at the time of the study. We cannot evaluate the extent to which the improvement conferred by the selected markers would be observed among

Table 3. *C*-statistics, integrated discrimination improvement (IDI) and net reclassification improvement (NRI), for endometrial cancer risk model alone, and including biomarker concentrations: EPIC cohort

Model	Full sample (247 cases; 469 controls)			Postmenopausal women (175 cases; 339 controls)		
	C	IDI	NRI	*C*	IDI	NRI
5-year EC-risk model	62.7 (58.5–67.0)			65.5 (60.5–70.4)		
Biomarkers alone	67.0 (62.9–71.1)			67.0 (62.2–71.9)		
Optimism[1]	4.7			4.7		
Adjusted performance	62.3 (58.2–66.4)			62.3 (57.5–67.2)		
Model 1: EC score + all biomarkers	68.5 (64.4–72.5)			70.2 (65.5–74.9)		
Difference from EC-risk score	5.8	0.26 (0.15–0.38)	37.4 (22.0–52.8)	4.7	0.29 (0.15–0.43)	40.2 (22.0–58.5)
Optimism[1]	3.8	0.10	15.5	3.8	0.10	15.5
Adjusted improvement	2.0	0.16 (0.05–0.28)	21.9 (16.5–37.3)	0.9	0.19 (0.05–0.33)	24.7 (6.5–43.0)
Model 2: EC score + selected biomarkers	67.7 (63.6–71.8)			69.1 (64.3–73.9)		
Difference from EC-risk score	5.0	0.16 (0.09–0.23)	33.5 (18.1–48.9)	3.6	0.18 (0.10–0.27)	37.8 (19.5–56.0)
Optimism[1]	3.3	0.08	14.5	3.3	0.08	14.5
Adjusted improvement	1.7	0.08 (0.01–0.15)	19.0 (3.6–34.4)	0.3	0.10 (0.02–0.19)	23.3 (5.0–41.5)

[1]Estimated in full study population.

exogenous hormone users. The study design may limit the accurate interpretation of the discriminative capacity of biomarkers due to case and control matching.[19,20] However, inclusion of the endometrial cancer risk estimate as a regression offset should provide appropriate adjustment to account for the nested case–control study design, as proposed by Pepe *et al.*[19] Moreover all biomarkers were adjusted for age, center and menopausal status, and therefore, potential confounding of our results from these important predictors and matching variables has been avoided. Another limitation is the large proportion of the measurements on IL1Ra (52%) and TNFa (18%) below the assay limit of detection. The effect of missing values was evaluated using the following three different methods: (*i*) inclusion of an additional categorical marker indicating values below detection limit, (*ii*) replacement of values below detection limit with the detection limit and (*iii*) exclusion of these markers from the common model. Overall, the risk estimates were similar from the three approaches, thus we present only results from the first approach. Finally, we used bootstrapping for internal validation in this study as it has been suggested as the most efficient technique to address optimism due to overfitting.[17] We did not have data available for external validation.

We observed only modest improvement in discrimination after incorporating biomarker concentrations in endometrial cancer risk prediction models. Future models should include hormones and etiologic markers on independent pathways and confirmed genetic markers to further improve discrimination.

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