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

Renée T. Fortner[1]*, Anika Hüsing[1]*, Tilman Kühn[1], Meric Konar[1,2], Kim Overvad[3], Anne Tjønneland[4], Louise Hansen[4], Marie-Christine Boutron-Ruault[5,6,7], Gianluca Severi[5,6,7,8], Agnès Fournier[5,6,7], Heiner Boeing[9], Antonia Trichopoulou[10,11], Vasiliki Benetou[10,11], Philippos Orfanos[10,11], Giovanna Masala[12], Claudia Agnoli[13], Amalia Mattiello[14], Rosario Tumino[15], Carlotta Sacerdote[16], H.B(as). Bueno-de-Mesquita[17,18,19], Petra H.M. Peeters[20,21], Elisabete Weiderpass[22,23,24,25], Inger T. Gram[22], Oxana Gavrilyuk[22,26], J. Ramón Quirós[27], José Maria Huerta[28,29], Eva Ardanaz[29,30,31], Nerea Larrañaga[29,32], Leila Lujan-Barroso[33],Emilio Sánchez-Cantalejo[29,34], Salma Tunå Butt[35], Signe Borgquist[36], Annika Idahl[37], Eva Lundin[38], Kay-Tee Khaw[39], Naomi E. Allen[40], Sabina Rinaldi[41], Laure Dossus[41],Marc Gunter[41], Melissa A. Merritt[42], Ioanna Tzoulaki[42], Elio Riboli[42], Rudolf Kaaks[1]

*contributed equally

1. Division of Cancer Epidemiology, German Cancer Research Center (DFKZ), Heidelberg, Germany
2. Department of Biostatistics, Hacettepe University, Ankara, Turkey
3. Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark
4. Unit of Diet, Genes and Environment, Danish Cancer Society Research Center, Copenhagen, Denmark
5. INSERM, Centre for Research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones and Women's Health team, F-94805, Villejuif, France
6. Université Paris Sud, UMRS 1018, F-94805, Villejuif, France
7. Gustave Roussy, F-94805, Villejuif, France
8. Human Genetics Foundation (HuGeF), Torino, Italy
9. Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
10. Hellenic Health Foundation, Athens, Greece
11. WHO Collaborating Center for Nutrition and Health, Unit of Nutritional Epidemiology and Nutrition in Public Health, Dept. of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Greece
12. Cancer Risk Factors and Life-Style Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence- Italy
13. Epidemiology and Prevention Unit , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan Italy
14. Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples Italy
15. Cancer Registry and Histopathology Unit, "Civic - M.p.Arezzo" Hospital, ASP Ragusa, Italy
16. Unit of Cancer Epidemiology, Città della Salute e della Scienza University-Hospital and Center for Cancer Prevention (CPO), Turin, Italy
17. Department for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

18. Department of Epidemiology and Biostatistics, The School of Public Health, Imperial College London, London, United Kingdom
19. Department of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
20. Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Netherlands
21. MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College, London, UK
22. Department of Community Medicine, University of Tromsø , The Arctic University of Norway, Tromsø, Norway
23. Department of Research, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway
24. Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
25. Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland
26. Department of Obstetrics and Gynecology University Hospital Northern Norway, Tromsø, Norway
27. Public Health Directorate, Asturias, Spain
28. Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain
29. CIBER Epidemiología y Salud Pública (CIBERESP), Spain
30. Navarra Public Health Institute, Pamplona, Spain
31. IdiSNA, Navarra Institute for Health Research, Pamplona, Spain
32. Public Health Division of Gipuzkoa, Regional Government of the Basque Country, Spain
33. Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain
34. Escuela Andaluza de Salud Pública. Instituto de Investigación Biosanitaria ibs.Granada. Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain
35. Division of Surgery, Clinical Sciences Malmö, Lund University, Sweden.
36. Division of Oncology and Pathology, Clinical Sciences, Lund University, Sweden
37. Department of Clinical Sciences, Obstetrics and Gynecology and Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Umeå, Sweden"
38. Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden
39. Cancer Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom
40. Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, UK
41. International Agency for Research on Cancer, Lyon, France
42. School of Public Health, Imperial College London, London, United Kingdom

Corresponding author:

Renée T. Fortner
German Cancer Research Center (DKFZ)
Division of Cancer Epidemiology
Im Neuenheimer Feld 280
D-69120 Heidelberg

Phone: +49 (0)6221 42 2241
Fax: +49 (0)6221 42 2203
E-Mail: r.fortner@dkfz.de

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Novelty and Impact: The investigation is the first to evaluate whether adding serum concentrations of sex steroid hormones, metabolic markers, growth factors, adipokines, and cytokines improves discrimination of an endometrial cancer risk prediction model. Using data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, we observed a 2 percentage point improvement in discrimination in models including all evaluated hormones and accounting for over-fitting.

Abstract

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.

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 U.S.-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 prediction, 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, 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. 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 ≤-150ºC, with the exception of Sweden, where samples are stored at -70 ºC.

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, and Naples, 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, HT), and with no reported hysterectomy or history of cancer (except non-melanoma skin cancer) were eligible for this study. Cases were restricted to incident epithelial endometrial cancers diagnosed during follow-up; non-epithelial 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, perimenopausal), age at enrolment (±6 months), time of day of blood collection (±1 hour), fasting status (<3 hours; 3–6 hours, >6 hours) and for premenopausal women, phase of menstrual cycle (follicular, periovulatory, 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 into 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 temperature for >24 hours 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-value 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.

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 Supplemental Table 1.

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. Further, 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 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 three different methods: 1) inclusion of an additional categorical marker indicating values below detection limit, 2) replacement of values below detection limit with the detection limit, and 3) 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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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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