





RESEARCH ARTICLE

Cancer Epidemiology

Prospective evaluation of 92 protein biomarkers for early detection of endometrial cancer

Victoria Cooley^{1,2} | Renée Turzanski Fortner^{1,3} | Trasia Mukama^{1,4}  | Sabine Naudin⁵ | Valeria Pala⁶  | Laure Dossus⁷ | Inger T. Gram⁸  | Karina Standahl Olsen⁸ | Maria-Jose Sánchez^{9,10,11} | Pernilla Israelsson¹² | Naomi Allen¹³ | Hilde Langseth^{3,14} | Rudolf Kaaks¹ 

¹Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

²Medical Faculty Heidelberg, Heidelberg University, Heidelberg, Germany

³Department of Research, Cancer Registry of Norway, Norwegian Institute of Public Health, Oslo, Norway

⁴Makerere University School of Public Health, Kampala, Uganda

⁵Paris-Saclay University, UVSQ, Inserm, Gustave Roussy, CESP, Villejuif, France

⁶Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy

⁷Nutrition and Metabolism Branch, International Agency for Research on Cancer, World Health Organization, Lyon, France

⁸Faculty of Health Sciences, Department of Community Medicine, UiT The Arctic University of Norway, Tromsø, Norway

⁹Escuela Andaluza de Salud Pública (EASP), Granada, Spain

¹⁰Instituto de Investigación Biosanitaria IBS.GRANADA, Granada, Spain

¹¹Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

¹²Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden

¹³Nuffield Department of Population Health, University of Oxford, Oxford, UK

¹⁴Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK

Correspondence

Rudolf Kaaks, Division of Cancer Epidemiology, German Cancer Research Center, Im Neuenheimer Feld 581, 69120 Heidelberg, Germany.
Email: r.kaaks@dkfz.de

Funding information

Barrie Dalglish Centre for Myeloma and Related Blood Cancers; Leukemia and Lymphoma Society

Abstract

The human epididymis protein 4 (HE4) remains the best available endometrial cancer (EC) biomarker; however, its discrimination between cases and cancer-free individuals is limited and might be improved when combined with other protein markers. We evaluated the discrimination capacity of 92 proteins as potential early detection biomarkers for EC in nested case-control studies in the European Prospective Investigation into Cancer and Nutrition (EPIC) (63 cases, 123 controls) and Janus (75 cases, 146 controls) cohorts, evaluating blood samples taken ≤ 2 years prior to diagnosis. Proteins were measured with the Olink Target 96 Oncology II panel assays. Areas under the receiver operating characteristic curves (AUCs) were calculated using

Abbreviations: AEH, atypical endometrial hyperplasia; aROCs, covariate-adjusted receiver operating curves; AUC, area under the curve; BMI, body mass index; CIs, confidence intervals; CRN, Cancer Registry of Norway; EC, endometrial cancer; EIN, endometrial intraepithelial neoplasia; EPIC, European Prospective Investigation into Cancer and Nutrition; HE4, human epididymis protein 4; PEA, proximity extension assay; SE95, sensitivities at 95% specificity; TVUS, transvaginal ultrasound.

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *International Journal of Cancer* published by John Wiley & Sons Ltd on behalf of UICC.

logistic regression. The discrimination between cases and controls of top-performing proteins was modest (EPIC: HE4, CA125, CAIX, and S100A4; Janus: HE4, CA125, FURIN, CXCL13, and IL6; AUC range: 0.65 [S100A4], 0.76 [HE4, EPIC] within 0 to <12 months of blood collection) and decreased as the time between blood draw and cancer diagnosis increased (12–24 months AUC range: 0.49 [S100A4], 0.69 [CA125, Janus]). The combination of these other markers with HE4 did not improve discrimination. HE4 and other candidate proteins had limited discrimination between EC cases and controls and hence do not appear to be useful for early detection of this disease in women at average population risk.

KEYWORDS

biomarkers, early detection, endometrial cancer

What's New?

In this prospective study, the authors evaluated the discrimination capacity of 92 cancer-related proteins as potential early detection biomarkers for endometrial cancer using blood samples from two large-scale European population cohorts (EPIC and Janus). The human epididymis protein 4 (HE4) and the antigen CA125 demonstrated the best—though limited—discrimination capacity between cases and controls across both cohorts up to two years prior to diagnosis. Discrimination waned as the time from blood draw to diagnosis increased, and the combination of other markers with HE4, currently the best endometrial cancer biomarker available, did not result in improvements in discriminative performance.

1 | INTRODUCTION

Endometrial cancer (EC) is the most common gynecological cancer in many high-income countries. The earlier detection of EC could improve survival outcomes, but currently, there is no population-based screening method recommended for this cancer. Current screening and targeted prevention strategies are restricted to high-risk women (i.e., Lynch syndrome) and include annual transvaginal ultrasound (TVUS) for screening and radical hysterectomy for prevention.¹ While the early recognition of symptoms, such as abnormal vaginal bleeding or discharge, often leads to diagnosis at an earlier stage (localized: 69% of cases; regional: 17% of cases) where the 5-year relative survival is favorable (localized: 95%; regional: 70%), the 5-year relative survival estimates for those with advanced disease are only 18%.^{2,3} In Norway, the 5-year relative survival for localized disease is 98.7%, 68.0% for regional, and 30.1% for distant disease.⁴

Postmenopausal bleeding, which occurs in the vast majority of women with EC, lacks sufficient specificity, and only 9% of women with bleeding have a resulting EC diagnosis following clinical investigation.⁵ Endometrial biopsies, which are often used in combination with TVUS as a diagnostic measure, are associated with varying degrees of pain and discomfort and have a failure rate of approximately 11%.⁶ The specificity of TVUS imaging is also lower, at 86% for an endometrial thickness cutoff of 3.0–5.9 mm.⁷ The use of diagnostic tests that rely upon results collected from blood, cervicovaginal fluid, and urine has the potential to provide a minimally invasive approach to the earlier detection of EC and also to reduce the

number of unnecessary endometrial biopsies.⁸ Such methods, however, are not available for clinical use, and additional prospective studies are needed to generate evidence to support their utility in clinical practice.⁸ The justification of mainstream screening practices is often not deemed worthy from a mortality reduction perspective when considering the possibility of producing unnecessary concern over many false-positive tests and the associated resourcing and cost implications.

The human epididymis protein 4 (HE4) is the best EC biomarker found so far in clinical studies,^{9–11} although other blood-based biomarkers such as antigens CA125, CA72-4, and CA19-9,¹² inflammation markers,¹³ angiogenesis factors,¹⁴ and other proteins¹⁵ have been identified as being associated with EC. HE4 concentrations, however, have also been shown to be elevated in association with other cancers (i.e., pancreatic and ovarian)¹¹ and this biomarker has been shown to provide only limited discrimination between EC patients and non-cases (area under the curve [AUC] range: 0.68–0.77).¹⁶

One way to potentially improve the specificity and overall discrimination capacity for the detection of EC is to use multi-protein biomarker panels that include HE4 plus other proteins. This motivates the search for the identification and/or validation of further candidate detection markers, using modern proteomics and multi-protein assay platforms. Two previous retrospective case-control studies utilized different multiplex assays of cancer-related markers based on the proximity extension assay (PEA) technology from Olink Proteomics (Target Oncology panels) and identified proteins (<10 proteins per

study from final analyses; overlapping protein: MMP-7) associated with EC.^{17,18} Furthermore, using the same panel, a study nested within the prospective European Prospective Investigation into Cancer and Nutrition (EPIC) cohort assessed and partially confirmed the potential of several of this panel's markers for the early detection of ovarian cancer, up to 9 months before diagnosis.¹⁹

A limitation of all of the studies on the identification or validation of early detection biomarkers for EC is that they have compared protein profiles between prevalent, clinically manifest EC samples and those without cancer. It thus remains unclear whether, or to what extent, protein markers may be used for detecting EC before the appearance of clinical symptoms. In considering the low relative 5-year survival rates for women diagnosed with advanced disease, there is a need to identify additional biomarkers that can aid in identifying this disease before the manifestation of major symptoms, in order to offer curative treatment. We here present findings from a first prospective evaluation of proteins as potential early detection biomarkers for EC, using nested case-control studies in two large-scale population cohorts. We used the Olink Target 96 Oncology II panel and assessed the discriminative capacity of a total of 92 cancer-related proteins, including HE4 and CA125, for the prospective detection of EC through measurements in plasma and serum samples that had been collected up to 2 years prior to diagnosis.

2 | METHODS

2.1 | Case-control studies nested within the EPIC and Janus cohorts

The sample for the current study includes nested case-control studies within the EPIC and the Norwegian Janus Serum Bank (Janus) cohorts. EPIC is an ongoing multi-center prospective cohort study that aims to investigate the relationship between nutrition and cancer.²⁰ The enrollment of 519,978 participants (226,673 women who provided a blood sample) took place between 1992 and 2000 across 23 centers in 10 countries. Data were collected via questionnaire and anthropometric measures, and blood samples were collected. Janus is a large prospective population-based biobank that was established in 1973 and includes blood samples from 318,628 Norwegians (152,491 women).^{21,22} Approximately 90% of the cohort participated in Norwegian Regional Health Studies (years 1972–2003) and has questionnaire-based data on lifestyle, as well as anthropometric, and biochemical data available; and the remainder consists of Red Cross blood donors.

2.2 | Sample selection and data collection

Cases were defined as women diagnosed with incident invasive EC (ICD-O code: C541) within 24 months of blood collection. Women

were excluded if they had a previous cancer diagnosis (with the exception of non-melanoma skin cancer) prior to blood draw. Incidence density sampling with a 1:2 case-control matching ratio was utilized. Tumors were classified as Type I or Type II in line with the classifications previously implemented.²³

2.2.1 | European Prospective Investigation into Cancer and Nutrition

Within the EPIC cohort, cases were identified through record linkages with regional cancer and pathology registries (all countries except France, Germany, and Naples, Italy) and active follow-up and verification of self-reports (France, Germany and Naples, Italy). Women were excluded if they had reported a hysterectomy at the time of blood draw. Cases and controls were matched on the following characteristics at the time of blood collection: study center, menopausal status (premenopausal, postmenopausal, unknown), exogenous hormone use, age at blood collection (± 6 months), time of the day of blood collection (± 1 h), time between blood draw and last consumption of foods or drinks (< 3 , $3-6$, > 6 h, unknown), and for premenopausal women, phase of menstrual cycle. Tumor characteristics such as stage (available as localized, metastatic regional, and metastatic categories), histology, and grade were obtained from pathology reports and cancer registries. Information on additional epidemiologic risk factors such as smoking status, body mass index (BMI), menopausal status, and oral contraceptive use was obtained from baseline questionnaires and measurements. We excluded women based on the following characteristics in sequential order: 21,205 with prevalent cancers, 35,970 with hysterectomy, 53 with incomplete data with no lifestyle or dietary information available, and 2385 with no follow-up data available. The final sample from EPIC included 63 cases and 123 matched controls.

2.2.2 | Janus

Cases were identified through linkage with the Cancer Registry of Norway (CRN), which has had mandatory reporting of cancer cases since 1953.²⁴ Cases and controls were matched on the following characteristics at the time of blood collection: source of blood collection (health examination, blood donors), age (within ± 1 year from age of case at blood draw), date of blood draw (within ± 3 months from date of blood draw of case), and county of residence. Information on tumor characteristics such as stage (available as localized, regional metastasis, and distant metastasis categories), histology, and grade was obtained from the CRN. Information on additional epidemiologic risk factors such as smoking status and BMI was obtained from the Norwegian Regional Health Studies.²¹ A total of 2105 women were excluded due to prevalent cancers (follow-up data available for all Janus members via registries). The final sample included 75 cases and 146 matched controls.

2.3 | Laboratory assays

The Olink Target 96 Oncology II panel assays were performed in an Olink® certified laboratory at the German Center for Environmental Health (Helmholtz Zentrum München), Neuherberg, Germany. Samples were analyzed in batches, with samples from matched case-control sets included in the same batch in a randomized and blinded order. Results were reported in “normalized protein expression” values on the log₂ scale. For small proportions of study participants, and for relatively few proteins, PEA measurements fell below the detection limit (EPIC: FADD [18.1%], VIM [3.0%], CEACAM5 [3.5%], CA125 [3.5%]; Janus: TXLNA: [2.5%], FADD [0.4%], VIM [0.4%], CEACAM5 [0.8%], CA125 [1.2%], FCRLB [5.8%]). When assay results were below the limit of detection, we assigned values equal to the midpoint between zero and the lower limit of detection.

2.4 | Statistical analysis

To assess the discrimination performance of the proteins, covariate-adjusted receiver operating curves (aROCs) were generated for protein-related risk scores developed from unconditional logistic regression models,^{25–27} and the AUC with 95% confidence intervals (CIs) was calculated for each protein, separately by cohort and lag-time interval (0 to <12 and 12 to 24 months). All aROCs were adjusted for age at blood draw, and EPIC aROCs were additionally adjusted for menopausal status. Proteins that had an AUC of ≥ 0.65 for EC cases in the 0 to <12 months lag-time interval compared to controls were selected for further evaluation for sensitivities at 95% specificity (SE95). Spearman's partial correlations, adjusting for age at blood draw and cohort, were calculated within case and control groups to assess relationships between the selected proteins (Supporting Information Figure F1). Locally Estimated Scatterplot Smoothing was used to produce fitted lines in the visualization of the top performing protein levels by time before EC diagnosis (Supporting Information Figure F3). Multi-marker models, in which the selected proteins were added singly to a model containing HE4, were developed to assess any improvement in discriminative performance. Likelihood ratio tests were utilized to assess any improvements in model fit. Optimism-corrected AUCs were calculated through 1000 bootstrap iterations, to account for overfitting in the multi-marker models. Statistical significance was evaluated at the 0.05 alpha level. All analyses were executed using R (4.3.0) for Windows.²⁸

3 | RESULTS

The characteristics of cases and controls in the EPIC and Janus cohorts are presented in Table 1. The median age at blood collection was 58 years for cases and controls (interquartile range IQR: 54, 63) in the EPIC cohort and 50 for cases (IQR: 43, 54) and 49 for controls (IQR: 43, 54) in the Janus cohort (Table 1). The median age at EC diagnosis was 59 (IQR: 55, 63) in EPIC and 51 (IQR: 43, 55) in Janus. In

both cohorts, the majority of cases were diagnosed at the localized stage (EPIC: 75%; Janus 79%).

The discrimination of HE4 and CA125 was similar between cohorts, with slightly higher AUC values across both lag-time intervals for HE4 in the EPIC cohort (EPIC: 0 to <12 months: 0.76 [0.66, 0.87] and 12 to 24 months: 0.66 [0.56, 0.77] compared with the Janus cohort: 0 to <12 months: 0.75 [0.65, 0.84] and 12 to 24 months: 0.62 [0.52, 0.71]). AUC values for CA125 were slightly higher in the Janus cohort across both lag-time intervals (Janus: 0 to <12 months: 0.74 [0.65, 0.83] and 12 to 24 months: 0.69 [0.6, 0.77]; EPIC: 0 to <12 months: 0.72 [0.6, 0.83] and 12 to 24 months: 0.59 [0.48, 0.7]). Overall, HE4 generally had the highest discrimination within both cohorts and across lag-times, with the exception of the 12–24 months lag-time in the Janus cohort where CA125 had a slightly higher AUC (Table 2; AUC [95% CI] values for all individual proteins found in Supporting Information Table T1).

In addition to HE4 and CA125, two other proteins in the EPIC cohort (CAIX and S100A4) and three other proteins in the Janus cohort (FURIN, CXCL13, and IL6) had an AUC of ≥ 0.65 for the discrimination between cases and controls in the 0 to <12 months lag-time interval; the distributions of the proteins by lag-time are shown in Figure 1. For both cohorts, the discrimination of these proteins substantially decreased as the time between blood draw and cancer diagnosis increased (i.e., HE4 in EPIC cohort: 0 to <12 months: 0.76 [0.66, 0.87] and 12 to 24 months: 0.66 [0.56, 0.77]; FURIN in the Janus cohort: 0 to <12 months: 0.68 [0.59, 0.77] and 12 to 24 months 0.58 [0.48, 0.69]) (Figure 2; further ROC curves presented in Supporting Information Figure F2). The SE95 values for these proteins also displayed a similar decline by lag-time within both cohorts, and sensitivities approached zero for the 12–24 months category (Table 3).

Multi-marker models featuring the addition of the top performing proteins (listed above) to a model with HE4 were developed (Table 2). In the 0 to <12 lag-time interval in the EPIC cohort, the separate addition of CAIX, S100A4, and CA125 to a model with HE4 alone resulted in statistically significant improvements in model fit, but only minor increases in AUC values (Table 2). A similar finding was observed in the Janus cohort for the addition of CA125 in both lag-times (i.e., Janus 0 to <12 months: HE4 AUC = 0.75, HE4 + CA125 overoptimism corrected AUC: 0.76; Table 2).

4 | DISCUSSION

In this first prospective study to evaluate 92 proteins on the Olink Oncology II panel as early detection biomarkers for EC, HE4 and CA125 demonstrated the best discrimination between cases and controls in the EPIC and Janus cohorts for cancers diagnosed within a year and between 1 and 2 years of a diagnosis. They were the only two proteins that consistently showed discrimination capacity (i.e., lower 95% confidence limits >0.50) in both cohorts, despite differences in the distributions of the age at diagnosis. These findings in the pre-diagnostic setting confirm those of previous, cross-sectional case-control comparisons based on prevalent cases and control

TABLE 1 Characteristics of cases and controls in the EPIC and Janus cohorts.

Characteristic	EPIC		Janus	
	Case, N = 63 ^a	Control, N = 123 ^a	Case, N = 75 ^a	Control, N = 146 ^a
Age at blood draw	58 (54, 63)	58 (54, 63)	50 (43, 54)	49 (43, 54)
BMI	26.8 (23.3, 30.3)	25.0 (23.0, 27.8)	25.3 (23.5, 30.1)	24.5 (22.4, 27.0)
Unknown			14	24
Smoking status				
Current	8 (13%)	18 (15%)	24 (39%)	41 (34%)
Former	14 (23%)	19 (16%)	8 (13%)	20 (16%)
Never	39 (64%)	82 (69%)	29 (48%)	61 (50%)
Unknown	2	4	14	24
Age at diagnosis	59 (55, 63)		51 (43, 55)	
Histology				
Type I	55 (87%)		64 (85%)	
Type II	8 (13%)		2 (2.7%)	
Sarcomas	0 (0%)		9 (12%)	
Grade				
Well differentiated	17 (35%)		5 (63%)	
Moderately differentiated	19 (40%)		2 (25%)	
Poorly differentiated/undifferentiated	8 (17%)		1 (13%)	
Not determined	4 (8.3%)		0 (0%)	
Unknown	15		67	
Stage				
Localized	30 (75%)		58 (79%)	
Regional/metastatic	10 (25%)		15 (21%)	
Unknown	23		2	
Lag-time category (months)				
0 to <12	27 (43%)		39 (52%)	
12 to 24	36 (57%)		36 (48%)	
Lag-time (months)	13 (8, 19)		12 (7, 19)	

Abbreviations: BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition.

^aMedian (IQR), n (%).

women, where both HE4⁹⁻¹¹ and CA125^{12,29} were also found to be the two best discriminating markers. For both HE4 and CA125, but also the other proteins with AUCs ≥ 0.65 in at least one of the cohorts for blood draws 0 to <12 months prior to diagnosis (CAIX, S100A4, FURIN, CXCL13, and IL6), discrimination waned with longer time between blood draw and diagnosis (i.e., 12–24 months). Finally, upon the addition of these proteins to a model with HE4, only minimal increases in discrimination were observed across both cohorts and lag-times, and again their joint discrimination capacity decreased for the 12–24 months lag-time.

Of the seven proteins suggesting discrimination potential in our study, only HE4 and IL6 were identified previously as being associated with EC in one¹⁷ of two cross-sectional studies in Sweden or Italy, also using the Olink technology.^{17,18} Other retrospective and nested case-control studies have utilized enzyme-linked and bead-based immunoassays for the serum and plasma quantification of IL6^{13,30} and

spectrophotometry-based methods for the uterine lavage measurement of pro-protein convertases, notably FURIN.³¹ IL6 is a pro-inflammatory cytokine that is released by macrophages within adipose tissue and has been found to be involved in tumorigenesis through multiple pathways.³² In these studies, IL6 exhibited potential in differentiating EC cases from controls, but associations became attenuated and did not persist after the adjustment for BMI.^{13,30} Most of the previous studies included plasma samples from prevalent EC cases and cancer-free control women, and the discrimination potential for proteins, as observed in these studies, might not necessarily translate well to pre-diagnosis settings, to identify cancer before the manifestation of any other symptoms.

While other proteins such as Mk¹⁷ and COL9A1¹⁸ were identified as being associated with EC on the Target 96 Oncology II¹⁷ or III¹⁸ panels, the study from Sweden utilized four other Olink Multiplex assays (CVD II, CVD III, INF I, NEU I; proteins PRSS8, ADM, MMP-7,

TABLE 2 Multi-marker models featuring the addition of top-performing proteins^a to a model with HE4 in the EPIC and Janus cohorts.

Model	EPIC				Janus			
	0 to <12, AUC (95% CI)	Optimism corrected AUC	12 to 24, AUC (95% CI)	Optimism corrected AUC	0 to <12, AUC (95% CI)	Optimism corrected AUC	12 to 24, AUC (95% CI)	Optimism corrected AUC
HE4	0.76 (0.66, 0.87)	NA	0.66 (0.56, 0.77)	NA	0.75 (0.65, 0.84)	NA	0.62 (0.52, 0.71)	NA
CA125	0.72 (0.6, 0.83)	NA	0.59 (0.48, 0.7)	NA	0.74 (0.65, 0.83)	NA	0.69 (0.6, 0.77)	NA
CAIX	0.66 (0.53, 0.79)	NA	0.58 (0.47, 0.69)	NA	–	–	–	–
S100A4	0.65 (0.54, 0.77)	NA	0.49 (0.38, 0.6)	NA	–	–	–	–
FURIN	–	–	–	–	0.68 (0.59, 0.77)	NA	0.58 (0.48, 0.69)	NA
CXCL13	–	–	–	–	0.67 (0.57, 0.77)	NA	0.59 (0.48, 0.7)	NA
IL6	–	–	–	–	0.66 (0.57, 0.74)	NA	0.59 (0.48, 0.69)	NA
Marker combinations								
HE4 + CA125	0.79 (0.68, 0.9)	0.76	0.67 (0.56, 0.77)	0.62	0.78 (0.69, 0.86)	0.76	0.7 (0.61, 0.78)	0.66
HE4 + CAIX	0.8 (0.7, 0.9)	0.77	0.69 (0.59, 0.8)	0.65	–	–	–	–
HE4 + S100A4	0.82 (0.74, 0.9)	0.79	0.67 (0.56, 0.78)	0.62	–	–	–	–
HE4 + FURIN	–	–	–	–	0.76 (0.67, 0.84)	0.74	0.63 (0.53, 0.73)	0.58
HE4 + CXCL13	–	–	–	–	0.75 (0.65, 0.84)	0.73	0.62 (0.52, 0.73)	0.58
HE4 + IL6	–	–	–	–	0.75 (0.66, 0.84)	0.73	0.62 (0.52, 0.72)	0.57

Note: All models are additionally adjusted for age at blood draw and additionally menopausal status at blood draw for the EPIC cohort. Optimism-corrected AUC values are presented to account for overfitting.

^aOnly proteins with AUCs ≥ 0.65 are presented.

ST2, VEGF-A, and HGF identified)¹⁷ and the study from Italy utilized the Target 96 Immuno-Oncology panel (proteins Gal-1, Gal-9, MMP7, and FASLG identified).¹⁸ The Target 96 Oncology II panel (used in our present study) and the Oncology III panel (used in the study from Italy) feature entirely different proteins, which also makes any direct comparisons of study findings impossible. Overall, the endometrial cases in our study were younger than those of the two earlier studies mentioned above. In addition, our study included a mix of endometrioid and other tumor types, while the study from Italy included endometrioid adenocarcinomas.¹⁸

When interpreting the results of this study, it is important to consider the temporal trajectory of the diagnosis of EC from the development of initial symptoms and precursor lesions. EC often develops from endometrial intraepithelial neoplasia (EIN) or atypical endometrial hyperplasia (AEH). During the development of AEH, the PTEN gene undergoes somatic mutations and then mutations in the ARID1A gene and inactivation of TGF- β are involved in the ultimate progression into invasive endometrioid carcinoma.^{33–35} A hysterectomy is typically the primary treatment unless a fertility-sparing pharmacologic treatment in the form of pro-gestational agents is desired.³⁶ Approximately 30%–50% of women with EIN-AEH who have a hysterectomy are found to have EC.³⁶ Endometrial sampling is used for women who desire to undergo fertility-sparing treatment to definitively rule out EC and a histologic assessment within 3–6 months of therapy is given.³⁶ The timeline that spans the transition from hyperplasia to AEH to EC is unclear. The relative timing of each interval is not fully defined, with the potential for reversion of the hyperplasia with the usage of progestin therapy. In considering the complexity of

the trajectories mentioned above, and the additional potential to dismiss intermenstrual and post-menopausal bleeding as a benign condition and delay diagnosis,^{5,37} additional investigation is needed to better understand the time interval between early symptom development and diagnosis. Such an improved understanding can inform future early detection work, helping to identify an optimal window of time following symptom presentation where a diagnosis is plausible and allowing the investigation of other marker pathways.

We observed a substantial decrease in discrimination between subsequent cases and controls, comparing those providing a blood sample proximate to diagnosis (i.e., 0–12 months) to those providing a sample more distant from diagnosis (i.e., 12–24 months). The lack of prospective studies in the current literature and the complexity around the length of the interval surrounding the experience of symptoms and development of precursor lesions to EC make it challenging to describe the relationship between the decline in discrimination and the biological changes occurring in the pre-diagnostic phase and progression of the development of neoplasm. One study featuring a follow-up period of 20 years of women with endometrial hyperplasia reported a 20-year progression to endometrial carcinoma risk of 28% among those with AEH.³⁸ Measurements fell below the detection limit for relatively few proteins and subjects in our study, and we would expect that this decline in discrimination over time would not be largely reflective of limitations in the protein assay used. The evaluation of future assays with lower limits of detection, where lower but meaningful levels of protein could be detected among cases, would be needed to fully elucidate this point.

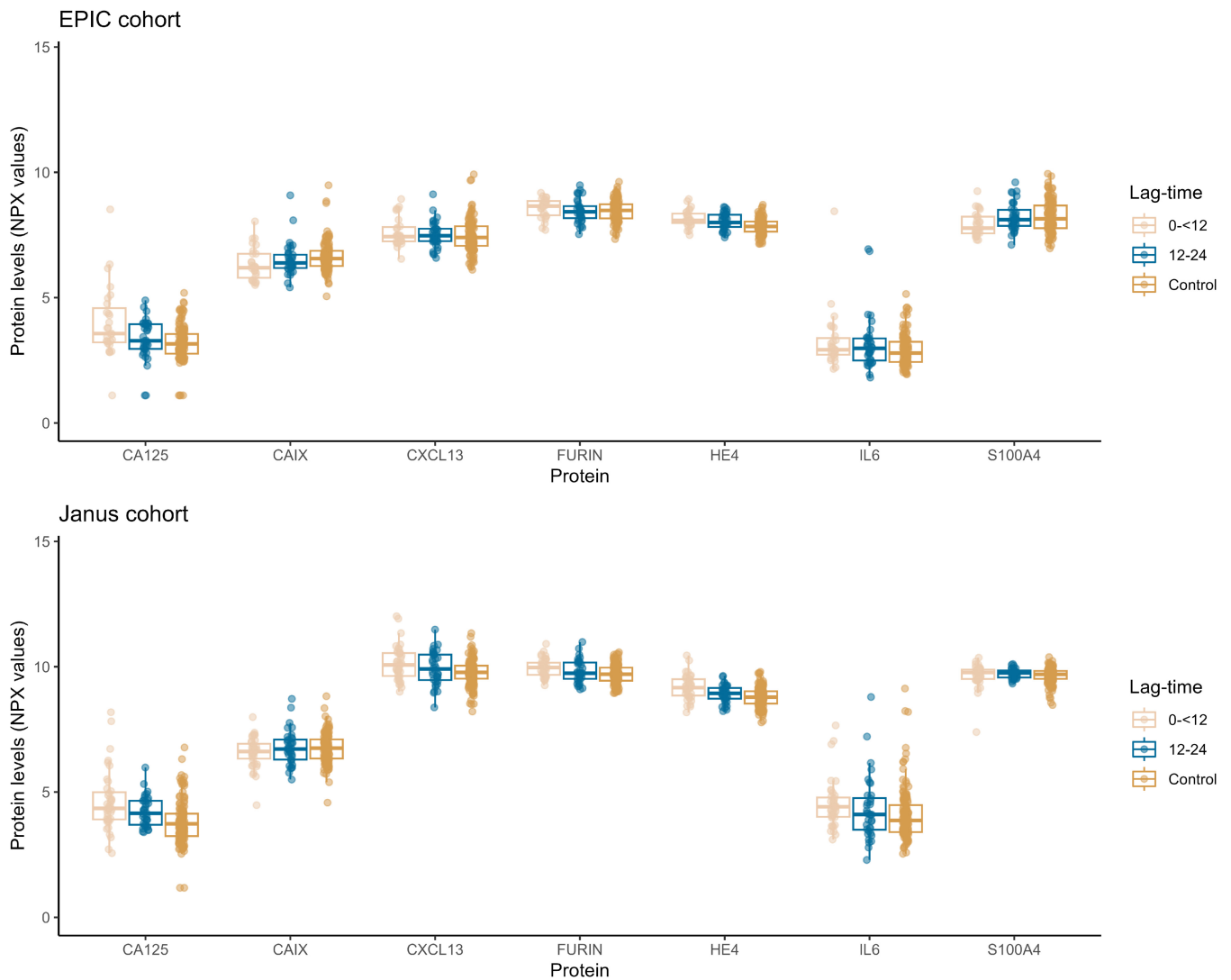


FIGURE 1 Distributions of proteins in the EPIC and Janus cohorts, stratified by lag-time category. The proteins above displayed an AUC value of ≥ 0.65 for cases in the 0 to <12 months lag-time interval and controls, and were selected for further analysis. AUC, area under the curve; EPIC, European Prospective Investigation into Cancer and Nutrition.

In previous work assessing the potential of early detection biomarkers for ovarian cancer, such a decrease in discrimination with increasing time between blood draw and diagnosis has also been observed.^{19,39} In a previous nested case-control study conducted within the EPIC cohort using the same protein panel, HE4 and CA125 were found to have the best discrimination in the 0 to ≤ 9 months lag-time interval (AUC [95% CI]: 0.86 [0.82, 0.89] and 0.84 [0.81, 0.87], respectively) but performance substantially decreased for the >9 –18 months lag-time.¹⁹ The detection of HE4 and CA125 as the best performing early detection biomarkers for both endometrial and ovarian cancer is expected, given the presence of the endometrioid histotype in ovarian cancer and that both cancers are gynecological in nature. Proteins such as FOLR1, KLK11, MDK, WISP1, CXCL13, MSLN, and ADAM8 were identified in this prior study, and none overlapped with the current study (with the exception of CXCL13 in Janus). Further studies featuring a larger number of cases are needed to confirm the specific utility of these proteins for ovarian cancer earlier detection.¹⁹

This study has a few limitations. There is a potential for between-person and between-study heterogeneity in diagnosis dates. Also, no data were available regarding the development and experience of symptoms and their timing relative to blood draw in either of the two cohorts. Future work incorporating symptom data into multi-marker discrimination models featuring HE4, CA125, and other promising proteins could be of potential benefit for the earlier detection of EC. This study was limited to cases diagnosed within the 2 years following blood collection, and the assessment of lag-time intervals longer than 2 years for additional marker pathways demonstrating potential would be of interest for future studies. The limited sample size and small number of cases in this study made it difficult to explore more granular lag-time categories and develop more complex multi-marker models, and further useful discriminatory proteins might have also been missed. Given that the discrimination potential of many proteins was assessed, the likelihood of a Type 1 error is increased, though our identification of HE4 and CA125 in both cohorts is consistent with previous literature.

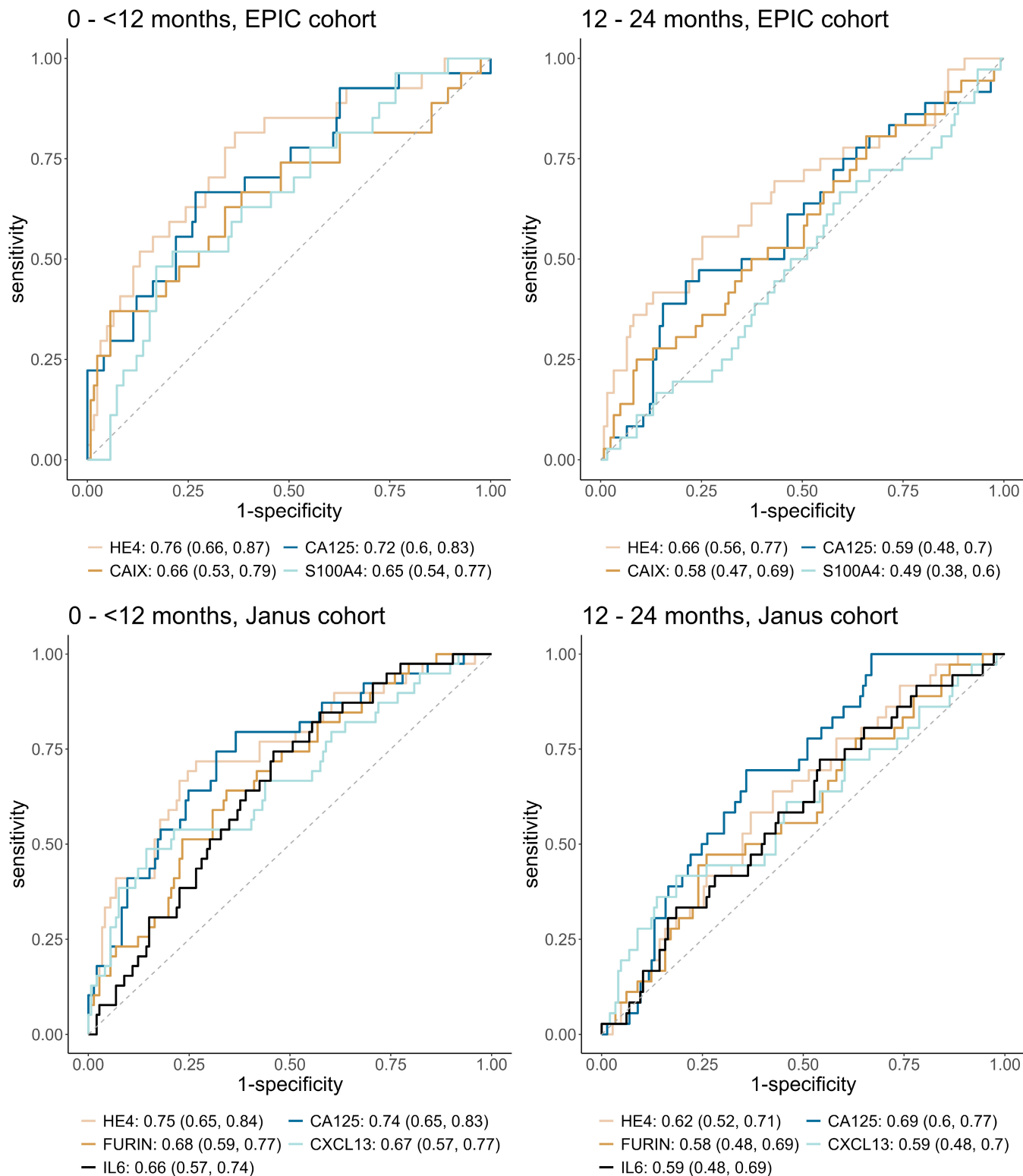


FIGURE 2 Cohort-specific ROC curves depicting the discriminative performance (AUC [95% CI]) of proteins with AUCs ≥ 0.65 for cases in the 0 to <12 months lag-time interval and controls. ROC curves for these top performing proteins in the 12–24 lag-time interval are also displayed. For both the EPIC and Janus cohorts, AUCs are adjusted for age at blood draw, and additionally menopausal status for the EPIC cohort only. AUC, area under the curve; EPIC, European Prospective Investigation into Cancer and Nutrition; ROC, receiver operating characteristics.

HE4 and CA125 only found in both of the EPIC and Janus cohorts displayed the highest, though limited, discrimination as EC early detection biomarkers across both of the lag-times assessed. Overall,

discrimination decreased with increasing lag-time, and the combination of single markers with HE4 did not result in any improvement in discriminative performance. Additional studies including larger sample

Protein	Sensitivity at 95% specificity by lag-time (months)			
	EPIC, 0 to <12	EPIC, 12 to 24	Janus, 0 to <12	Janus, 12 to 24
HE4	0.33 (0.11, 0.52)	0.22 (0.08, 0.47)	0.33 (0.08, 0.51)	0.08 (0, 0.19)
CA125	0.26 (0.11, 0.44)	0.06 (0, 0.17)	0.18 (0.08, 0.41)	0.03 (0, 0.14)
CAIX	0.26 (0.11, 0.52)	0.14 (0, 0.31)	0.15 (0.05, 0.28)	0.06 (0, 0.19)
S100A4	0 (0, 0.3)	0.06 (0, 0.14)	0.08 (0, 0.21)	0.06 (0, 0.17)
FURIN	0.04 (0, 0.19)	0.03 (0, 0.11)	0.15 (0.05, 0.33)	0.08 (0, 0.19)
CXCL13	0.15 (0, 0.3)	0.03 (0, 0.14)	0.18 (0.08, 0.46)	0.19 (0, 0.33)
IL6	0.07 (0, 0.3)	0.11 (0.03, 0.22)	0.08 (0, 0.21)	0.03 (0, 0.14)

Note: Non-overlapping proteins are included for comparability purposes.

sizes, longer lag-time intervals, and most importantly the investigation of alternative marker pathways, together with risk factor, symptom, and imaging data where available, are needed to identify biomarkers to improve the EC diagnosis lead time.

AUTHOR CONTRIBUTIONS

Victoria Cooley: Writing – original draft; visualization; formal analysis; data curation; software. **Renée Turzanski Fortner:** Conceptualization; writing – review and editing; supervision; resources; investigation; methodology. **Trasias Mukama:** Writing – review and editing. **Sabine Naudin:** Writing – review and editing; resources. **Valeria Pala:** Writing – review and editing; resources. **Laure Dossus:** Writing – review and editing; resources. **Inger T. Gram:** Writing – review and editing; resources. **Karina Standahl Olsen:** Writing – review and editing; resources. **Maria-Jose Sánchez:** Writing – review and editing; resources. **Pernilla Israelsson:** Writing – review and editing; resources. **Naomi Allen:** Writing – review and editing; resources. **Hilde Langseth:** Writing – review and editing; resources. **Rudolf Kaaks:** Conceptualization; funding acquisition; writing – review and editing; resources; supervision; investigation; methodology; validation; project administration.

ACKNOWLEDGEMENTS

We acknowledge the use of data and biological samples from the EPIC-Asturias cohort, PI Ramón Quirós, the EPIC-Bilthoven cohort, PI W. M. Monique Verschuren, the EPIC-France cohort, PI Gianluca Severi, the EPIC-Utrecht, PI Roel Vermeulen, and the EPIC-Norfolk cohort, PI Nick Wareham. We thank the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands, and the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands, for their contribution and ongoing support to the EPIC Study. We are grateful for the continuous participation of our cohort participants—without their commitment, this work would not have been possible. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

The article is supported by Barrie Dagleish Centre for Myeloma and Related Blood Cancers and Leukemia and Lymphoma Society.

TABLE 3 Sensitivities at 95% specificity for top performing proteins by lag-time (selection criteria is mentioned in the methods section) in the EPIC and Janus cohorts.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All EPIC study participants have given written consent for future analyses of their blood samples for research purposes. This study was approved by the International Agency for Research on Cancer (IARC, Lyon, France) Ethics Committee. The use of Janus in this study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics (REC no. 509876). The Janus donors have given a broad consent for the use of the samples in cancer research.

ORCID

Trasias Mukama  <https://orcid.org/0000-0002-6520-5272>

Valeria Pala  <https://orcid.org/0000-0001-5438-970X>

Inger T. Gram  <https://orcid.org/0000-0002-0031-4152>

Rudolf Kaaks  <https://orcid.org/0000-0003-3751-3929>

REFERENCES

- Dellino M, Cerbone M, Lagana AS, et al. Upgrading treatment and molecular diagnosis in endometrial cancer-driving new tools for endometrial preservation? *Int J Mol Sci.* 2023;24(11):9780. doi:10.3390/ijms24119780
- Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin.* 2023;73(1):17-48. doi:10.3322/caac.21763
- Wild, CP, Weiderpass, E, Stewart, B. *World Cancer Report: Cancer Research for Cancer Prevention.* Taylor & Francis Ltd; 2020. <http://publications.iarc.fr/586>.
- Cancer Registry of Norway, Norwegian Institute of Public Health. *Cancer in Norway 2023—Cancer Incidence, Mortality, Survival and Prevalence in Norway.* Cancer Registry of Norway; 2024.
- Clarke MA, Long BJ, Del Mar Morillo A, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of endometrial cancer risk with postmenopausal bleeding in women: a systematic review and meta-analysis. *JAMA Intern Med.* 2018;178(9):1210-1222. doi:10.1001/jamainternmed.2018.2820
- van Hanegem N, Prins MM, Bongers MY, et al. The accuracy of endometrial sampling in women with postmenopausal bleeding: a systematic review and meta-analysis. *Eur J Obstet Gynecol Reprod Biol.* 2016; 197:147-155. doi:10.1016/j.ejogrb.2015.12.008

7. Vitale SG, Riemma G, Haimovich S, et al. Risk of endometrial cancer in asymptomatic postmenopausal women in relation to ultrasonographic endometrial thickness: systematic review and diagnostic test accuracy meta-analysis. *Am J Obstet Gynecol*. 2023;228(1):22-35. doi:10.1016/j.jajog.2022.07.043
8. Baker-Rand H, Kitson SJ. Recent advances in endometrial cancer prevention, early diagnosis and treatment. *Cancer*. 2024;16(5):1028. doi:10.3390/cancers16051028
9. Angioli R, Miranda A, Aloisi A, et al. A critical review on HE4 performance in endometrial cancer: where are we now? *Tumour Biol*. 2014;35(2):881-887. doi:10.1007/s13277-013-1190-4
10. Li J, Wang X, Qu W, Wang J, Jiang SW. Comparison of serum human epididymis protein 4 and CA125 on endometrial cancer detection: a meta-analysis. *Clin Chim Acta*. 2019;488:215-220. doi:10.1016/j.cca.2018.11.011
11. Degez M, Caillon H, Chauvire-Drouard A, et al. Endometrial cancer: a systematic review of HE4, REM and REM-B. *Clin Chim Acta*. 2021;515:27-36. doi:10.1016/j.cca.2020.12.029
12. Bian J, Sun X, Li B, Ming L. Clinical significance of serum HE4, CA125, CA724, and CA19-9 in patients with endometrial cancer. *Technol Cancer Res Treat*. 2017;16(4):435-439. doi:10.1177/1533034616666644
13. Trabert B, Eldridge RC, Pfeiffer RM, et al. Prediagnostic circulating inflammation markers and endometrial cancer risk in the prostate, lung, colorectal and ovarian cancer (PLCO) screening trial. *Int J Cancer*. 2017;140(3):600-610. doi:10.1002/ijc.30478
14. D'Andrilli G, Bovicelli A, Paggi MG, Giordano A. New insights in endometrial carcinogenesis. *J Cell Physiol*. 2012;227(7):2842-2846. doi:10.1002/jcp.24016
15. Romano A, Rizner TL, Werner HMJ, et al. Endometrial cancer diagnostic and prognostic algorithms based on proteomics, metabolomics, and clinical data: a systematic review. *Front Oncol*. 2023;13:1120178. doi:10.3389/fonc.2023.1120178
16. Alblas M, Velt KB, Pashayan N, Widschwendter M, Steyerberg EW, Vergouwe Y. Prediction models for endometrial cancer for the general population or symptomatic women: a systematic review. *Crit Rev Oncol Hematol*. 2018;126:92-99. doi:10.1016/j.critrevonc.2018.03.023
17. Enroth S, Berggrund M, Lycke M, et al. A two-step strategy for identification of plasma protein biomarkers for endometrial and ovarian cancer. *Clin Proteomics*. 2018;15:38. doi:10.1186/s12014-018-9216-y
18. Ura B, Capaci V, Aloisio M, et al. A targeted proteomics approach for screening serum biomarkers observed in the early stage of type I endometrial cancer. *Biomedicine*. 2022;10(8):1857. doi:10.3390/biomedicines10081857
19. Mukama T, Fortner RT, Katzke V, et al. Prospective evaluation of 92 serum protein biomarkers for early detection of ovarian cancer. *Br J Cancer*. 2022;126(9):1301-1309. doi:10.1038/s41416-021-01697-z
20. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 2002;5(6B):1113-1124. doi:10.1079/PHN2002394
21. Hjerkind KV, Gislefoss RE, Tretli S, et al. Cohort profile update: the Janus serum Bank cohort in Norway. *Int J Epidemiol*. 2017;46(4):1101-1102. doi:10.1093/ije/dyw302
22. Langseth H, Gislefoss RE, Martinsen JI, Dillner J, Ursin G. Cohort profile: the Janus serum bank cohort in Norway. *Int J Epidemiol*. 2017;46(2):403-404. doi:10.1093/ije/dyw027
23. Setiawan VW, Yang HP, Pike MC, et al. Type I and II endometrial cancers: have they different risk factors? *J Clin Oncol*. 2013;31(20):2607-2618. doi:10.1200/JCO.2012.48.2596
24. Larsen IK, Smastuen M, Johannesen TB, et al. Data quality at the Cancer Registry of Norway: an overview of comparability, completeness, validity and timeliness. *Eur J Cancer*. 2009;45(7):1218-1231. doi:10.1016/j.ejca.2008.10.037
25. Pepe MS, Fan J, Seymour CW, Li C, Huang Y, Feng Z. Biases introduced by choosing controls to match risk factors of cases in biomarker research. *Clin Chem*. 2012;58(8):1242-1251. doi:10.1373/clinchem.2012.186007
26. Pepe MS, Fan J, Seymour CW. Estimating the receiver operating characteristic curve in studies that match controls to cases on covariates. *Acad Radiol*. 2013;20(7):863-873. doi:10.1016/j.acra.2013.03.004
27. Janes H, Pepe MS. Adjusting for covariates in studies of diagnostic, screening, or prognostic markers: an old concept in a new setting. *Am J Epidemiol*. 2008;168(1):89-97. doi:10.1093/aje/kwn099
28. R Core team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. 2023. <https://www.R-project.org/>.
29. Barr CE, Njoku K, Jones ER, Crosbie EJ. Serum CA125 and HE4 as biomarkers for the detection of endometrial cancer and associated high-risk features. *Diagnostics*. 2022;12(11):2834. doi:10.3390/diagnostics12112834
30. Dossus L, Rinaldi S, Becker S, et al. Obesity, inflammatory markers, and endometrial cancer risk: a prospective case-control study. *Endocr Relat Cancer*. 2010;17(4):1007-1019. doi:10.1677/ERC-10-0053
31. Singh H, Heng S, Nicholls PK, et al. Proprotein convertases in postmenopausal endometrial cancer: distinctive regulation and non-invasive diagnosis. *Biochem Biophys Res Commun*. 2012;419(4):809-814. doi:10.1016/j.bbrc.2012.02.111
32. Ellis PE, Barron GA, Bermano G. Adipocytokines and their relationship to endometrial cancer risk: a systematic review and meta-analysis. *Gynecol Oncol*. 2020;158(2):507-516. doi:10.1016/j.ygyno.2020.05.033
33. Gao Y, Lin P, Lydon JP, Li Q. Conditional abrogation of transforming growth factor-beta receptor 1 in PTEN-inactivated endometrium promotes endometrial cancer progression in mice. *J Pathol*. 2017;243(1):89-99. doi:10.1002/path.4930
34. Rahmanto SY, Shen W, Shi X, et al. Inactivation of Arid1a in the endometrium is associated with endometrioid tumorigenesis through transcriptional reprogramming. *Nat Commun*. 2020;11(1):2717. doi:10.1038/s41467-020-16416-0
35. Joshi A, Ellenson LH. PI3K/PTEN/AKT genetic mouse models of endometrial carcinoma. *Adv Exp Med Biol*. 2017;943:261-273. doi:10.1007/978-3-319-43139-0_9
36. Management of endometrial intraepithelial neoplasia or atypical endometrial hyperplasia: ACOG clinical consensus no. 5. *Obstet Gynecol*. 2023;142(3):735-744. doi:10.1097/aog.0000000000005297
37. Crosbie EJ, Kitson SJ, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial cancer. *Lancet*. 2022;399(10333):1412-1428. doi:10.1016/S0140-6736(22)00323-3
38. Lacey JV Jr, Sherman ME, Rush BB, et al. Absolute risk of endometrial carcinoma during 20-year follow-up among women with endometrial hyperplasia. *J Clin Oncol*. 2010;28(5):788-792. doi:10.1200/JCO.2009.24.1315
39. Terry KL, Schock H, Fortner RT, et al. A prospective evaluation of early detection biomarkers for ovarian cancer in the European EPIC cohort. *Clin Cancer Res*. 2016;22(18):4664-4675. doi:10.1158/1078-0432.CCR-16-0316

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Cooley V, Fortner RT, Mukama T, et al. Prospective evaluation of 92 protein biomarkers for early detection of endometrial cancer. *Int J Cancer*. 2025;157(3):480-489. doi:10.1002/ijc.35428