GYNECOLOGY

Biomarker panel for early detection of endometrial cancer in the Prostate, Lung, Colorectal, and Ovarian cancer screening trial

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BACKGROUND: Endometrial cancer is the most common gynecological cancer in the United States. However, no early detection test exists for asymptomatic women at average risk for endometrial cancer.

OBJECTIVE: We sought to identify early detection biomarkers for endometrial cancer using prediagnostic serum.

STUDY DESIGN: We performed a nested case-control study of postmenopausal women in the Prostate, Lung, Colorectal, and Ovarian cancer screening trial (n = 78,216), including 112 incident endometrial cancer cases and 112 controls. Prediagnostic serum was immunodepleted of high-abundance proteins and digested with sequencing grade porcine trypsin via pressure cycling technology. Quantitative proteomics and phosphoproteomics was performed using high-resolution liquid chromatography—tandem mass spectrometry and highly multiplexed isobaric mass tag combined with basic reversed-phase liquid chromatography. A set of proteins able to predict cancer status was identified with an integrated score assessed by receiver-operator curve analysis.

RESULTS: Mean time from blood draw to endometrial cancer diagnosis was 3.5 years (SD, 1.9 years). There were 47 differentially abundant proteins between cases and controls ($P < .05$). Protein alterations with high predictive potential were selected by regression analysis and compiled into an aggregate score to determine the ability to predict endometrial cancer. An integrated risk score of 6 proteins was directly related to disease incidence in cases with blood draw ≤2 years, >2 years to ≤5 years or >5 years prior to cancer diagnosis. The integrated score distinguished cases from controls with an area under the curve of 0.80 (95% confidence interval, 0.72—0.88).

CONCLUSION: An integrated score of 6 proteins using prediagnostic serum from the Prostate, Lung, Colorectal, and Ovarian cancer screening trial distinguishes postmenopausal endometrial cancer cases from controls. Validation is needed to evaluate whether this test can improve prediction or detection of endometrial cancer among postmenopausal women.

Key words: biomarkers, early detection, endometrial cancer, prediagnostic serum

The American Cancer Society estimates 63,230 new endometrial cancer cases will be diagnosed in 2018 and 11,350 will succumb to their disease.[1] Endometrial cancer is also projected to become the third most common cancer among women in the United States by 2030.[2] Despite being the most common gynecological malignancy, there are currently no early detection screening tests available among asymptomatic women at average risk for endometrial cancer. While 5 year survival for early-stage disease is 69—90%, 5 year survival decreases to 17—58% for stages III—IV and 15% for women whose disease has spread beyond the pelvis.[3]

Although 90% of patients diagnosed with endometrial cancer report abnormal uterine bleeding, only 10% of patients with postmenopausal uterine bleeding have cancer, making it a nonspecific predictor of disease. In a report of 219 endometrial cancer patients, investigators found that the interval from the onset of bleeding symptoms to diagnosis of endometrial cancer was 9.7 weeks and was not associated with stage, histology, or race.[4]

Other meta-analyses of endometrial cancer patients with bleeding have not provided evidence that symptomatic patients present with early-stage disease earlier than those with advanced stage disease or with more aggressive histological.[5] Approximately 25% of women who develop endometrial cancer are diagnosed with endometrial hyperplasia, which can be present 1—20 years prior to cancer diagnosis.[6—8]

This lengthy interval from the development of precursor lesions to the onset of symptoms prompting clinical intervention suggests the need for a screening test for earlier detection of endometrial cancer in asymptomatic patients. The increasing incidence and mortality of endometrial cancer over the past 20 years further suggests the need for this approach, particularly among our underserved populations at higher risk for poor cancer related outcome.

We conducted a nested case-control study using prediagnostic serum from postmenopausal women enrolled in the screening arm of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. The primary objective was to identify serum proteins able to distinguish endometrial cancer cases from controls ≤2 years of diagnosis and to determine whether there was a

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AJOG at a Glance

Why was this study conducted?

Despite being the most common gynecological malignancy, there are currently no early detection screening tests available among asymptomatic women who are at average risk for endometrial cancer. Using prediagnostic serum from both endometrial cancer cases and controls, we sought to investigate for early detection serum biomarkers.

Key findings

A serum biomarker panel containing 6 proteins (complement factor B, serotransferrin, catalase, proteasome subunit beta type 6, beta-2-microglobulin, and protocadherin-18) was able differentiate endometrial cancers from controls within 2 years of cancer diagnosis using serum from patients enrolled in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.

What does this add to what is known?

The results of this study are preliminary and require further validation to determine whether these biomarkers can be used clinically to predict or detect endometrial cancer among postmenopausal women.

temporal relationship in abundance of these proteins among cases as time of cancer diagnosis approached.

Materials and Methods

We performed a nested case-control study within the screening arm of the PLCO Cancer Screening Trial.[9] Briefly, between 1993 and 2001, 78,216 women aged 55 to 74 years were recruited from 10 screening centers across the United States. Participants were randomized to either a screening or nonscreening arm; participants randomized to the screening arm provided serum samples upon enrollment and at 5 subsequent medical examinations.

Incident cancers were determined by participant self-report and confirmed by review of the participant's medical record and pathology report. All participants provided written informed consent and the PLCO Cancer Screening Trial was approved by the Institutional Review Board of the National Cancer Institute.

We evaluated postmenopausal women randomized to the screening arm of the PLCO Cancer Screening Trial who met the following inclusion criteria: intact uterus, available serum sample, no previous cancer diagnosis, and completion of an intake questionnaire. Participants also had to provide written consent allowing biochemical study of their serum.

Endometrial cancer cases were defined as having a primary diagnosis of an invasive epithelial tumor of the uterus between the initial screening visit and Jan. 1, 2010. The participants with a preexisting malignancy at enrollment or a mesenchymal uterine tumor were excluded.

Controls were matched 1:1 based on age, race, study site, year of blood draw, and year of randomization. Controls were restricted to women with no history of a hysterectomy and were required to be alive at the time of diagnosis of their matched case.

Serum samples from 552 participants were provided by the PLCO central bank and were stored at −80°C until use. No more than 2 freeze-thaw cycles were allowed for each sample. Samples were blinded and randomized for processing and analysis. Serum samples were thawed on ice and highly abundant proteins were immunodepleted using Multiple Affinity Removal Human-14 Spin Cartridge (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. The unbound protein fraction was eluted and buffer exchanged with 100 μL × 6 of 100 mM triethylammonium bicarbonate in a Microcon-10 (MilliporeSigma, Burlington, MA) spin-column filter.

A total of 22.5 μL of protein retentate was then combined with 2.5 μL of 100% acetonitrile. Samples were then transferred to 0.5 mL MicroTubes (Pressure Biosciences, Easton, MA) and incubated at 99°C for 30 minutes, after which they were cooled to ambient temperature and 1 μL of SMART Digest Trypsin (Thermo Scientific, Waltham, MA) was added to each.

The MicroTubes were capped with MicroPestles (Pressure Biosciences) and digestion was performed in a Barocycler 2320EXT (Pressure BioSciences) by cycling between 45 kpsi for 50 seconds and atmospheric pressure for 10 sconds for 60 cycles at 50°C. Peptide digests were dried by vacuum centrifugation, resuspended in 100 mM triethylammonium bicarbonate, and quantified using the Pierce BCA protein assay kit (ThermoFisher Scientific).

Equivalent amounts of peptide were labeled with tandem-mass tag (TMT) isobaric labels (TMT11plex Isobaric Label Reagent Set; ThermoFisher Scientific) as per the manufacturer's recommendations. TMT-labeled plexes were fractionated using high-pH, reversed-phase liquid chromatography (1260 Infinity II; Agilent) into 96 fractions through the development of a linear gradient of acetonitrile (0.69% minutes). The 96 fractions were concatenated into 12 in a serpentine manner.

The TMT-labeled peptide digest fractions were analyzed by nanoflow liquid chromatography–tandem mass spectrometry using a nanoflow LC system (EASY-nLC 1200; ThermoFisher Scientific) coupled online with a tribrid Orbitrap MS (Fusion Lumos; Thermo Fisher Scientific). Peptide identifications were generated by searching raw liquid chromatography–mass spectrometry data files with a publicly available, nonredundant human proteome database (Swiss-Prot; *Homo sapiens* [http://www.uniprot.org/]) appended with porcine trypsin (Uniprot: P00761) with Mascot (Matrix Science, Boston, MA) and Proteome Discoverer (PD).

The resulting peptide spectral matches (PSMs) were filtered using a false-discovery rate <1% (q value <0.01), as determined by the Percolator module of the PD. TMT reporter ion intensities were extracted using PD at a mass tolerance of 20 ppm, and PSMs

lacking a TMT reporter ion signal in the pooled study reference channel, lacking TMT reporter ion intensity in all TMT channels, or PSMs exhibiting an isolation interference of ≥50% were excluded from downstream analyses.

The abundance of proteins identified by unique PSMs were determined by calculating the median log2 abundance ratios of all PSMs corresponding to a unique protein accession. The abundance of PSMs mapping to multiple proteins were compared with the corresponding unique protein abundances using a mean squared error approach to enable their assignment to unique proteins based on comparative abundance analyses.

Protein-level abundance was calculated from normalized, median log2-transformed TMT reporter ion ratio abundances from a minimum of 2 PSMs corresponding to a single protein accession. Normalized log2-transformed protein-level abundance for each TMT-11 multiplex was merged and protein-level abundance for proteins not quantified in all patient samples, but in at least ≥50%, were imputed using a k-nearest neighbor strategy in the pamr prediction analysis for microarrays R-package.

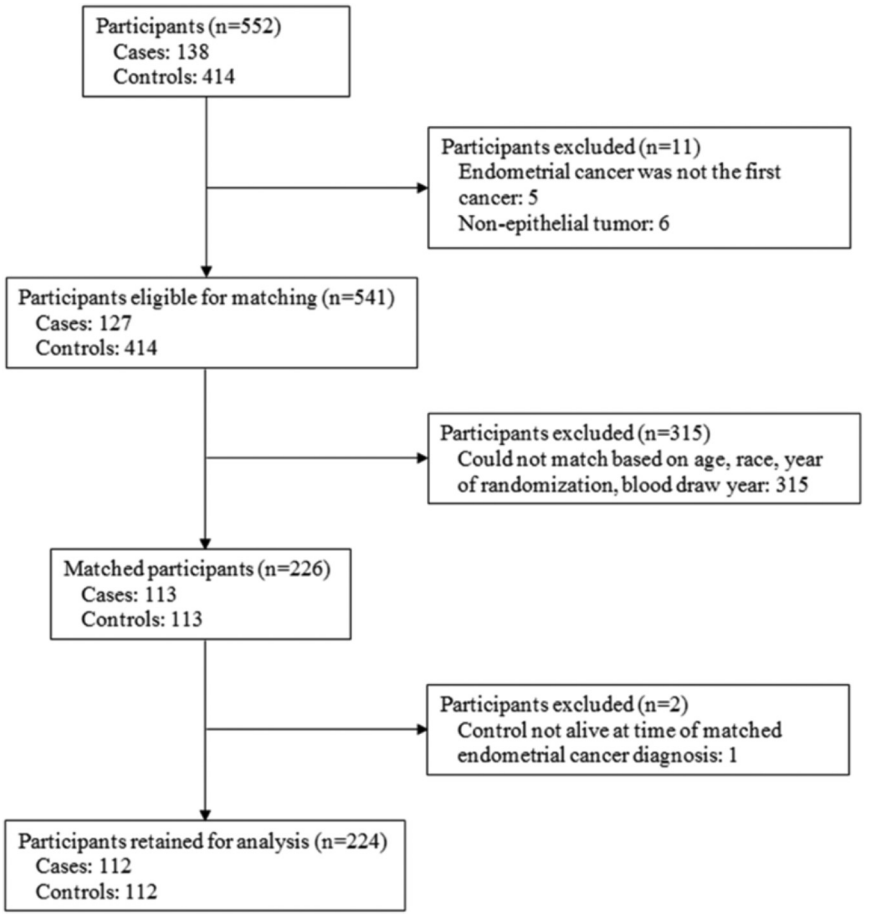
Differences in baseline demographics were determined by χ^2. Cases were separated into 3 groups based on time of blood draw prior to endometrial cancer diagnosis: case group 1 (≤2 years), case group 2 (between 2 years and ≤5 years), case group 3 (>5 years). We assumed early detection serum biomarkers are a subset of the differentially abundant proteins between case group 1 and matched controls.

The differentially abundant proteins for case group 1 vs matched controls were identified using the linear models for microarray data (LIMMA) method implemented in the *limma* bioconductor package (version 3.28.21) with $P < .05$. Proteins significantly altered in case group 1 and matched controls exhibiting identical fold-change trends in comparative analyses of cases and controls (ie, case group 2 vs matched controls, case group 3 vs matched controls, case group 1 vs case group 2, and case group 2 vs case group 3) were prioritized for integrated risk score analyses. Furthermore, prioritized protein abundance alterations were assessed relative to serum albumin protein abundance across cohorts to confirm that alteration trends were not confounded by differential efficiency of immunodepletion column performance.

The least absolute shrinkage and selection operator (LASSO) regression analysis was applied to prioritized protein alterations using the *glmnet* R package (version 2.0-5) and by further selecting the optimal tuning parameter with the lowest mean squared error by running 10-fold cross-validation 100 times.

This analysis yielded a predictive set of proteins highly associated with cancer status, and this integrated score was calculated for each sample by summing the relative abundance of the selected proteins weighted by their respective LASSO coefficients. The performance of the integrated score was evaluated using the receiver-operator curve (ROC) analysis (ROCR package, version 1.0-7, and pROC package, version 1.9.1 in R) among case group 1 and all controls.

FIGURE 1
Study selection process



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Results

There were 112 cases and 112 matched controls included in the analysis (Figure 1). Demographics were compared between cases and controls (Table 1). Mean age for cases was 62.3 years (SD, 5.0) and 62.1 years (SD, 4.8) for controls. Mean time from initial blood draw to endometrial cancer diagnosis was 3.5 years (SD, 1.9).

Of the 224 patients analyzed, 212 (94.6%) were white. Cases were more

TABLE 1
Demographic characteristics of endometrial cancer cases and matched controls

Characteristic	Cases (n = 112)	Controls (n = 112)	P[a]
Age, y (at randomization)[b]			.903
<60	35 (31.3)	34 (30.4)	
60–64	41 (36.6)	45 (40.2)	
65–69	27 (24.1)	23 (20.5)	
≥70	9 (8.0)	10 (8.9)	
Race[b]			1.000
White, non-Hispanic	106 (94.6)	106 (94.6)	
Black, non-Hispanic	2 (1.8)	2 (1.8)	
Asian	4 (3.6)	4 (3.6)	
Body mass index, kg/m^2			.002
<25	37 (33.0)	57 (50.9)	
≥25–29.9	31 (27.7)	34 (30.4)	
≥30	44 (39.3)	21 (18.8)	
Number of live births			.518
0	16 (14.3)	12 (10.7)	
1–2	33 (29.5)	40 (35.7)	
≥3	63 (56.3)	60 (53.6)	
Highest education level attained			.149
Less than high school	1 (0.9)	0 (0.0)	
Some high school	3 (2.7)	7 (6.3)	
Completed high school	18 (16.1)	33 (29.5)	
Post high school, not college	13 (11.6)	11 (9.8)	
Some college	34 (30.4)	26 (23.2)	
College graduate	24 (21.4)	17 (15.2)	
Postgraduate	19 (17.0)	18 (16.1)	
Menopausal hormone use			.435
Current use	52 (46.4)	49 (43.8)	
Former use	17 (15.2)	22 (19.6)	
Never use	41 (36.6)	41 (36.6)	
Unsure	2 (1.8)	0 (0.0)	
Smoking status			.013
Never	69 (61.6)	56 (50.0)	
Current	5 (4.5)	18 (16.1)	
Former	38 (33.9)	38 (33.9)	

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likely to be obese ($P = .002$) and less likely to have used oral contraceptives ($P = .022$). Controls were more likely to have been current or former smokers ($P = .013$). There were no differences between groups among other known risk or protective factors for endometrial cancer.

Among cases, 97 (86.6%) were endometrioid endometrial cancer. There were 5 (serous 4.5%), 4 carcinosarcomas (3.6%), 3 mucinous (2.7%), 2 clear cell (1.8%), and 1 mixed tumor (0.9%). Among the endometrioid tumors, 52 (53.6%) were grade 1, 32 (33.0%) were grade 2, 5 (5.2%) were grade 3, and 8 (8.2%) were missing grade data. Thirteen cases (11.6%) were deceased at the time of last contact. Stage data were available for only 24 (21.4%) cases (20 stage IA, 3 stage IB, and 1 stage IIIC1).

Quantitative proteomic analyses resulted in the identification of 1100 total proteins, 565 of which were coquantified across all patient samples (Supplemental Table 1). Endometrial cancer cases were then separated into 3 groups based on time of blood draw prior to an endometrial cancer diagnosis. There were 31 cases in case group 1 (diagnosed <2 years from blood draw), 52 cases in case group 2 (diagnosed within >2 but <5 years), and 29 cases in case group 3 (diagnosed >5 years).

Forty-seven proteins were identified as significantly altered (LIMMA value of $P < .05$) between case group 1 and their matched controls. Ten proteins were further prioritized because they exhibited consistent abundance alterations in cases compared with controls, regardless of time of sampling prior to endometrial cancer diagnosis (Table 2).

We then used a LASSO regression analysis on this set of 10 prioritized, case vs the control protein alterations, resulting in the selection of 6 proteins: complement factor B, serotransferrin, catalase, proteasome subunit beta type-6, beta-2-microglobulin, and protocadherin-18 (Figure 2).

The abundance trends for these 6 proteins were then assembled into an aggregate score to predict the relative risk of having endometrial cancer (Figure 3). The integrated diagnostic score per sample was calculated using the following equation: (1.823 × complement factor B) − (0.011 × serotransferrin) + (0.123 × catalase) + (0.827 × proteasome subunit beta type-6) − (0.253 × beta-2-microglobulin) − (0.417 × protocadherin-18).

TABLE 1
Demographic characteristics of endometrial cancer cases and matched controls *(continued)*

Characteristic	Cases (n = 112)	Controls (n = 112)	P[a]
Oral contraceptive use	42 (37.5)	59 (52.7)	.022
Family history of endometrial cancer	1 (0.9)	1 (0.9)	1.000
History of hypertension	45 (40.2)	33 (29.5)	.092
History of diabetes	9 (8.0)	7 (6.3)	.604

All data are n (percentage).

[a] P values are based on χ^2 statistics; [b] Matching factors. Other matching factors not included in the table are study center, year of randomization, and year of blood draw; there was no statistical difference in distribution between cases and controls.

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The performance of the integrated diagnostic score was evaluated using ROC analysis among case group 1 and all controls (Figure 4). The area under the curve (AUC) of the performance in this cohort was 0.80 [95% confidence interval, 0.72−0.88]. A cutoff of 0.5 for this score provided 45.2% sensitivity, 96.4% specificity, and a negative- and positive-predictive value of 86.4% and 77.8%, respectively.

We also evaluated the integrated diagnostic score stratified by body mass index (BMI): BMI $<$ 25 kg/m^2, BMI 25−29 kg/m^2, and BMI $\geq$ 30 kg/m^2. The discriminative capability was supported in all 3 subgroups (AUC, 0.70, 0.89, and 0.89, respectively; $P < .05$). Moreover, there was better performance of this integrated risk score among women with a BMI of 25−29 kg/m^2 or a BMI of $\geq$30 kg/m^2. Overall, there was a slight increase in the predictive accuracy based on integrated score alone compared with using an integrated score with BMI (AUC, 0.80 vs 0.82, $P < .05$).

Comment

Principal findings

In this case-control study nested within the PLCO Cancer Screening Trial, we analyzed prediagnostic serum from postmenopausal women to investigate for biomarkers that could aid in prediction or detection of endometrial cancer. Forty-seven proteins were significantly altered between cases and controls $\leq$2 years of diagnosis.

A predictive protein set of 6 proteins (complement factor B, serotransferrin, catalase, proteasome subunit beta type-6, beta-2-microglobulin, and protocadherin-18) distinguished cases from controls with an ROC analysis showing an AUC of 0.80 among patients with blood draw $\leq$2 years from cancer diagnosis.

Results

The results of this study are consistent with previous publications. The most highly abundant proteins among cases $\leq$2 years of diagnosis corresponded to different subunits of the cellular proteasome. Chymotrypsin-like activity of proteasomes from multiple types of cancers is higher among tumor compared with normal tissue with the largest proteasome activity seen within endometrial cancer.[10]

Additionally, the abundance of serotransferrin and apolipoprotein A-1 were both lower among cases $\leq$2 years of diagnosis compared with controls, which is consistent with previous investigation that focused on validating serum biomarkers for early detection of endometrial cancer.[11]

This study also provides clarification in which there are conflicting data in the literature. The excess production of reactive oxygen species or inadequate antioxidant protection can lead to oxidative stress, believed to play a role in carcinogenesis.[12] Catalase is an important enzyme that protects the cell from oxidative stress, but there are conflicting data on whether catalase activity in endometrial cancer differs from benign endometrium.[12,13] Although our study did not investigate tissue, we show that serum levels of catalase was 19% higher among cases.

There are also conflicting data on whether human serum amyloid A (SAA) is higher among endometrial cancer patients. Cocco et al,[14,15] using a bead-based immunoassay, showed higher concentrations of serum SAA among endometrioid and serous tumors compared with controls. They also demonstrated higher SAA levels among grade 3 compared with grade 1 or 2 endometrioid tumors.[15] However, another study showed no difference in serum SAA levels among endometrioid cancer vs healthy patients.[16] Our investigation demonstrated no difference in SAA concentration between cases or controls $\leq$2 years of diagnosis (logFC [−0.25], FC = 0.841, $P = .189$).

Our findings differ significantly with previous studies that have examined serum from endometrial cancer patients and controls.[17−24] Previous reports have shown serum concentrations of DJ-1, visfatin, OVX1, sperm-associated antigen 9, and circulating soluble Fas are higher among endometrial cancer cases vs controls[15,23,25,26]; however, our study did not detect these proteins.

Preoperative serum levels of YKL-40 have been shown to be higher among endometrial cancer cases compared with controls,[21,22] but our analysis suggested no difference in the abundance of YKL-40 among cases $\leq$2 years of diagnosis and controls (logFC [0.26], FC, 1.20, $P = .173$).

Moreover, Trabert et al[18] also analyzed serum from both endometrial cases and controls from the PLCO Cancer Screening Trial in which they measured serum levels of 64 inflammation-related biomarkers, with 22 of these markers being associated with endometrial cancer risk. None of the biomarkers seen in this study are consistent with our findings; however, this study used immunoassays to evaluate the levels of targeted inflammation-related biomarkers, and their analysis did not stratify cases by the timing of the blood draw prior to cancer diagnosis.[18]

There are numerous reasons that our investigation did not identify previously

TABLE 2
Significantly altered proteins among cases vs controls ≤2 years to diagnosis

Serum protein	Gene	logFC	FC	*P*[a]
Hornerin	HRNR	0.78	1.72	.036
Cystatin-A	CSTA	0.74	1.67	.042
Proteasome subunit beta type-4[b]	PSMB4	0.70	1.62	< .001
Gamma-glutamylcyclotransferase	GGCT	0.64	1.56	.011
Zymogen granule protein 16	ZG16B	0.52	1.43	.042
Keratinocyte proline-rich protein	KPRP	0.50	1.41	.032
Serine protease inhibitor Kazal type	SPINK5	0.49	1.40	.029
Myoglobin OS	MB	0.44	1.36	.033
Leukocyte elastase inhibitor	SERPINB1	0.38	1.31	.021
Proteasome subunit beta type-6[b]	PSMB6	0.34	1.27	.019
Desmocollin-3	DSC3	0.34	1.27	.049
Proteasome subunit alpha type-6	PSMA6	0.30	1.23	.022
Leucine-rich alpha-2-glycoprotein	LRG1	0.29	1.22	0.014
Contactin-1	CNTN1	0.27	1.21	0.021
Catalase[b]	CAT	0.25	1.19	0.046
Glutathione synthetase	GSS	0.23	1.18	0.021
Hepatocyte growth factor receptor	MET	0.23	1.18	0.010
Laminin subunit beta-1	LAMB1	0.23	1.17	0.021
Proteasome subunit alpha type 7[b]	PSMA7	0.23	1.17	0.049
Insulin-like growth factor-binding protein 4	IGFBP4	0.21	1.16	.031
Aspartate aminotransferase	GOT1	0.21	1.15	.044
Complement factor B[b]	CFB	0.19	1.14	.027
Exostosin-2[b]	EXT2	0.17	1.13	.047
Coagulation factor XIII B chain[b]	F13B	−0.19	0.88	.024
Out at first protein homolog	OAF	−0.20	0.87	.009
Apolipoprotein B-100	APOB	−0.22	0.86	.042
Phosphatidylinositol glycan−specific phospholipase D	GPLD1	−0.25	0.84	.021
Protocadherin-18[b]	PCDH18	−0.26	0.84	.014
Apolipoprotein E	APOE	−0.29	0.82	.029
Protein Z-dependent protease inhibitor	SERPINA10	−0.30	0.82	.006
Clusterin	CLU	−0.31	0.81	< .001
Alpha-mannosidase	MAN2A2	−0.31	0.81	.002
Complement factor H-related protein 5	CFHR5	−0.36	0.78	.037
Beta-2-microglobulin[b]	B2M	−0.38	0.77	.050
Apolipoprotein	APOL1	−0.39	0.76	.019
Apolipoprotein M	APOM	−0.42	0.75	.046
Coagulation factor XIII A chain	F13A1	−0.43	0.74	.002
Apolipoprotein A-IV	APOA4	−0.44	0.74	.005
C4b-binding protein alpha chain	C4BPA	−0.49	0.71	.016

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(continued)

TABLE 2
Significantly altered proteins among cases vs controls ≤2 years to diagnosis *(continued)*

Serum protein	Gene	logFC	FC	P[a]
Cathelicidin antimicrobial peptide	CAMP	−0.55	0.68	.024
Apolipoprotein A-I	APOA1	−0.60	0.66	.033
Ficolin-2	FCN2	−0.62	0.65	.039
Apolipoprotein C-IV	APOC4	−0.62	0.65	.005
Serotransferrin[b]	TF	−0.84	0.56	.005
Apolipoprotein C-III	APOC3	−0.93	0.52	.004
Apolipoprotein C-I	APOC1	−0.95	0.52	.009
Apolipoprotein C-II	APOC2	−1.22	0.43	.005

logFC, logarithmic fold change; *FC*, fold change.

[a] Paired LIMMA was adjusted by serum albumin protein abundance; [b] Ten proteins were significantly different between cases and controls ≤2 years of diagnosis and also showing the same rate of change in concentration of protein over 3 time categories.

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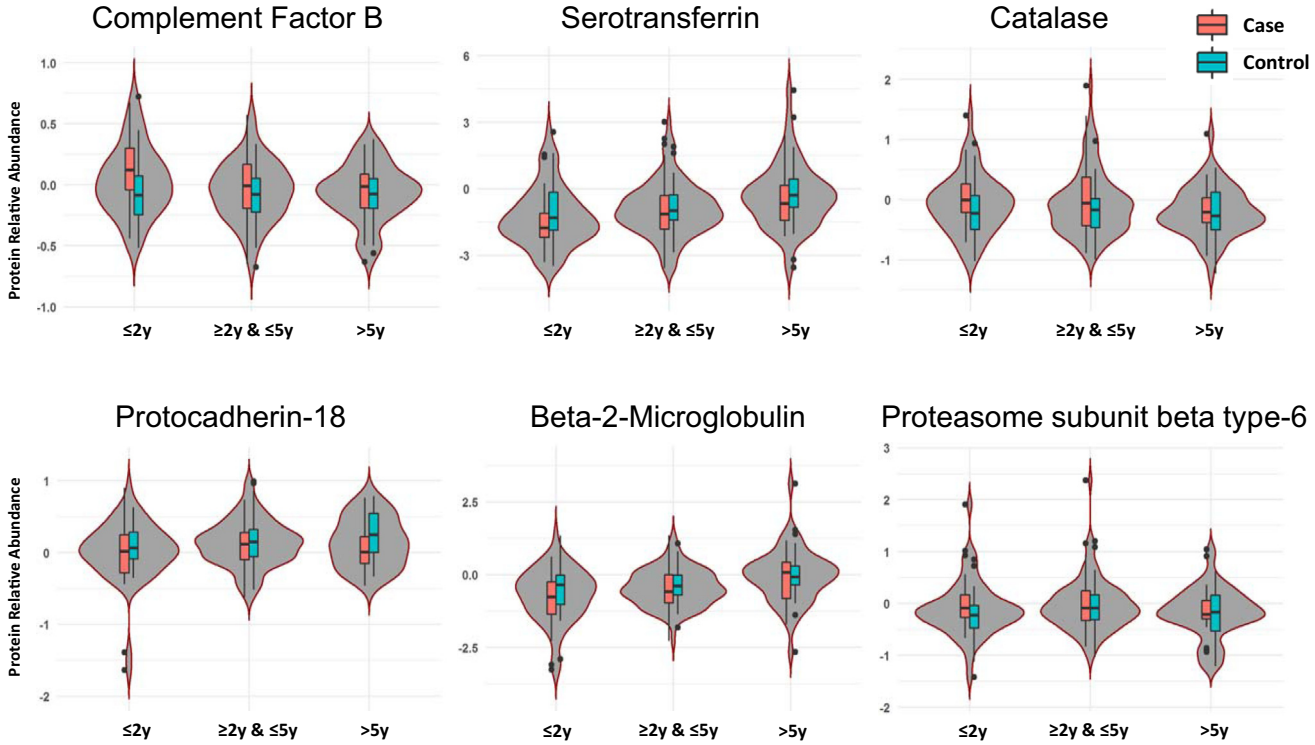
FIGURE 2
Prioritization of protein alterations with high diagnostic potential



Among cases in which the blood draw occurred ≤2 years of endometrial cancer diagnosis, there are 47 proteins significantly altered between cases and controls ($P < .05$). Comparative analyses of these abundance trends for cases diagnosed >2 years to ≤5 years with matched controls and >5 years with matched controls resulted in the prioritization of 10 protein candidates reflecting identical abundance trends as observed in cases diagnosed within 2 years from blood draw. Six high-confidence candidates were selected from the 10 by LASSO regression analysis; figure depicts case vs control abundance trends for these 6 candidates in patients diagnosed with endometrial cancer <2 years, >2 years to ≤5 years, or >5 years from initial blood draw.

LASSO, least absolute shrinkage and selection operator.

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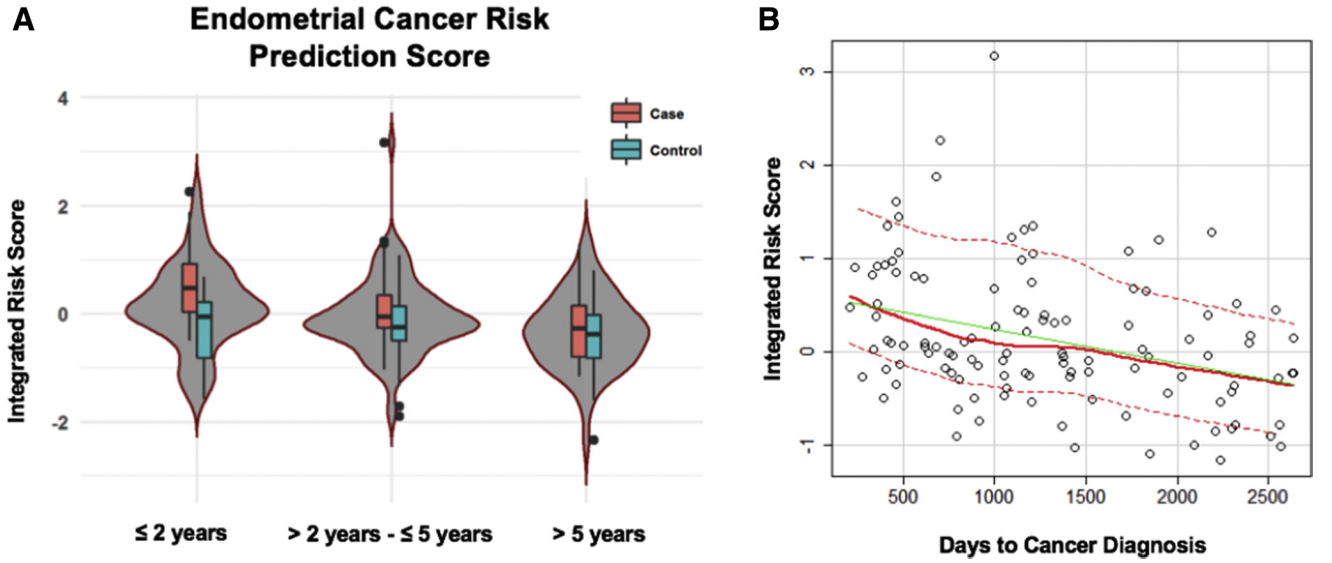
FIGURE 3
A serum-based, integrated risk score to predict endometrial cancer



Protein alterations with high diagnostic potential were selected by LASSO regression analysis and assembled into an aggregate score calculated for each case to predict the relative risk of having endometrial cancer. **A,** Integrated risk scores are directly related to disease incidence in patients with blood draw ≤2 years, >2 years to ≤5 years, or >5 years prior to endometrial cancer diagnosis. **B,** Integrated risk scores exhibit a linear relationship from time of blood draw to receiving a diagnosis of endometrial cancer.

LASSO, least absolute shrinkage and selection operator.

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published biomarkers in endometrial cancer. First, we conducted an exploratory global proteomic analysis of prediagnostic serum, whereas previous investigations performed targeted analyses for specific biomarkers using immunoassay approaches on serum drawn from patients at the time of diagnosis.[17-24]

Additionally, protein abundance, serum depletion, and the dynamic range in serum also likely played a role. Depending on the protein, there can be several factors that also might not make them amenable to liquid chromatography—mass spectrometry. Also, the abundance of these proteins within the individual samples that comprise each plex may have an impact on how well they are represented in the pool and therefore may not be selected during the tandem mass spectrometry process. In short, the levels measured from an enzyme-linked immunosorbent assay or other assay may not necessarily reflect the detectability by a global proteomics approach.

Clinical implications

There is currently no available screening test for asymptomatic women at average risk for endometrial cancer. CA125 and CA 15-3 have been evaluated as potential screening biomarkers for endometrial cancer. CA 15-3 levels, however, are found to be elevated in only 36% of endometrial cancer patients, and CA125 has been shown to be elevated in 19—40% of endometrial cancer patients.[27,28] Moreover, increased levels of CA125 may also be related to inflammatory conditions not related to endometrial cancer. Identifying biomarkers that can be used for early detection of endometrial cancer may lead to improvements in practice and outcomes.

Research implications

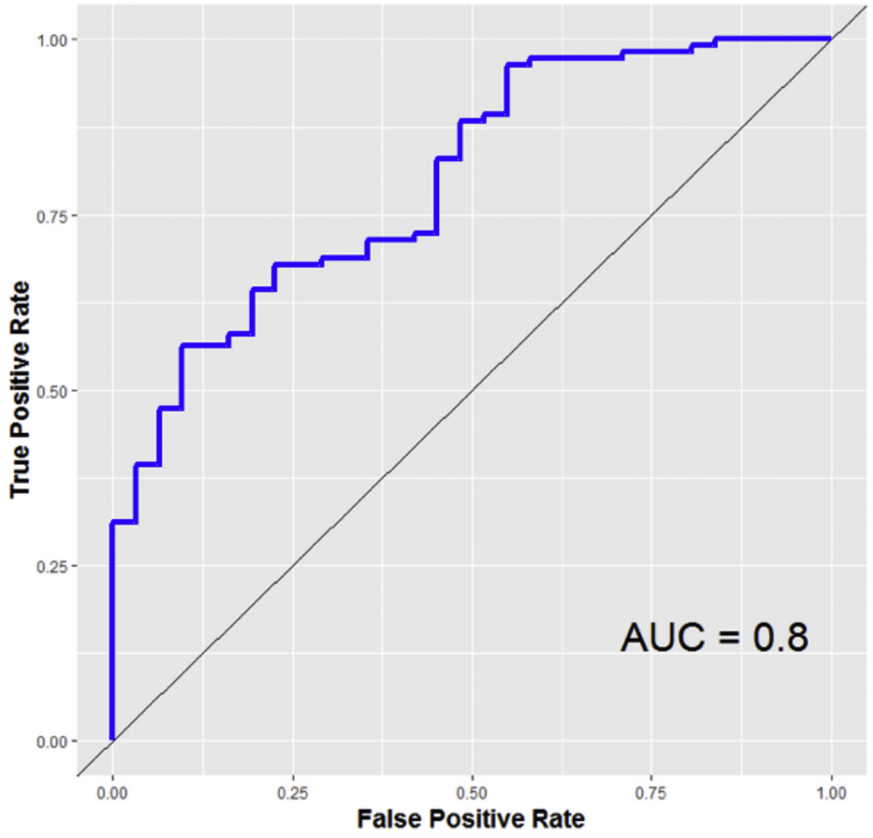
Our exploratory proteomic investigation of prediagnostic serum from the PLCO Cancer Screening Trial revealed a biomarker panel of 6 proteins able to differentiate cases from controls. Our next step is to confirm these findings in a separate cohort. Validation of these findings with an independent prediagnostic serum set would provide justification to evaluate this panel in a clinical setting.

Strengths and limitations

Strengths of this study include that the PLCO Cancer Screening Trial prospectively collected serum samples prior to endometrial cancer diagnosis with uniform collection and storage protocols. Reported cases in the PLCO Cancer Screening Trial were confirmed by a review of medical records and pathology reports. The population of the PLCO Cancer Screening Trial is geographically diverse with follow-up data spanning 2 decades.

Limitations of this study include that 94.6% of the study population was white, limiting generalizability to other racial groups. Additionally, 86.6% of cases consisted of endometrioid histology. We had stage data for only 21.4% of cases and had minimal information on the depth of myometrial invasion and lymphovascular space invasion, preventing

FIGURE 4
Evaluation of serum-based, integrated risk score to predict endometrial cancer



Receiver-operator curve evaluating integrated score for 6 high-confidence proteins (complement factor B, serotransferrin, catalase, proteasome subunit beta type-6, beta-2-microglobulin, and protocadherin-18) among case group 1 and all controls. The ROC score predicted a diagnosis of endometrial cancer in patients diagnosed in ≤2 years from initial blood draw with an AUC of 0.8. Performance of this risk score for endometrial cancer cases diagnosed >2 years to ≤5 years exhibited an AUC of 0.64 and for cases diagnosed >5 years following blood draw, an AUC of 0.51.

AUC, area under the cxurve; *ROC*, receiver-operator curve.

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us from evaluating intermediate-risk cases. Lastly, this was an exploratory analysis of prediagnostic serum and requires further validation before definitive conclusions may be drawn.

Conclusions

This prospective analysis of prediagnostic serum from participants of the PLCO Cancer Screening Trial introduces a new biomarker panel that can differentiate endometrial cancer cases from controls ≤2 years from diagnosis. There is also a trend in the change in concentration of these proteins beginning at >5 years prior of diagnosis, warranting further investigation to determine the possible utility as an early screening test for endometrial cancer. The results of this exploratory study are preliminary and require validation in an independent prediagnostic serum set to determine whether this biomarker panel may be investigated in a clinical setting. ■

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