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Development of multimarker panel for early detection of endometrial cancer. High diagnostic power of prolactin

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Abstract

Objective—Endometrial carcinoma is the most common gynecologic cancer. Although the prognosis for endometrial cancer is generally good, cancers identified at late stages are associated with high levels of morbidity and mortality. Therefore, prevention and early detection may further reduce the burden of this challenging disease.

Methods—A panel of 64 serum biomarkers was analyzed in sera of patients with stages I-III endometrial cancer and age-matched healthy women, utilizing a multiplex xMAP™ bead-based immunoassay. For multivariate analysis, four different statistical classification methods were used: logistic regression (LR), separating hyperplane (SHP), k nearest neighbors (KNN), and Classification Tree (CART). For each of these classifiers a diagnostic model was created, based on the cross-validation set consisting of sera from 115 patients with endometrial cancer and 135 healthy women.

Results—Our data have demonstrated that patients with endometrial cancer have significantly different expression patterns of several serum biomarkers as compared to healthy controls. Prolactin was the strongest discriminative biomarker for endometrial cancer providing 98.3% sensitivity and 98.0% specificity alone. Our results have revealed that serum concentration of cancer antigens, including CA 125, CA 15-3, and CEA are higher in patients with Stage III endometrial cancer as compared to those with Stage I. In addition, we have shown that the expression of CA 125, AFP and ACTH is elevated in women with tumor grade 3 vs. grade 1. Furthermore, 5-biomarker panel

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(prolactin, GH, Eotaxin, E-selectin, and TSH) identified in this study, was able to discriminate endometrial cancer from ovarian and breast cancers with high sensitivity and specificity.

Conclusions—The ability of prolactin to accurately discriminate between cancer and control groups indicates that this biomarker could potentially be used for development of blood-based test for the early detection of endometrial cancer in high-risk populations. Combining the information on multiple serum markers using flexible statistical methods allows for achieving high cancer selectivity.

Keywords

endometrial cancer; cancer markers; early detection; multiplex profiling; prolactin

Introduction

Endometrial adenocarcinoma is the most common malignant neoplasm of the female genital tract. Despite the advances that have been made in other cancers, both annual incidence of and death rate associated with endometrial cancer appear to be rising [1]. In the United States, approximately 41,200 cases are diagnosed and about 7,350 women die from the disease each year [2]. Endometrial cancer, which originates from the lining of the uterus, accounts for approximately 90% of uterine cancers. The incidence of endometrial cancer in women in the U.S. is 2–3%. According to the American Cancer Society, the incidence peaks between the ages of 60 and 70 years, but 10–25% of cases may occur before the age of 50 [2]. Increased risk of developing endometrial cancer has been noted in women who take tamoxifen and women with genetic susceptibility to the disease [3]. Continuing challenges of endometrial cancer treatment include the need to improve screening and prevention efforts. The 5-year survival for early stage localized endometrial cancer is 75–95% however prognosis is poor for cancers found at stages III–IV. Five-year survival rate falls to 66% if cancer has spread regionally at the time of diagnosis. For women with disease that has spread beyond pelvis (stage IV) survival is less than 20% [2].

At present, there are no early detection tests for endometrial cancer in women without symptoms who are at average endometrial cancer risk. The Pap test, which is very effective for early detection of cervical cancer, can find some early endometrial cancers, but most cases are not found by this test [2]. Results from pelvic examination are frequently normal, especially in the early stages of disease, thus limiting the ability to identify early disease. Changes in size, shape or consistency of the uterus and/or its surrounding supporting structures may exist when the disease is more advanced. Although not recommended as a general screening test, the American Cancer Society does advocate endometrial sampling or biopsy in high-risk women at the time of menopause [4].

Several biomarkers have shown association with endometrial cancer. For example, p53, hypoxia-inducible factor 1 α (HIF-1 α , HIF-2 α), Ki-67, VEGF, EGFR, and HER2/neu correlate with development or progression of endometrial cancer [5]. Elevated levels of CA 125, CA 15-3 and CA 19-9 are significantly associated with shorter survival time in endometrial cancer patients [6]. CA 125 correlates with tumor size and stage of endometrial cancer [7–9] and is also a significant independent predictor of the extrauterine spread of disease [10]. At present, no serum biomarkers are available for screening for endometrial carcinoma or for monitoring recurrence in endometrial carcinoma survivors. Patients with recurrent disease are detected only following the development of symptoms or abnormalities in imaging assessments [11].

In this study, we examined multiple cancer-associated serum proteins in order to identify those with high association with endometrial cancer as potential biomarkers for screening or disease monitoring.

Materials and Methods

Patients

Serum samples from 115 women with stage I–III endometrial cancer and 135 age-matched healthy individuals were collected and provided by Dr. Karen Lu (Table 1). Sera were annotated with information regarding gynecologic diagnosis, endometrial cancer stage, grade and patient's age (Table 1). Serum samples from women with ovarian (N=70), breast (N=91) cancers and matched healthy controls were provided by Gynecologic Oncology Group (Cleveland Clinic, Cleveland, OH), Dr. Jeffrey Marks (Duke University, Durham, SC), and Dr. Jill Siegfried (UPCI, Pittsburgh, PA). Written informed consent was obtained from each subject or from her guardian. The local institutional review boards approved the appropriate protocols for each sample collection procedure.

Collection and storage of blood serum

Blood processing was similar for all samples collected at the contributing centers. Samples were obtained from cancer patients prior to surgery and before administration of anesthesia. Ten ml of peripheral blood was drawn using standardized phlebotomy procedures. Blood samples were collected without anticoagulant and allowed to coagulate for up to 2 hrs at room temperature. Sera were separated by centrifugation, immediately aliquoted, frozen and stored at -80°C . No more than 2 freeze-thaw cycles were allowed for any sample.

Multiplex bead-based immunoassay

The xMAP™ bead-based technology (Luminex Corp., Austin, TX) permits simultaneous analysis of numerous analytes in one sample. Sixty-four bead-based xMAP™ immunoassays for most known or potential endometrial cancer serum biomarkers were utilized in this study (Table 2). Assays for CA 15-3, CA 19-9, CA 125, CEA, CA 72-4, AFP, ErbB2, EGFR, human kallikreins (hK) 8,10, Cyfra 21-1, IGFBP1, S100, mesothelin, SCC, angiostatin, serum amyloid A (SAA), MHC class I chain-related gene A (MICA), and transthyretin (TTR) were developed in the UPCI Luminex Core Facility [12]. The inter-assay variability of each assay was 5–16%. Intra-assay variability was 2–11%. Each bead-based assay was validated in comparison with appropriate standard ELISA based on the same pair of capture and detection antibody and has demonstrated 97–99% correlation [12]. Assays for Eotaxin, IL-6R, IL-2R, and TNFR1 were purchased from Invitrogen (Camarillo, CA), assays for MMPs were from R&D Systems (Minneapolis, MN), remaining assays were obtained from Millipore/Linco Research (St. Charles, MO). Overall, 11 different multiplexed panels were used.

Multiplex analysis

Assays were performed according to manufacturers' protocols. Luminex Core assays were performed as previously described [13]. Samples were analyzed using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA). For each analyte, 100 beads were analyzed and means were calculated. The concentrations of analytes were quantitated using standard curve that was generated using Bio-Rad five-parametric curve fitting [14] to the series of known concentration of analytes.

Univariate analysis of data

The Kruskal-Wallis One Way Analysis of Variance on Ranks was used to evaluate the significance of differences in marker expression between each disease state. All Pairwise Multiple Comparison Procedures (Dunn's Method) was also incorporated to quantify the relationships between groups for each marker. The level of significance was taken as $P<0.05$.

Multivariate analyses

Four different statistical classification methods were used, logistic regression (LR) [15], separating hyperplane (SHP), k nearest neighbors (KNN), and Classification Tree (CART) [16–18]. For each of these classifiers a diagnostic model was created. The Statistical Analysis System (SAS version 9: Cary, NC) was used to fit the logistic regressions using PROC LOGISTIC. The best subset for each size panel of analytes was identified through the branch and bound algorithm of Furnival and Wilson (1974) [19]. This algorithm maximizes the score function over all possible combinations of analytes for any given size panel. The Statistical Analysis System was also used to fit the logistic regressions and to identify best subsets for each size panel of biomarkers. Panels were generated from size 1 to 10. Sensitivities were estimated for specificities of 90%, 95% and 98% by ranking the predicted fit for each control subject, determining the cut-points corresponding to these levels of specificity, and applying the cut-points to the ranked predictions for the endometrial cancer cases. To minimize overfitting bias, leave one out cross-validation was used. The separating hyperplane (SHP) method attempts to find a linear classifier to separate the two groups of data. When no perfect linear separation exists, the linear classifier is selected to minimize the total violation. The k nearest neighbors (KNN) was performed using the 5 nearest neighbors: a point is classified based on the classification of the majority of its five nearest neighbors. We have used in house software for the SHP and KNN methods, For the Classification Tree (CART) the MATLAB routines *treefit* and *treeval* were used. The selected panel for SHP, KNN and CART was done as follows. Markers were selected incrementally. Given an existing subset of the markers, each marker was considered as a potential addition to the panel. The marker that provided the best addition separation in SHP was then added to the panel. We began with no markers and added until little additional progress was made.

Results

Multiplex analysis of serum concentrations of different biomarkers in endometrial cancer patients

A bead-based 64-biomarker panel, including most potential endometrial cancer serum biomarkers, was utilized to screen sera from 115 patients with endometrial cancer and 135 age-matched healthy controls. The biomarkers included cancer antigens, growth/angiogenic factors, apoptosis-related molecules, metastasis-related molecules, adhesion molecules, adipokines, cytokines, chemokines, hormones, and other proteins (Table 2).

Serum concentrations of IL-6, MIP-1 α , MIP-1 β , TNFRI, IL-2R, IGFBP-I, TSH, Prolactin, GH, ACTH, CA 125, CA 19-9, TGF α , MMP-7, MICA, SCC, and SAA were significantly elevated in patients with endometrial cancer as compared to healthy controls ($p < 0.05$ – $p < 0.001$; Table 3). In contrast, serum levels of Eotaxin, VEGF, ErbB2, EGFR, AFP, mesothelin, FSH, LH, CD40L, sVCAM-1, sICAM-1, tPAI-1, MPO, adiponectin, MMP-2,3,8,9, ULBP-1,3, TTR, and sFasL were significantly lower in patients with endometrial cancer compared to healthy individuals ($p < 0.05$ – $p < 0.001$; Table 3). Serum levels of other proteins were not statistically different in tested clinical groups.

To analyze potential association of individual biomarkers with stage and grade, Kruskal-Wallis test was performed. Serum prolactin concentrations did not correlate with tumor stage or grade ($p > 0.1$). By Welch Two Sample T-test, CI for grade 1 vs. 2 was (–0.38, 0.51), for grade 2 vs. 3, CI was (–0.45, 0.052), and for early (I+II) vs. late (III) stages, CI was (–0.26, 0.33). Therefore, we rule out any differences outside confidence intervals for the means of log (prolactin) between groupings (by stage and by grade) with 95% confidence, meaning that prolactin expression is stage- and grade-independent.

Cancer antigens, CA 125, CA 15-3, and CEA showed a significantly increased concentrations in Stage III as compared to Stage I ($p < 0.05$, Figure 1). Serum levels of CA 125, AFP and ACTH were higher in Grade 3 as compared to Grade 1 (Figure 1). Taken together, our data revealed that concentrations of several biomarkers vary between grades and stages in patients with endometrial cancer.

Statistical analysis of serum prolactin as the endometrial cancer marker

Prolactin was the strongest discriminative biomarker for endometrial cancer providing alone 98.3% sensitivity at 98.0% specificity. The classification accuracy of other individual biomarkers was not higher than 40.5% sensitivity at 95.0% specificity (data not shown). Prolactin correctly classified 60/64 (93.8%) patients with stages IA–IB, 12/14 (85.8%) patients with stage II, and 25/26 (96.2%) patients with stage III endometrial cancer. Thus, prolactin potentially offers high sensitivity and specificity for early detection of endometrial cancer.

Analysis of prolactin selectivity for endometrial cancer

To examine whether prolactin is highly selective for endometrial cancer, serum prolactin concentrations were measured in age- and menopausal status matched women with ovarian ($N=70$) and breast ($N=91$) cancers (Figure 2A). Prolactin levels were significantly different ($p < 0.001$, Figure 2B) in endometrial cancer as compared to two other female cancers. Despite significant differences in prolactin concentrations in endometrial cancer as compared with ovarian and breast cancers, prolactin alone was not able to reliably discriminate endometrial cancer cases from two other cancers (Figure 3A). Consequently, we have performed multivariate analysis to identify a combination of biomarkers that would further improve cancer selectivity of diagnostic test. The resultant panel was comprised of 5 biomarkers: prolactin, Eotaxin, GH, E-selectin, and TSH. The identified panel offered significantly higher classification power of endometrial cancer patients from two other cancers (Figure 3B). Logistic regression algorithm demonstrated slightly higher sensitivity and specificity for distinguishing endometrial cancer from ovarian and breast cancers as compared to other statistical algorithms (Table 4). All utilized statistical methods demonstrated a high degree of cancer-selectivity of the identified panel. These results indicate that addition of several biomarkers to prolactin results in generation of a diagnostic panel with high accuracy for detection of early stages endometrial cancer combined with high selectivity for endometrial cancer vs. other women's cancers.

Discussion

Endometrial cancer, one of the most common gynecological malignancies, is a challenging disease that currently cannot be detected early with a non-invasive and reliable screening test. In the present study, we used multiplexing approach to investigate serum proteins with high association with endometrial cancer. Our results revealed that prolactin was the strongest discriminative biomarker for endometrial cancer (98.3% sensitivity 98% specificity). Addition of Eotaxin, GH, E-selectin, and TSH to prolactin improves its classification efficiency of endometrial cancer patients from other cancers.

Elevated levels of CA 125, CA 19-9, IL-6, TGF α , MMP-7, SCC [7,20–23] and decreased expression of VEGF, AFP, adiponectin, [24–26] in endometrial cancer have been previously reported. Although endometrial cancer group has a significantly higher CA 125 as compared with control group, in both groups, CA 125 is below the cut-off clinically defined for ovarian cancer. This is in agreement with published evidence of stage- and grade-dependency of serum CA 125 [7]. The involvement of MMP-9, adiponectin, and FSH in endometrial cancer development was previously reported [27–29]. To our knowledge, this is the first report which describes an abnormal expression of serum MIP-1 α , MIP-1 β , TNFRI, IL-2R, IGFBP-I, MICA,

SAA, TTR, Eotaxin, mesothelin, sVCAM-1, sICAM-1, tPAI-1, CD40L, MPO, ULBP-1,3, and MMP-2,3,8,9 in endometrial adenocarcinoma. Chemokine MIP-1 β is a powerful chemoattractant for immune cells, including monocytes, T, NK and dendritic cells [30]. Overexpression of this protein may contribute to the induction of antitumor response in endometrial cancer. In contrast, soluble TNFRI, an extracellular domain of TNFRI, is a potent inhibitor of TNF which is involved in the down-regulation of a host immune response towards tumor and promotion of tumor survival [31]. Also, elevated levels of serum SAA, suggested to be of liver origin, were measured in patients with several cancer types including gastric [32], colon [33] and lung [34]. Increase in serum SAA protein was linked to the mechanism of tumor cell invasion and metastasis [33]. Hence, overexpression of TNFRI and SAA could potentially contribute to cancer progression. Transthyretin is a biomarker for nutritional status of the individual [35] and is also reduced during the acute phase response associated with inflammation [36]. A truncated form of TTR has been shown to be part of a set of biomarkers for the diagnosis of ovarian cancer [37]. Eotaxin is a powerful and selective eosinophil chemoattractant. It may play a complex role in modulating of tumor growth through the recruitment of eosinophils to the tumor site. The down-regulation of Eotaxin may promote tumor growth. CD40L plays an important role in regulation of B and T cells maturation and function. The decreased concentration of this marker might be associated with decreased induction and maintenance of anti-tumor immunity since CD40-ligation on dendritic and B cells play a crucial role in antigen processing and presentation, including tumor antigens.

In contrast to published evidences, we detected low levels of several serum MMPs. MMP activity can be abolished by binding of tissue inhibitors of metalloproteinases (TIMPs). In this study we used antibody which can only detect free MMPs. Therefore, the decreased levels of MMP-2,3,8,9 in sera of patients with endometrial cancer may be explained by inability to detect bound form of these proteinases. Furthermore, since MMP-9 is involved in shedding of both ICAM-1 and VCAM-1 [38,39], we suggest that low levels of MMP may contribute to decrease of soluble forms of adhesion molecules detected in this study. On the other hand, deficient immunity against cancer cells could be attributed to a decreased adhesion of immune cells [40], which could result from decreased expression of adhesion molecules [41]. Down-regulated serum sICAM-1 and sVCAM-1 were observed in this study. Expression of serum MPO, ULBP-1,3, and tPAI-1 in tumor patients has not been fully investigated and their role in human malignancies has not been analyzed.

Hormones have been shown to be significant contributors to growth of several cancer types, including breast, prostate, endometrial, and thyroid [42]. Our data confirmed the importance of several hormones in the development of screening panel for endometrial cancer. Our results demonstrated elevated levels of prolactin, TSH, ACTH and decreased expression of FSH in patients with endometrial cancer compared to healthy controls. Down-regulation of FSH levels in endometrial cancer observed in this study was corroborated by previous research [43,44], however, this is the first study demonstrating increased expression of TSH and ACTH in endometrial cancer patients. These hormones play an important role in communication and regulation of the immune cells [45] and have a direct effect on an immune function [46]. For instance, TSH induces cytokine secretion by lymphocytes [47]. Therefore, hormones might play an important role in cancer development, growth and progression.

This study demonstrated that prolactin, a single-chain protein closely related to growth hormone, is the strongest discriminative biomarker for endometrial cancer with high diagnostic power for early stage disease. Although previous case report suggested that prolactin may be an endometrial tumor marker for recurrent disease [48], to our knowledge, this study is the first one to report the possible usefulness of prolactin as a biomarker for screening for endometrial cancer. Most of the prolactin comes from the pituitary gland, but stromal cells of the endometrium produce prolactin during the secretory phase, as well. Significantly elevated

levels of prolactin in endometrial cancer could be due to increased prolactin secretion by stromal cells in response to tumor growth and differentiation. The main reported function of prolactin is to control breast development and lactation in women. However, prolactin also acts as a cytokine and plays an important role in immune and inflammatory responses [49, 50]. In addition, prolactin can act both as circulating hormone and as a paracrine/autocrine factor to either stimulate or inhibit various stages of the formation and remodeling of new blood vessels [51,52]. The formation of a new blood supply, angiogenesis, is an essential component of carcinogenesis and unrestricted tumor growth. Therefore, increased levels of serum prolactin can play an important role in growth and progression of endometrial and other cancers. In fact, we observed elevated prolactin levels in ovarian, pancreatic and lung (our unpublished observations) cancers. Further studies on the role of prolactin in endometrial cancer development and its early detection are warranted.

Our data showed that CA 125, CA 15-3, and CEA serum levels were significantly higher in endometrial patients with Stage III cancer compare to Stage I. The increase in size and tumor aggressiveness with stage may stimulate shedding of cancer antigens, resulting in their higher concentrations in the blood stream. Furthermore, with higher tumor grade, increase in tumor invasiveness and metastasis may induce production of various proteins, including cancer antigens, hormones and others. We have observed an abnormal expression of CA 125, ACTH and AFP in endometrial cancer.

The presented data demonstrate that prolactin alone sufficiently discriminates endometrial cancer from healthy controls and that adding more biomarkers does not significantly increase classification efficiency of this marker. However, since we have observed elevated levels of prolactin in several other cancers, prolactin appears to be not cancer-selective and alone is not sufficient for diagnosis of endometrial cancer. We, therefore, have identified a panel consisting of five biomarkers, prolactin, Eotaxin, GH, E-selectin, and TSH that allowed classification of endometrial cancer from ovarian and breast cancers with high sensitivity and specificity. This multi-marker panel may present a prototype of a clinical screening test for women with endometrial cancer who do not have common endometrial cancer-associated symptoms. Further validation of these results in an independent case-control set followed by validation in The Prostate, Lung, Colorectal and Ovarian (PLCO) Screening Trial retrospective longitudinal samples is required before this assay can be used in clinical trials. Additionally, we plan to evaluate molecular profile of different histologic subtypes of endometrial cancers and of non-cancerous endometrial pathology cases.

This study is one of the first to demonstrate the importance of utilization of the multi-marker approach for the early detection of endometrial cancer. Our previous studies (summarized in [13,53]), which investigated the roles of multi-marker panels in the development of ovarian cancer, another type of gynecologic cancer, came out with promising results. Future studies in this area should concentrate on examining the longitudinal changes in serum concentrations of these biomarkers and investigating their associations with treatment response, relapse, complications, and survival. Increasing our understanding of the role of biomarkers in the etiology and the course of endometrial cancer has a great potential to facilitate the development of new early detection and treatment modalities for this challenging disease.

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Figure 1. Serum levels of endometrial cancer biomarkers in women with different stage and grade endometrial cancer

Comparison of serum markers expression in women with **A.** Stage I (N=75), Stage II (N=14), Stage III (N=26), and **B.** Grade 1 (N=18), Grade 2 (N=59), Grade 3 (N=38) endometrial cancer. Horizontal lines indicate mean values. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Figure 2. Serum prolactin expression in endometrial, ovarian, and breast cancers

Sera were collected from patients with endometrial (N=115), ovarian (N=70), breast (N=91) cancers and age-matched healthy controls; *** - $p < 0.001$. **A.** Prolactin levels in each cancer type compared to matched controls. **B.** Serum prolactin expression in cancers.

Fig. 3. Evaluation of selectivity of endometrial cancer panel

Cumulative ROC curve for endometrial cancer vs ovarian and breast cancers. **A.** Prolactin alone. **B.** Five-marker panel (prolactin, GH, eotaxin, E-Selectin, TSH).

Table 1

Patient characteristics

Patient Groups	Age	Histology			Grade			Stage				
		Endo	MT	CC	Ser	O	I	2	3	IA	IB	IC
Controls N=135	Range 22–84											
	Median 57											
	Mean 60.2											
EndoCA N=115	Range 25–92											
	Median 58.5	92	2	4	10	7	18	59	38	17	47	11
	Average 62.4											

EndoCA – endometrial cancer; Endo – endometrioid; MT – Malignant Mixed Mullerian Tumor; CC – clear cell, Ser – endometrioid/serous/clear cell; O – others

Table 2

Available multiplex kits for testing serum biomarkers

Biological groups	Proteins
Cytokines/Chemokines/Receptors	IL-6, IL-8, TNF α , TNF-R1, CD40L, IL-2R, RANTES, MIP-1 α , MIP-1 β , MCP-1, Eotaxin, MIF, IP-10
Growth/angiogenic factors	VEGF, bFGF, G-CSF, HGF, ErbB2, EGFR, IGFBP-1, TGF- α , angiostatin
Cancer Antigens	CA 125, CA 15-3, CA 19-9, CA 72-4, S100, CEA, AFP, SCC
Apoptotic proteins	Cyfra 21-1, sFas, sFasL
Proteases	human Kallikrein-8 (hK8), hK10, MMPs 1,2,3,7,8,9,12,13
Adhesion molecules	sVCAM-1, sICAM-1, sE-selectin
Hormones	prolactin, TSH, β HCG, LH, ACTH, GH, FSH
Adipokines	adiponectin, resistin
Other markers	tPAI-1, active PAI-1, MHCclass I-related chains A (MICA), serum amyloid A (SAA), transthyretin (TTR), mesothelin, myeloperoxidase (MPO)

Table 3

Expression of serum biomarkers in healthy controls and patients with endometrial cancer.

Serum markers	Controls	EndoCA	Units	P-value
Prolactin	9.8±0.86 7.7 (2.2–112.0)	169.3±9.65 150.3 (22.1–562.9)	ng/ml	P<0.0001
TSH	2.4±0.65 1.4 (0.1–85.8)	3.9±0.36 2.6 (0.2–22.5)	ng/ml	P<0.0001
ACTH	24.7±2.06 16.6 (0.7–133.6)	40.5±4.73 26.7 (0.7–348.2)	pg/ml	P=0.031
FSH	65.9±2.93 62.9 (1.7–170.9)	43.5±2.68 40.9 (0.3–135.2)	ng/ml	P<0.0001
GH	0.6±0.06 0.2 (0.0–3.4)	1.2±0.19 0.4 (0.0–11.9)	ng/ml	P=0.0017
LH	25.3±1.30 23.9 (0.0–87.9)	17.9±1.08 16.9 (0.4–63.4)	ng/ml	P<0.0001
CA 125	7.1±1.51 3.3 (0.1–155.0)	14.5±2.58 7.1 (0.1–228.8)	U/ml	P<0.0001
CA 19-9	0.3±0.09 0.2 (0.0–11.7)	0.9±0.28 0.3 (0.0–25.1)	U/ml	P=0.0003
AFP	2912.3±146.65 2524.9 (223.9–10630.1)	2337.9±144.08 1933.7 (111.9–8481.8)	U/ml	P<0.0005
IL-6	23.2±6.10 6.6 (0.2–681.6)	38.1±5.53 15.9 (0.2–420.1)	pg/ml	P<0.0001
CD40L	5.3±0.37 4.5 (0.1–26.1)	4.0±0.49 2.1 (0.0–31.3)	ng/ml	P<0.0001
sFasL	133.9±14.56 71.2 (1.9–876.8)	70.2±7.47 63.9 (3.8–469.9)	pg/ml	P=0.0010
IL-2R	173.8±15.83 128.4 (1.9–1029.3)	256.8±31.69 178.9 (1.9–2654.9)	pg/ml	P=0.0104
Eotaxin	150.4±6.58 136.6 (27.5–446.4)	99.4±4.81 82.9 (14.7–269.8)	pg/ml	P<0.0001
MIP-1α	66.5±17.14 9.5 (0.2–1732.6)	75.9±13.64 9.5 (0.2–872.7)	pg/ml	P=0.0402
MIP-1β	116.9±39.80 16.7 (8.1–4554.2)	245.6±52.08 16.7 (8.1–4656.1)	pg/ml	P=0.0018
TNFR-I	2090.5±82.69 1877.8 (616.9–8265.4)	2909.5±103.51 2674.9 (352.2–5732.0)	pg/ml	P<0.0001
IGFBP-I	3.6±0.42 2.1 (0.1–70.7)	4.8±0.59 2.7 (0.0–38.8)	ng/ml	P=0.036
EGFR	35.5±0.68 34.5 (11.6–70.7)	31.9±0.88 29.5 (13.0–59.3)	ng/ml	P<0.0001
ErbB2	5.0±0.39 4.6 (1.0–54.9)	4.1±0.12 4.1 (1.4–9.8)	ng/ml	P=0.0007
TGFα	302.7±140.26 14.4 (0.0–11000.0)	459.3±268.03 8.5 (0.0–26881.1)	pg/ml	P=0.0145
VEGF	163.1±20.67 122.1 (3.8–2567.9)	125.9±14.52 85.9 (0.3–995.8)	pg/ml	P=0.0051
sVCAM-1	1887.3±41.25 1865.7 (703.0–4052.2)	1524.4±48.04 1531.8 (0.0–3010.8)	ng/ml	P<0.0001
sICAM-1	246.7±10.52 219.9 (77.7–981.8)	210.9±9.30 204.4 (0.0–577.3)	ng/ml	P=0.0292
MMP-2	190.5±3.89 188.0 (97.5–332.2)	165.4±4.65 166.2 (0.0–324.6)	ng/ml	P=0.0002
MMP-3	11.0±0.49 9.4 (0.2–38.0)	9.8±0.63 8.1 (0.0–59.8)	ng/ml	P=0.017
MMP-7	2.5±0.13 2.1 (0.0–9.0)	4.4±0.33 3.1 (0.0–17.7)	ng/ml	P<0.0001
MMP-8	10.0±0.88 7.9 (0.0–78.3)	4.3±0.40 2.7 (0.0–24.2)	ng/ml	P<0.0001
MMP-9	290.4±13.2 260.9 (15.6–791.3)	196.2±17.6 148.1 (0.0–1248.6)	ng/ml	P<0.0001
Mesothelin	7.6±0.69 5.5 (1.4–59.2)	4.7±0.21 4.2 (0.3–15.3)	ng/ml	P<0.0001
Adiponectin	23.3±0.68 26.5 (4.0–41.5)	19.2±0.89 17.1 (0.0–44.8)	μg/ml	P<0.0001
MICA	5.9±0.69 4.0 (0.0–56.8)	8.2±1.25 5.9 (0.0–127.9)	pg/ml	P=0.0401
SAA	29.1±6.79 13.9 (0.4–890.4)	61.3±19.15 20.5 (0.7–2015.6)	ng/ml	P=0.0118
tPAI-1	50.1±1.70 48.1 (7.9–141.9)	40.9±2.07 40.2 (0.0–105.4)	ng/ml	P=0.0010
MPO	76.7±8.33 44.8 (0.1–660.7)	25.1±3.63 11.4 (0.0–232.2)	ng/ml	P<0.0001

Serum markers	Controls	EndoCA	Units	P-value
TTR	387.6±10.63 376.5 (0.5–890.9)	315.9±11.29 318.3 (40.9–656.6)	ng/ml	P<0.0001
SCC	1.4±1.1 0.3 (0.0–149.9)	1.5±0.48 0.8 (0.0–54.9)	ng/ml	P<0.0001

Table 4

Evaluation of endometrial cancer panel selectivity.

	Ovarian Cancer		Breast Cancer	
	Sensitivity	Specificity	Sensitivity	Specificity
SHP	91%	95%	98%	95%
KNN	89%	91%	95%	97%
CART	83%	89%	90%	94%
LR	97%	96%	97%	96%

SHP, separating hyperplane

KNN, k nearest neighbors

CART, classification trees

LR, logistic regression