Influence of Ovarian Cancer Risk Status on the Diagnostic Performance of the Serum Biomarkers Mesothelin, HE4, and CA125

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Abstract

Objective: **To evaluate the effect of ovarian cancer risk on the performance of the serum biomarkers mesothelin, human epididymis protein 4 (HE4), and CA125.**
Methods: **We measured mesothelin, HE4, and CA125 levels from women with invasive ovarian cancer (*n* = 143), benign gynecologic conditions (*n* = 124), and controls (*n* = 344). Demographic, epidemiologic, reproductive, medical, and family history data were collected using a standardized questionnaire. Pedigree and *BRCA 1/2* test results were used to stratify women into average and high-risk groups. The diagnostic accuracy of each biomarker was characterized using receiver operating characteristic curve methods.**
Results: **Baseline characteristics did not vary by risk or case status. The distribution of stage and histology was similar in average and high-risk women. All three markers discriminated ovarian cancer cases from risk-matched healthy and benign controls. Marker performance did not vary by risk status. The sensitivity at 95% specificity for discriminating cases from risk-matched healthy control women in the average and high-risk groups, respectively, was 53.9% and 39.0% for mesothelin, 80.4% and 87.8% for HE4, and 79.4% and 82.9% for CA125. The performance of the markers was not as robust when cases were compared with benign controls. Area under the curve values for cases versus healthy and benign controls did not vary by risk status.**
Conclusions: **The ability of serum mesothelin, HE4, and CA 125 levels to discriminate ovarian cancer cases from healthy and benign controls is not influenced by risk status. Our findings support the pursuit of additional studies evaluating the early detection potential of these markers in high-risk populations.** (Cancer Epidemiol Biomarkers Prev 2009;18(5):1365–72)

Introduction

Ovarian cancer biomarker research is motivated by a strong desire to identify a panel of complementary biomarkers that will be useful for early detection of the disease. Diagnostic markers that can discriminate ovarian cancer cases at clinical diagnosis from cancer-free controls with good sensitivity at high levels of specificity are candidate markers for early detection. Characterizing the performance of candidate early detection markers in women at increased ovarian cancer risk is of particular interest because women at high or elevated risk are ideal candidates for initial studies of new screening tests due to the low incidence of ovarian cancer in the general population.

The application of genomic and proteomic technologies has led to the identification of a number of ovarian cancer biomarkers that perform well when tested in the general population as diagnostic markers (1). Mesothelin and human epididymis protein 4 (HE4) are two of the most intensively studied of the novel biomarkers. Mesothelin is a 40-kDa polypeptide cell surface protein present on normal mesothelial lining cells. Mesothelin expression is increased in ovarian cancer tissues and a soluble form is detectable in blood (2-6). Elevated serum levels of mesothelin are detectable in 40% to 67% of patients with ovarian cancer (3). Mesothelin enhances the diagnostic performance of CA125, the only currently available and best validated ovarian cancer biomarker, at high levels of specificity that are relevant for early detection. In the general population, a composite marker that includes both CA125 and mesothelin increased the sensitivity at 98% specificity from 78.8% for CA 125 alone to 86.5% (7). HE4 is an 11-kDa protein that is a precursor to the epididymal secretory protein E4 and resides on human chromosome 20q12-13.1. HE4 is overexpressed in 93% of serous epithelial ovarian cancers (8-10). In the general population, serum levels of HE4 are elevated in over 90% of women with ovarian cancer at diagnosis (11). Compared with CA125, HE4 is less frequently elevated in patients with benign gynecologic disease (11).

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Table 1. Criteria used to classify women as high-risk for ovarian cancer

The woman's family contained at least two ovarian or breast cancer cases among the subject or her first or second-degree relatives. This condition was satisfied by multiple primary cancers in the same person. In situations where breast cancer was used to meet this criterion, at least one breast cancer must have been diagnosed before menopause. If menopausal status was unknown, women under the age of 50 y were considered to be premenopausal; or

The woman was of Ashkenazi Jewish ethnicity with one first-degree relative or two second-degree relatives with breast or ovarian cancer, or the woman was of Ashkenazi ancestry and had a personal history of breast cancer. As explained above, in situations where breast cancer was used to meet this criterion, at least one breast cancer must have been diagnosed before menopause; or

The probability of carrying a *BRCA I* or *BRCA II* mutation given family pedigree of breast and ovarian cancer exceeded 20%. This was determined by the BRCAPRO 95% posterior probability interval. This criterion included women who tested positive for a *BRCA I* or *BRCA II* mutation and women who had a first- or second-degree relative with a *BRCA I* or *BRCA II* mutation.

Because mesothelin and HE4 complement CA125, they are of particular interest for early detection.

Women at increased risk for developing ovarian cancer are ideal candidates for studies investigating novel early detection tests. Ovarian cancer is a relatively uncommon disease with an incidence of roughly 45 per 100,000 postmenopausal women. The lifetime risk of developing ovarian cancer among unselected women in the United States is roughly 1 in 65. A family history of ovarian cancer is the most important risk factor for developing the disease. Women with a single first-degree relative with ovarian cancer have a 5% lifetime risk for developing the disease. Approximately 7% to 10% of ovarian cancer cases occur in women with a strong family history of ovarian and/or breast cancer. A substantial proportion of the cancer prone phenotype in these high-risk families is explained by mutations in the *BRCA1* or *BRCA2* genes. The lifetime risk for developing ovarian cancer for mutation positive women ranges from 15% to 40%, depending on the specific mutation. Interestingly, mutational testing of cohorts of ovarian cancer patients unselected for family history suggest that germline *BRCA* mutations contribute to ovarian cancer development in a larger proportion of cases than previously thought. Mutation rates as high as 11% to 15% have been observed in 2 recent population-based studies of unselected women with invasive ovarian cancer (12, 13). Furthermore, it seems that germline mutations in *BRCA1* and *BRCA2* in unselected women confer a high risk of ovarian cancer similar to those women with multiple affected family members (14).

Tumors arising in women at increased genetic risk are molecularly distinct from sporadic ovarian cancer and have a different clinical behavior (15-18). Compared with sporadic cases, *BRCA*-associated hereditary ovarian cancers are more likely to be serous subtype, of higher grade, contain more solid areas and to accumulate *p53* (19). Even after controlling for clinical-pathologic features, *BRCA*-associated cancers have distinct gene expression profiles and respond better to standard therapies (20, 21). Differences in the clinical and molecular characteristics of sporadic and hereditary ovarian cancers raise the possibility that diagnostic performance of ovarian cancer biomarkers may differ in average and high-risk women. We undertook this study to determine if there are differences in the diagnostic performance of CA125 and the novel candidate early detection markers HE4 and mesothelin in

Table 2. Summary of the baseline characteristics for the average and high risk controls and cases

Characteristic	Healthy controls (*n* = 444)			Benign controls (*n* = 124)			Cases (*n* = 143)		
	Average-risk (*n* = 150)	High-risk (*n* = 294)	*P**	Average-risk (*n* = 110)	High-Risk (*n* = 14)	*P**	Average-risk (*n* = 102)	High-risk (*n* = 41)	*P**
Age, median (range)	52 (30-75)	50 (31-83)	0.09	54 (27-83)	51 (25-71)	0.14	58 (35-86)	57 (19-80)	0.83
BMI, median (range)	24.5 (18.5-42.0)	24.2 (16.2-43.9)	0.82	25.8 (18.8-51.6)	26.6 (17.8-50.6)	0.92	25.6 (19.2-42.9)	24.5 (20.6-53.7)	0.90
Race									
White	128 (85.3)	269 (91.5)		95 (86.4)	13 (92.9)		84 (82.4)	37 (90.3)	
Asian	3 (2.0)	3 (1.0)		4 (3.6)	0		5 (4.9)	1 (2.4)	
Black	0	0		1 (0.9)	0		0	1 (2.4)	
Other †	0	6 (2.0)		3 (2.7)	0		4 (3.9)	0	
Unknown	19 (12.7)	16 (5.5)	0.01	7 (6.4)	1 (7.1)	0.90	9 (8.8)	2 (4.9)	0.26
Menopause Status, *n* (%)									
Premenopause	53 (35.3)	96 (32.7)		17 (15.5)	3 (21.4)		13 (12.7)	2 (4.9)	
Postmenopause	97 (64.6)	198 (67.3)	0.57	93 (84.5)	11 (78.6)	0.57	89 (87.3)	39 (95.1)	0.17
Hormone replacement therapy use (ever), *n* (%)									
No	32 (21.3)	178 (60.6)		48 (43.6)	6 (42.9)		42 (41.2)	14 (34.2)	
Yes	45 (30.0)	110 (37.4)		53 (48.2)	8 (57.1)		47 (46.1)	24 (58.5)	
Unknown	73 (48.7)	6 (2.0)	<0.001	9 (8.2)	0	0.51	13 (12.7)	3 (7.3)	0.36

Abbreviation: BMI, body mass index.

**P* values were obtained by Wilcoxon rank-sum test for continuous variables and χ^2 for categorical variables.

† Other race includes American/Alaskan Indians and women who reported more than one race.

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Table 3. Summary of the tumor characteristics for ovarian cancer cases by risk status

	Average-risk cases (*n* = 102)	High-risk cases (*n* = 41)	*P**
Histology, *n* (%)			
Clear cell	8 (7.8)	2 (4.9)	
Endometrioid	10 (9.8)	4 (9.8)	
Mucinous	6 (5.9)	2 (4.9)	
Serous	66 (64.7)	24 (58.5)	
Other	12 (11.8)	9 (21.9)	*P* = 0.62
Stage, *n* (%)			
Stage 1	29 (29.3)	6 (17.1)	
Stage 2	7 (7.1)	3 (8.6)	
Stage 3	53 (53.5)	22 (62.9)	
Stage 4	10 (10.1)	4 (11.4)	*P* = 0.58

NOTE: Missing data on stage for three average-risk cases and six high-risk cases.
**P* values were obtained from χ^2.

women at average and high-risk for ovarian cancer. Because our long-term goal is applying these markers for use in early detection, we focused on testing the ability of the markers to discriminate ovarian cancer cases from healthy controls at high levels of specificity. We also included a benign ovarian tumor control group as benign tumors are a potential source of false positive screening tests.

Diagnostic performance is a necessary first step in evaluating candidate ovarian cancer early detection markers. However, assessment of their early detection potential requires validation in samples collected before clinical diagnosis when women are asymptomatic. This study provides evidence that the markers we studied have performance characteristics that support further testing in women at increased risk for ovarian cancer. This is the first study to report information on the relative diagnostic performance of these novel markers in average- and high-risk populations. Our goal was to determine if these novel markers merit further study in screening programs designed for targeting women who are at high-risk for ovarian cancer and to determine if findings from screening programs targeting high-risk women are likely to be generalizable to women in the general population. Although high-risk women are ideal candidates for ovarian cancer screening trials and programs adequate performance in average-risk women will be necessary to have a major effect on ovarian cancer mortality.

Materials and Methods

Study Population. Women included in this report were enrolled between 1999 and 2003 in multiple Institutional Review Board–approved protocols associated with the Seattle-based Pacific Ovarian Cancer Research Consortium, a National Cancer Institute–funded Ovarian Cancer Specialized Program of Research Excellence. All participants completed a standardized baseline questionnaire that queried women regarding a broad range of demographic, epidemiologic, and reproductive factors as well as personal or family cancer history. Risk for developing ovarian cancer was ascertained by self-report of personal and family history of cancer and *BRCA* gene mutation test results in study enrollment questionnaires. Women were specifically queried regarding the occurrence and age at diagnosis for breast, ovarian, colon, prostate, and other cancers in first and second-degree relatives. The criteria used to classify women as high risk for ovarian cancer are outlined in Table 1. We selected these criteria because they identify women with at least a 10% lifetime risk of developing ovarian cancer. Women with a less

Table 4. Marker levels for the controls and cases by risk status

	Average-risk		High-risk		*P**
	n	Mean (SE)	*n*	Mean (SE)	
Healthy controls	150		294		
CA125		0 (1.0)		−0.8 (0.8)	<0.001
Mesothelin		0 (1.0)		0.1 (1.0)	0.63
HE4		0 (1.0)		−0.2 (1.0)	0.10
Benign controls	110		14		
CA125		0.7 (1.4)		0.5 (1.1)	0.67
Mesothelin		0.02 (1.2)		0.1 (1.1)	0.79
HE4		0.4 (1.3)		0.3 (1.3)	0.95
CASES	102		41		
CA125		4.2 (2.6)		3.6 (2.6)	0.17
Mesothelin		2.6 (2.8)		2.2 (2.8)	0.38
HE4		5.2 (3.9)		5.5 (3.6)	0.63
Cases (by histology)					
Clear cell	8		2		
CA125		1.8 (2.0)		0.6 (0.1)	0.15
Mesothelin		0.3 (1.1)		−1.0 (0.5)	0.09
HE4		0.7 (1.3)		2.1 (0.8)	0.17
Endometrioid	10		4		
CA125		4.7 (3.1)		3.5 (4.2)	0.61
Mesothelin		1.2 (2.2)		0.9 (3.2)	0.85
HE4		4.9 (3.4)		5.7 (4.8)	0.77
Mucinous	6		2		
CA125		3.0 (1.9)		3.4 (1.5)	0.80
Mesothelin		−0.2 (0.6)		1.8 (0.2)	<0.001
HE4		1.6 (1.4)		6.7 (0.8)	0.006
Serous	66		24		
CA125		4.8 (2.4)		4.4 (2.1)	0.50
Mesothelin		3.7 (2.6)		2.8 (2.6)	0.16
HE4		6.4 (3.8)		6.3 (3.5)	0.95
Other			9		
CA125		3.1 (2.9)		2.1 (2.8)	0.42
Mesothelin		1.0 (2.5)		1.9 (3.3)	0.50
HE4		3.8 (3.5)		3.9 (3.7)	0.93
Cases (by stage)					
Stage 1	29		6		
CA125		2.5 (2.6)		2.8 (2.5)	0.85
Mesothelin		0.4 (1.4)		0.2 (1.7)	0.82
HE4		1.7 (2.0)		3.9 (3.2)	0.15
Stage 2	7		3		
CA125		3.4 (2.3)		2.8 (2.4)	0.73
Mesothelin		1.0 (1.3)		2.3 (3.7)	0.62
HE4		5.3 (3.0)		6.2 (2.1)	0.63
Stage 3	53		22		
CA125		5.3 (2.1)		4.4 (2.2)	0.12
Mesothelin		3.8 (2.7)		3.0 (2.9)	0.26
HE4		7.1 (3.5)		6.8 (3.1)	0.72
Stage 4	10		4		
CA125		4.6 (2.8)		5.8 (1.6)	0.38
Mesothelin		3.4 (3.0)		3.2 (2.6)	0.88
HE4		4.5 (3.3)		7.9 (2.1)	0.05

NOTE: The marker values have been log transformed and standardized to a mean of 0 and an SD of 1 in the average risk controls.
**P* values were obtained from the Student's *t* test.

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significant family history are classified as average-risk. Specimen collection and processing protocols associated with each study are detailed below.

Ovarian Cancer Cases and Women with Benign Gynecologic Disease (Benign Control Group). The Pacific Ovarian Cancer Research Consortium Surgical Specimen Donation protocol identifies women undergoing gynecologic surgery for ovarian-related conditions including benign and malignant disorders. These women are recruited and enrolled at the time of their preoperative clinical visit. Up to 50 mL of blood are collected in the operating room after induction of anesthesia but before the onset of surgery. Serum is collected using Serum Separator Tubes (DB Vacutainer, Becton Dickinson and Company). The blood is allowed to coagulate at room temperature for up to 4 h. The serum is then aliquoted and stored at −80° until analysis. The current study focuses on a representative sample of 143 women with invasive epithelial ovarian cancer and 124 women with benign ovarian tumors who were enrolled during the study interval. Diagnoses are confirmed by standardized review of medical records and examination of paraffin-embedded tissue by a research pathologist.

Healthy Women. Healthy control women include participants in a local Seattle-based high-risk ovarian cancer screening program and women undergoing routine screening mammography at Swedish Medical Center—a large community-based hospital located in downtown Seattle. The ovarian cancer screening program focused on women at increased risk based on personal and family history of cancer and/or *BRCA 1* and *2* gene mutation status as outlined in Table 1. Participants were screened quarterly with serum CA125 levels and transvaginal sonography on an annual basis. Blood collection occurred in the outpatient setting at the time of ovarian cancer screening or mammography. Serum was collected and processed by the same staff and using an identical protocol as for Surgical Donation Protocol participants. All participants were determined to be free from all invasive cancers for a period of at least 5 y before and 2 y after the date of blood sample collection.

Laboratory Analysis. CA125 and HE4 serum levels were assessed using bead-based immunoassays done as described by Scholler (9, 22). Anti-CA125 X52 mouse monoclonal antibody and anti-HE4 3D8 monoclonal antibody were biotinylated using the EZ-Link-sulfo-NHS-biotinylation kit (Pierce) according to the manufacturer's instructions and dialyzed against Phosphate Buffered Saline (PBS; Fisher BioReagents) using a dialysis slide (Slide-a-Lyzer 7 kDa MWCO; Pierce). All incubations were carried out for 30 min, except as otherwise specified, in PBS supplemented with 1% Bovine Serum Albumim (Sigma-Aldrich). Washes were done with PBS supplemented with 0.05% Tween 20 (Sigma-Aldrich). Anti-CA125 monoclonal antibody X306 (5 μg/mL; Research Diagnostics, Inc.) was coupled to carboxy-coated beads. Antibody-coated beads were incubated with 4-fold diluted patient sera, and captured antigen was detected with 2 μg/mL of biotinylated anti-CA125 monoclonal antibody X52 (Research Diagnostics, Inc.) followed by phycoerythrin-conjugated streptavidin (Bio-Rad Laboratories, Inc.). Bead-based assays were carried out in 96-well MultiScreen GV filter plates (Millipore Corporation) using a vacuum manifold (Millipore) to drain assay reagents. Plates were analyzed with the Bio-Plex Array reader (Bio-Rad). This procedure has been found to yield values that are strongly correlated ($r > 0.90$) with the research standard CA125II RIA from Fujirebio Diagnostics, Inc. (9).

Anti-HE4 monoclonal antibodies 3D8 and 2H5 were kind gifts from Dr. Ingegerd Hellstrom. Anti-HE4 2H5 was coupled to beads at a concentration of 10 μg/mL with the following buffer modifications: bead activation buffer was made with 0.1 mol/L Sodium Phosphate (pH 6.2; Sigma-Aldrich). 1-Ethyl-3-[3dimethylamino-propyl] carbodiimide hydrochloride (Pierce) and N-hydroxysulfosuccinimide (Pierce) were diluted to 38 and to 109 mg/mL, respectively, in activation buffer. The coupling buffer was made with 0.05 mol/L 2[N-Morpholino] ethanesulfonic acid (pH 5.0; Sigma-Aldrich). PBS 1% bovine serum albumin was used for bead blocking and storage buffers. Antibody-coated beads were incubated with 10-fold diluted sera. Captured antigens were detected with 2 μg/mL of biotinylated 3D8 followed by a 10-min incubation with 1,000-fold diluted phycoerythrin-conjugated streptavidin (Becton Dickinson Pharmingen).

Mesothelin serum levels were assessed using a novel bead-based immunoassay done as described by Scholler (22). Briefly, carboxy-coated beads were conjugated to antimesothelin polyclonal antibody (R&D Systems) at a concentration of 50 μg/mL. To capture antigen, antibody-coated beads were incubated with 5-fold diluted sera. Antimesothelin biobody (Bb) #7 at a concentration of 1 μg/mL (23) was preincubated with 2,000-fold diluted PJ31S PhycoLink Streptavidin-R-Phycoerythrin (Prozyme) on ice and in the dark. Bead-captured antigens were detected with Bbs preincubated with PJ31S streptavidin. Buffers used for anti-mesothelin polyclonal antibody bead conjugations were the same as the buffers used for anti-HE4 bead conjugations.

Statistical Analyses. Descriptive statistics were calculated for baseline characteristics of the healthy and benign control and ovarian cancer case groups for each risk category. Differences in the distribution of the baseline characteristics between the average and high-risk women within each case-control group were compared using the Wilcoxon rank-sum test for continuous variables and χ^2 for the categorical variables.

Serum levels of CA125, mesothelin, and HE4 were log transformed and standardized to have mean 0 and SD 1 in the average-risk controls. This transformation induces the same measurement scale for all three markers, which promotes comparison among the three markers because their scales are the same (24).

The mean and SD of the three biomarkers were calculated separately for the healthy controls, benign controls, and the cases. These values were also calculated by histology and stage within the cases. The student's t test was used to determine if there were statistically significant differences in the mean values of the markers between the average and high-risk women.

The diagnostic accuracy of the three markers was assessed separately for the average-risk and high-risk women by estimating the receiver operating characteristic

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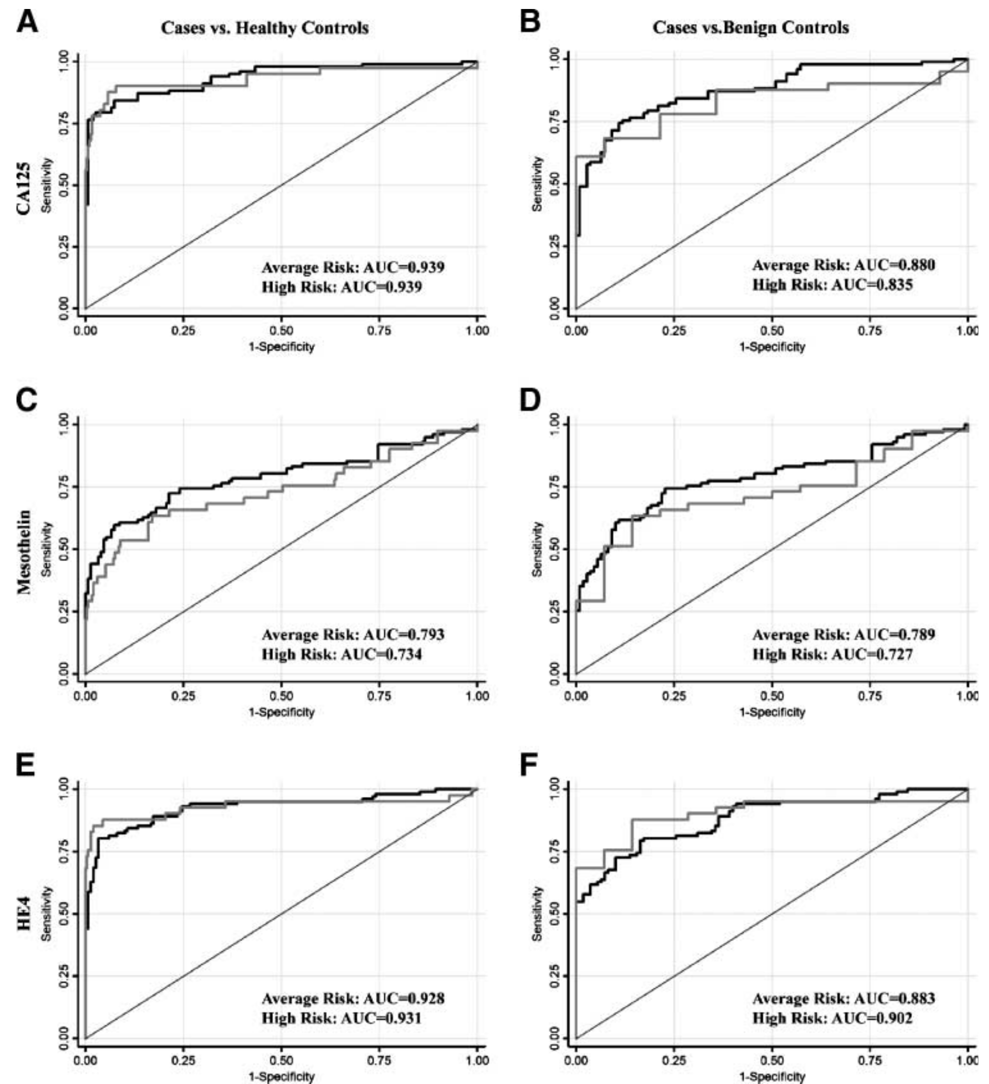

Figure 1. ROC curves for women at high risk (*gray line*) and average risk (*black line*) for ovarian cancer. **A.** CA125 for cases versus healthy controls. **B.** CA125 for cases versus benign controls. **C.** Mesothelin for cases versus healthy controls. **D.** Mesothelin for cases versus benign controls. **E.** HE4 for cases versus healthy controls. **F.** HE4 for cases versus benign controls.

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(ROC) curves and area under the curve (AUC) statistics for cases versus healthy controls and cases versus benign controls (25). These ROC curves display the misclassification rate (1-Specificity) in the controls, and the true classification rate in the cases (Sensitivity; ref. 11). An AUC value of 1.0 represents perfect performance of the marker and 0.50 indicates a level of performance that is expected by chance alone. The aforementioned methods used to standardize the marker levels leave ROC curves and their P values unchanged (7). We then compared the classification performance and equivalency of the three biomarkers by risk status using the nonparametric methods developed by DeLong and colleagues (26).

The STATA statistical software package (27) was used for these analyses. All statistical tests were 2-sided and considered to be statistically significant at P value of <0.05. No adjustments were made for multiple comparisons.

Results

The baseline characteristics for the study population are presented in Table 2. The median age of cases was 58 and 57 for average- and high-risk women, respectively. Most features including age, body mass index, or menopausal status did not vary by risk status in the either the case or control subgroups. Compared with average-risk healthy controls, a larger proportion of high-risk healthy controls reported having ever used hormone replacement therapy ($P < 0.001$). Average-risk healthy controls were more likely to report their ethnicity as unknown ($P < 0.01$).

As shown in Table 3, the majority of the ovarian cancer cases were of serous histology and advanced stage. Only 36% of the average-risk and 26% of the high-risk cases were stage I or II. The distribution of histologic subtypes and stage of ovarian cases was similar in the high and average-risk groups.

Log-transformed and standardized mean levels of biomarkers for average and high-risk cases and benign and healthy controls are presented in Table 4. Mean CA125 levels in high-risk healthy controls were lower than corresponding levels in average-risk women ($P < 0.001$). Although the total number of mucinous tumors was low, the mean HE4 and mesothelin levels in these tumors were higher in high-risk women than in average-risk women, 6.7

Table 5. Diagnostic accuracy of the biomarkers for average and high-risk women

	Cases vs healthy controls			Cases vs benign controls		
	AUC	Sensitivity at 95% specificity	Sensitivity at 98% specificity	AUC	Sensitivity at 95% specificity	Sensitivity at 98% specificity
CA125						
Average-risk	0.939	79.41	78.43	0.880	58.82	49.02
High-risk	0.939	82.93	78.05	0.835	63.41	60.98
AUC differences P*	0.98			0.45		
Mesothdin						
Average-risk	0.793	53.92	44.12	0.789	43.14	37.25
High-risk	0.734	39.02	31.71	0.727	34.15	29.27
AUC differences P*	0.36			0.42		
HE4						
Average-risk	0.928	80.39	68.63	0.883	61.76	57.84
High-risk	0.931	87.80	82.93	0.902	75.61	70.73
AUC differences P*	0.94			0.69		

*The AUC differences between the average- and high-risk women were compared for each biomarker using the nonparametric methods developed by DeLong (29).

versus 1.6 (P = 0.006) for HE4 and 1.8 versus −0.2 for mesothelin (P < 0.001).

ROC curves that summarize the sensitivity across all levels of specificity for detecting ovarian cancer cases relative to healthy and benign controls in average and high-risk women are presented in for each marker Fig. 1. The AUC numerically describes the overall performance of the marker, with an AUC of 1 indicating perfect sensitivity and specificity. Table 5 summarizes key features of the ROC curves including the AUC and marker performance at high levels of specificities that are relevant for early detection. Overall, the diagnostic performance of CA125 and HE4 was roughly equivalent and better than that of mesothelin. All markers did better when cases were compared with healthy as opposed to benign tumor controls. There were no differences in the ability of any of the markers to discriminate cases from healthy or benign controls between risk groups. HE4 tended to perform better and mesothelin tended to perform worse in high-risk women, but these differences were not significant. The sensitivity at 98% specificity for discriminating ovarian cancer cases from healthy controls in average- and high-risk women, respectively, was 78.4% and 78.0% for CA 125, 44.1% and 31.7% for mesothelin, and 68.6% and 82.9% for HE4.

Discussion

Mesothelin and HE4 are novel ovarian cancer biomarkers that may have utility for early detection. When tested as diagnostic markers using samples collected at clinical diagnosis from patients unselected for ovarian cancer risk both markers discriminate ovarian cases from healthy and benign controls at high levels of specificity. We undertook this study to evaluate if the diagnostic performance of CA125, mesothelin, and HE4 was affected by the ovarian cancer risk status as high-risk women are ideal candidates for early evaluation of new ovarian cancer screening tests. We recognize that although high performance in diagnostic samples is a necessary characteristic, the true utility of an early detection marker depends on its behavior before clinical diagnosis. We sought to determine if the markers were suitable for further testing in women at high-risk because women who are at high-risk for ovarian cancer are most likely to participate in a screening program for ovarian cancer. Our findings suggest that further studies evaluating early detection potential of the markers we tested in high-risk women are warranted and that the findings from these studies may be generalizable to women in the general population.

We used self-report of personal and family cancer history to a standardized questionnaire to obtain information for risk classification. Women were asked an extensive series of questions about diagnosis and age at diagnosis of ovarian, breast, colon, and other cancers in first- and second-degree family members. We also asked women if they had been tested for *BRCA 1* and *2* mutations and if a deleterious mutation or variant of uncertain significance was identified. However, only 48 (7%) of the women included herein reported they had been tested for the mutations and 28 of these women (58%) reported having a positive test.

A limitation of our study is that we were not able to validate self-report of information used to characterize risk status. It is possible that either direct interview or review of personal and family medical records might have provided more complete or accurate information and better risk classification. Misclassification could have occurred if a participant believed a relative's cancer was ovarian when in fact, it arose in another site. It is also possible women may have been inappropriately classified as being average risk if they chose to withhold information about genetic testing because of concerns about confidentiality.

The classification system we used to characterize women at high-risk identifies a heterogeneous group with respect to their true risk of ovarian cancer. Ovarian cancer risk in this group spans from roughly a 10% lifetime risk for developing ovarian cancer for women meeting minimal criteria to perhaps as high as 60% lifetime risk for *BRCA1* mutation carriers. A more homogeneous population, particularly one limited to mutation carriers, might be more informative. However, our criteria for identifying women as high-risk are generally accepted clinically and are consistent with eligibility criteria used to select women for enrollment into large multicenter, prospective ovarian cancer screening studies targeting women at high-risk (28, 29).

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The average- and high-risk groups in this study are well-balanced for most of the baseline demographic information that was collected. Self-report of use of hormone replacement therapy among healthy control women did vary by risk status ($P < 0.01$). The difference remained significant even after women responding ''unknown'' to having ever used hormone replacement therapy were removed from the analysis, suggesting most of the difference is related to a higher proportion of high-risk women reporting having ever used hormone replacement therapy. More frequent reported use of hormone replacement therapy among the high-risk women is probably explained by the fact that a greater proportion of these women were postmenopausal (67% versus 35%). We also identified differences in ethnicity between high- and average-risk healthy controls; however, these differences were not significant when women who reported their race as unknown were removed from the analysis.

Interestingly, mean levels of CA125 were lower in high-risk compared with average-risk healthy controls. Mean CA125 levels decrease substantially after menopause (30), and the lower CA levels in high-risk controls may be explained by the high proportion of postmenopausal women in the high-risk group. In a prior report, we noted that CA125 levels in healthy postmenopausal high-risk women were affected by ovarian cancer risk factors including talc use and parity, although the effects were minor; these parameters were not evaluated in the current report (31). Pauler (30) reported that CA125 levels in healthy women vary based on personal characteristics including age, race, smoking, caffeine intake, age at menarche, and menopause status. Factors evaluated in this report including age and race did not vary by risk group.

We found that for mucinous ovarian cancer cases, mesothelin and HE4 levels were higher in high-risk compared with average-risk women. This result could be spurious as the total number of mucinous cancers in the cohort was small ($n = 6$), and there were only 2 mucinous cancers in the high-risk group. Differences in marker levels could not be explained by either tumor volume or stage as these factors vary between the two groups (data not shown).

The overall diagnostic performance of the markers estimated using the area under the ROC curve did not vary by risk status. When ovarian cancer cases were compared with healthy controls, the AUC values for average- and high-risk women were nearly identical (0.939 versus 0.939 for CA 125, 0.793 versus 0.734 for mesothelin, and 0.928 and 0.931 for HE4). The findings were similar although the AUC values were lower when cases were compared with benign controls. The reduction in AUC is not surprising, as some benign tumors are known to emit low levels of the markers. At high levels of specificity, mesothelin seemed to perform better in average-risk women and HE4 did better in high-risk women, although the differences were relatively minor, not statistically significant, and may be influenced by the sparseness of the data in this portion of the curve. For mesothelin, the sensitivity at 98% specificity for ovarian cases versus healthy controls was 44.1% and 31.71% for average- and high-risk women, respectively. For HE4, the values were 68.3% for average and 82.9% for high-risk women. The direction and magnitude of the differences did not change when the analysis was limited to serous cancers (data not shown). We chose not to evaluate marker combinations because we believe that optimizing marker panels using diagnostic samples collected from symptomatic patients is not likely to be relevant for early detection. However, based on our data, it is unlikely that the diagnostic performance of a combination marker that includes CA125, mesothelin, and/or HE4 will vary by patient risk status.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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