Serum protein markers for early detection of ovarian cancer

Gil Mor*†, Irene Visintin*, Yinglei Lai‡, Hongyu Zhao§, Peter Schwartz*, Thomas Rutherford*, Luo Yue¶, Patricia Bray-Ward¶‖, and David C. Ward¶‖**

Departments of *Obstetrics and Gynecology, §Epidemiology and Public Health, and ¶Genetics, Yale University School of Medicine, New Haven, CT 06510; ‡Department of Statistics, The George Washington University, Washington, DC 20052; and ‖Nevada Cancer Institute, Las Vegas, NV 89135

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Early diagnosis of epithelial ovarian cancer (EOC) would significantly decrease the morbidity and mortality from this disease but is difficult in the absence of physical symptoms. Here, we report a blood test, based on the simultaneous quantization of four analytes (leptin, prolactin, osteopontin, and insulin-like growth factor-II), that can discriminate between disease-free and EOC patients, including patients diagnosed with stage I and II disease, with high efficiency (95%). Microarray analysis was used initially to determine the levels of 169 proteins in serum from 28 healthy women, 18 women newly diagnosed with EOC, and 40 women with recurrent disease. Evaluation of proteins that showed significant differences in expression between controls and cancer patients by ELISA assays yielded the four analytes. These four proteins then were evaluated in a blind cross-validation study by using an additional 106 healthy females and 100 patients with EOC (24 stage I/II and 76 stage III/IV). Upon sample decoding, the results were analyzed by using three different classification algorithms and a binary code methodology. The four-analyte test was further validated in a blind binary code study by using 40 additional serum samples from normal and EOC cancer patients. No single protein could completely distinguish the cancer group from the healthy controls. However, the combination of the four analytes exhibited the following: sensitivity 95%, positive predictive value (PPV) 95%, specificity 95%, and negative predictive value (NPV) 94%, a considerable improvement on current methodology.

insulin-like growth factor-II | leptin | osteopontin | prolactin

Epithelial ovarian cancer (EOC) is the fourth leading cause of cancer-related death in women in the U.S. and the leading cause of gynecologic cancer death. EOC is characterized by few early symptoms, presentation at an advanced stage, and poor survival. Despite being one tenth as common as breast cancer, EOC is three times more lethal. This year ≈22,220 women will be newly diagnosed with ovarian cancer, and 16,210 will die from the disease (1). The high mortality rate is due to the difficulties with the early detection of ovarian cancer. Indeed, ≈80% of patients are diagnosed with advanced staged disease. In patients who are diagnosed with early disease (stage I or II), the 5-yr survival ranges from 60% to 90%, depending on the degree of tumor differentiation (2, 3). In patients with advanced disease, 80–90% will initially respond to chemotherapy, but <10–15% will remain in permanent remission (4). Although advances in treatment have led to an improved 5-yr survival rate approaching 45%, overall survival has not been enhanced (2, 5).

Two alternative strategies have been reported for early detection by using serum biomarkers. One approach is the analysis of serum samples by mass spectrometry to find proteins or protein fragments of unknown identity that detect the presence/absence of cancer (6–8). Alternatively, analysis of the presence/absence/abundance of known proteins/peptides in the serum using antibody microarrays, ELISA, or other approaches has yielded a number of biomarker combinations with increased specificity and sensitivity for ovarian cancer relative to CA-125 alone (9–11). Serum biomarkers that are often elevated in women with EOC include carcinoembryonic antigen, ovarian cystadenocarcinoma antigen, lipid-associated sialic acid, NB/70, TAG 72.3, CA-15.3, and CA-125. The most commonly used biomarker is CA-125, which identifies a group of cell surface glycoproteins of uncertain biological significance. Although CA-125 is elevated in 82% of women with advanced EOC, it has very limited clinical application for the detection of early stage disease, exhibiting a positive predictive value (PPV) of <10%. Even the addition of ultrasound screening to CA-125 measurement improves the PPV to only ≈20% (6). By efficiently combining information on CA-125II (containing antibody M11 in addition to OC-125), CA-72-4, and macrophage-colony stimulating factor (M-CSF), preoperative early-stage sensitivity was significantly increased from 45% with CA-125II alone to 70%, while maintaining 98% specificity in patients with diagnosed active disease (12). Unfortunately, although this approach has increased the sensitivity and specificity of early detection, published biomarker combinations still fail to detect a significant percentage of stage I/II EOCs (3). The lack of specific markers for EOC makes it difficult to achieve the clinical objective of early detection using noninvasive screening methods. Thus, the identification of other cancer-specific markers for early detection of EOC is essential to improve our ability to accurately detect premalignant changes or early stage EOC in asymptomatic women. A method that is rapid, sensitive, specific, quantitative (at least relative to known standards), and reproducible is required.

In the present study, we describe the characterization of a blood test based on four analytes [leptin, prolactin, osteopontin (OPN), and insulin-like growth factor-II (IGF-II)] that can discriminate between disease-free and cancer patients, including patients diagnosed with stage I/II disease. The process used in developing this panel of protein markers involves several different screening steps using samples obtained from different patient populations and validation with different techniques. A final evaluation was performed in a blind manner with a different cohort and analyzed by multiple statistical approaches including a simple binary assay based on single, statistically derived analyte split points. The final results of the test have shown a sensitivity of 95%, a specificity of 95%, a PPV of 95%, and a negative predictive value (NPV) of 94%.

Materials and Methods

Sample Collection. Ten milliliters of blood was collected from each individual and centrifuged at 800 × *g* for 10 min and the serum fraction was separated, aliquotted, and stored at −80°C in the OB/GYN Tissue bank at Yale University School of Medicine

Abbreviations: PPV, positive predictive value; EOC, epithelial ovarian cancer; RCA, rolling circle amplification; MIF-1, macrophage inhibitory factor-1; OPN, osteopontin; IGF-II, insulin-like growth factor-II; OVCA, ovarian cancer.

†To whom correspondence may be addressed at: Department of Obstetrics and Gynecology, Reproductive Immunology Unit, Yale University School of Medicine, P.O. Box 208063, 333 Cedar Street, New Haven, CT 06520-8063. E-mail: gil.mor@yale.edu.

**To whom correspondence may be addressed at: Nevada Cancer Institute, 10000 West Charleston Boulevard, Las Vegas, NV 89135. E-mail: dward@nvcancer.org.

until further use. Collection, preparation, and storage of the blood samples were done by using guidelines set by the National Cancer Institute Inter-group Specimen Banking Committee. Qualified personnel obtained informed consent from each individual participating in this study.

Patient Population. Serum samples for antibody microarray analysis were collected from 86 individuals as part of a phase I clinical trial to determine the efficacy of phenoxodiol as an EOC therapy. Twenty-eight study participants were healthy and disease-free, 18 were newly diagnosed stage III/IV EOC, and the remaining 40 were patients with recurrent disease (stage III/IV). The average age of the disease-free individuals was 60.8 yr, whereas the average age of the EOC patients was 57.1 yr. None of the patients and age-matched normal controls were receiving hormone replacement therapy (HRT) at the time of sample collection. Any person who had received any type of HRT or EOC therapy <6 months before the enrollment was disqualified for this study. Serum samples used in the blinded ELISA cross-validation study were collected from 100 patients and 106 healthy/disease-free individuals as part of the Yale New Haven Hospital Early Detection program (Human Investigation Committee no. 10425). The age- and weight-matched control group consisted of 66 healthy/disease-free women with no history of disease (average age, 58.4 yr) and 40 high-risk women (having one or more first-degree relatives diagnosed with ovarian cancer) who were disease-free at the time of sample collection (average age, 57.6 yr). Of the 100 patients with EOC, 24 women were diagnosed with stage I/II (average age, 59.5 yr) and 76 women with stage III/IV disease (average age, 63 yr). Finally, an additional cohort of 40 individuals (8 healthy, 32 EOC cancer patients, including 3 with stage I-II) was recruited for blind validation of the marker set with criteria developed by using prior samples.

Serum Analysis Using Cytokine Rolling Circle Amplification (RCA) Microarrays. Antibody microarray screening was done under a paid research subcontract by Molecular Staging (a biotechnology company based in New Haven, CT) by using proprietary technology (13). A total of 169 proteins were analyzed in this RCA study. The

Table 1. Analytes showing a significant ($P < 0.05$) difference in expression between ovarian cancer patients and normal controls

	Healthy			Ovarian cancer			Healthy-ovarian cancer			
Analyte	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	Effsize	*P* value
6Ckine	9.18	0.52	28	9.67	0.69	51	−0.49	0.64	−0.76	0.001813
ACE	12.09	0.43	28	11.67	0.61	51	0.42	0.56	0.76	0.001763
BDNF	13.7	0.92	28	12.82	1.21	51	0.88	1.12	0.79	0.001293
CA125	7.05	0.42	28	11.3	2.45	51	−4.25	1.99	−2.13	<0.000001
E-Selectin	13.83	0.62	28	13.4	0.76	51	0.44	0.71	0.61	0.011176
EGF	8.41	1.63	28	10.14	1.57	51	−1.73	1.59	−1.09	0.000015
Eot2	13.12	1.11	28	12.55	1.2	51	0.57	1.17	0.49	0.04228
ErbB1	11.79	0.39	28	11.36	0.55	51	0.44	0.5	0.87	0.000383
Follistatin	10.26	0.63	28	10.76	1.02	51	−0.49	0.9	−0.55	0.0225
HCC4	13.93	0.59	28	14.17	0.45	51	−0.25	0.5	−0.49	0.04178
HVEM	8.33	0.67	28	8.75	0.7	51	−0.42	0.69	−0.61	0.011777
IGF-II	13.53	0.46	28	13.04	0.53	51	0.49	0.51	0.97	0.000094
IGFBP-1	13.24	1.58	28	13.97	1.34	51	−0.73	1.43	−0.51	0.033016
IL-17	8.78	0.56	28	8.24	0.55	51	0.53	0.55	0.96	0.000105
IL-1srII	9.96	0.6	28	9.48	0.69	51	0.48	0.66	0.72	0.002983
IL-2sRα	13.14	0.67	27	13.77	0.57	51	−0.63	0.6	−1.04	0.00004
Leptin	12.77	1.62	27	10.83	2.78	51	1.94	2.44	0.79	0.00134
M-CSF R	12.98	0.35	28	12.78	0.37	51	0.19	0.37	0.53	0.027136
MIF	10.75	0.75	28	11.82	0.75	51	−1.07	0.75	−1.42	<0.000001
MIP-1a	6.85	0.69	28	6.45	0.73	51	0.4	0.71	0.56	0.020757
MIP3b	7.55	0.73	28	7.92	0.8	51	−0.37	0.77	−0.48	0.043303
MMP-8	13.92	1.03	28	14.53	0.82	51	−0.61	0.9	−0.68	0.004956
MMP7	11.57	0.48	28	12	0.58	51	−0.43	0.55	−0.79	0.001262
MPIF-1	9.27	0.6	28	9.9	0.7	51	−0.63	0.67	−0.94	0.000155
OPN	12.62	0.79	28	13.81	0.69	51	−1.2	0.73	−1.64	<0.000001
PARC	14.21	0.2	28	14.38	0.23	51	−0.17	0.22	−0.78	0.001318
PDGF Rb	10.74	0.97	28	10.13	1.13	50	0.61	1.08	0.56	0.019795
Prolactin	11.01	0.51	28	11.75	1.12	51	−0.74	0.95	−0.78	0.001445
ProteinC	13.59	0.31	28	13.24	0.38	51	0.35	0.36	0.97	0.000089
TGF-b RIII	10.46	1.15	28	11.46	1.12	51	−1	1.13	−0.88	0.000344
TNF-R1	10.14	1.23	28	10.73	1.18	50	−0.59	1.2	−0.5	0.039197
TNF-a	7.06	0.97	28	6.3	0.7	51	0.75	0.8	0.94	0.000152
VAP-1	14.06	0.28	24	13.78	0.65	44	0.29	0.55	0.52	0.042888
VEGF R2	8.84	0.38	28	8.59	0.49	51	0.26	0.46	0.56	0.0189
VEGF R3	10	0.55	28	9.51	0.67	51	0.49	0.63	0.78	0.001388

6Ckine; ACE, angiotensin I-converting enzyme; CA125, cancer antigen 125; Eot2, eotaxin 2; ErbB1, epidermal growth factor receptor 1; HCC4, hemofiltrate CC chemokine 4; HVEM, herpesvirus entry mediator; IGFBP-1, IGF-binding protein-1; IL-1srII, IL-1 soluble receptor II; IL-2sRα, IL-2 soluble receptor α; M-CSFR, macrophage colony-stimulating factor receptor; MIP, macrophage-inflammatory protein; MMP, matrix metalloproteinase; MPIF, myeloid progenitor inhibitory factor; PARC, pulmonary and activation-regulated chemokine; PDGF, platelet-derived growth factor; VAP, vesicle-associated membrane protein-associated protein A.

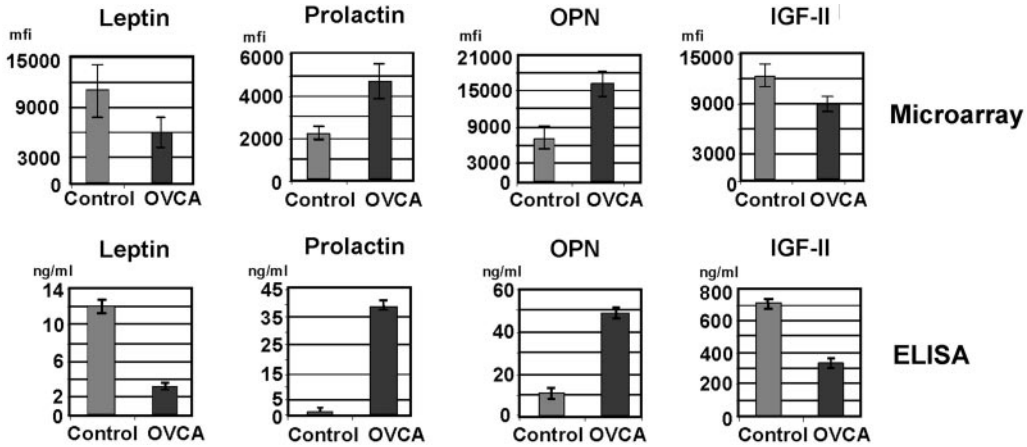

Fig. 1. Four tumor markers identified for which the RCA microarray data and ELISA data were completely concordant in classifying serum from normal and EOC individuals in the training cohort. Microarray data are expressed as median fluorescence intensity (mfl); ELISA data are expressed as average protein concentration (ng/ml).

full list of these proteins and the cognate antibodies used on the microarray are considered proprietary information of Molecular Staging and must be obtained directly from the company.

Serum Analysis Using ELISA Analysis. ELISA assay kits for each of the analytes selected for further analysis were purchased from Diagnostic Systems Laboratories (Webster, TX) or Assay Designs (Ann Arbor, MI). These analytes were as follows: EGF, macrophage inhibitory factor-1 (MIF-1), TNF-α, leptin, prolactin, IL-17, OPN, and IGF-II. Assays were performed following kit instructions. Plates were read on a Spectra Max M2 Microplate Reader (Molecular Devices) with the appropriate baseline correction for each assay.

Statistical Analysis. ANOVA was used to test the significance of the protein expression differences between ovarian cancer patients and healthy controls, by using the General Linear Mode procedure of SAS. Sample size effects were measured as the difference in mean between the two groups, normalized by within-group standard deviation: Effect Size = (Mean_Group1-Mean_Group2)/Std_Group1_Group2.

To differentiate between normal/high risk and ovarian cancer patients, three commonly used classification methods were used: support vector machine (SVM), k-nearest neighbors (k-NN), and classification trees (14). We used either leave-one-out or a 10-fold cross-validation procedure to evaluate the initial classification accuracy. To obtain a distribution of classification accuracy, the 10-fold cross-validation was run 1,000 times.

In addition to these three classification methods, we used split-point analysis to produce a score-based classification method that is more biologically interpretable. Split points are derived as follows. Suppose there are n samples classified into two groups. For each marker X, let $x_1, x_2, \ldots, x_n$ be the observed measurements. We screen $n-1$ split points $y_1, y_2, \ldots, y_{n-1}$, where $y_k = (x_k + x_{k+1})/2$ for $k = 1, 2, \ldots, n-1$. For each split point y_k, there are a_1 and a_2 observed measurements less than y_k in the first and the second groups, respectively; and there are b_1 and b_2 observed measurements greater than y_k in the first and the second groups, respectively. If the left and the right sides of y_k are assigned to the first and the second groups, respectively, then there are $a_2 + b_1$ misclassified samples (i.e., as either a false positive or a false negative). If the left and the right sides of y_k are assigned to the second and the first groups, respectively, then there are $a_1 + b_2$ misclassified samples. We choose the assignment of a single split point that minimizes the number of misclassified samples. For each marker, the split point defines two intervals: one for normal and another for cancer. Score 0 is assigned to an individual if the related observation falls in the normal interval; otherwise, score 1 is assigned. Overall, an individual is assigned a score as the sum of these assigned scores from m different markers. Therefore, the range of such scores is between 0 and m. A given threshold t is chosen to optimally separate cancer from healthy individuals; e.g., a given individual with a total score $\leq t$ is predicted to have normal status, whereas an individual with a score $>t$ will be diagnosed to have disease. By using these four biomarkers, a score of 1 or below is required for healthy status, whereas a score of 2 or above indicates cancer.

Results

We first analyzed the expression levels of 169 proteins in serum samples from 18 untreated EOC patients and 28 healthy, age-matched controls by means of RCA immunoassay microarray (13). In this initial screen, 35 proteins were differentially expressed between healthy women and newly diagnosed EOC patients based on ANOVA tests, with P values of <0.05. These results are shown in Table 1.

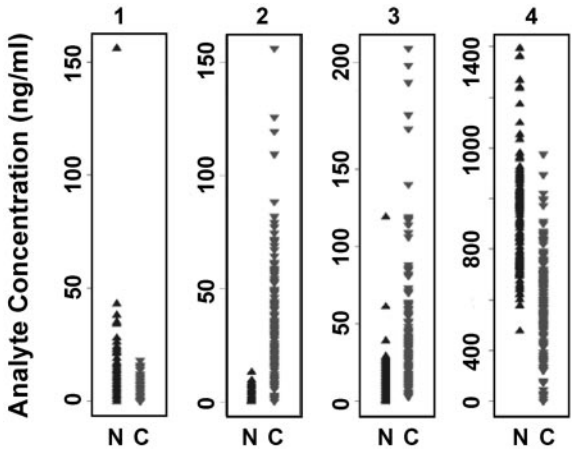

Fig. 2. Analyte concentration distributions in ELISA assays of leptin (graph 1), prolactin (graph 2), OPN (graph 3), and IGF-II (graph 4) from 106 normal (N) and 100 EOC (C) individuals. The average concentrations were as follows: leptin, 12 ng/ml (N) and 3 ng/ml (C); prolactin, 1 ng/ml (N) and 40 ng/ml (C); OPN, 11 ng/ml (N) and 49 ng/ml (C); and IGF-II, 716 ng/ml (N) and 350 ng/ml (C). No single assay can completely distinguish the cancer group from the normal group.

Table 2. Statistical analysis results using three different methods

Classification methods	Leave-one-out, %	1,000 10-fold cross-validation, %				
		Min	Max	Median	Mean	95% C.I.
SVM	96.6	96.1	97.6	96.6	96.5	(96.1, 97.1)
3-NN	93.2	91.3	95.1	93.2	93.2	(91.7, 94.2)
Tree	94.2	91.7	96.1	94.6	94.5	(93.2, 95.6)

SVM, support vector machine; 3-NN, 3-nearest neighbors; Tree, classification trees.

A subset of proteins were selected for independent ELISA testing because (*i*) they exhibited significant differences in protein expression between the 28 individuals used as the control group for microarray analysis and 40 additional patients with recurrent EOC, (*ii*) similar levels of expression were observed in newly diagnosed patients and patients with recurrent disease, (*iii*) ELISA tests were available commercially for these analytes, and (*iv*) they had compelling biological reasons for their evaluation. These analytes were: EGF, MIF-1, TNF-α, leptin, prolactin, IL-17, OPN, and IGF-II. Based on ELISA testing of 50 subjects from the original sample set (25 control, 25 EOC), EGF, TNF-α, and IL-17 did not provide consistent differentiation between the cancer and control serum samples. Although MIF-1 was a promising marker, we were unable to identify ELISA kits that were reliably available to continue testing; thus, MIF-1 was not examined further.

As shown in Fig. 1, four proteins (leptin, prolactin, OPN, and IGF-II) showed perfect correlation between the RCA microarray immunoassays and the ELISA assays and were able, when used together, to completely discriminate the control and cancer training group samples.

This combination of four biomarkers (leptin, prolactin, OPN, and IGF-II) was then assayed in a blinded cross-validation study consisting of 206 serum samples, which included samples from 106 healthy subjects and 100 ovarian cancer subjects stages I-IV. The concentration ranges for each analyte in serum from the normal individuals and EOC patients are shown in Fig. 2. As can be seen, both prolactin and OPN are significantly elevated in EOC serum, whereas leptin and IGF-II levels are reduced.

To differentiate between subjects with ovarian cancer and healthy subjects after sample decoding, statistical cluster analysis was performed. None of the four markers could reliably separate the normal and cancer groups using the least squares fit in a traditional binary analysis of the data set in Fig. 2, although pair plots of the four markers showed better separation between subjects in these two groups (data not shown).

Three commonly used classification methods [support vector machine (SVM), 3-nearest neighbors (3-NN), and classification trees (Tree)] were used to evaluate the classification accuracy with either leave-one-out or 10-fold cross-validation procedure. For 10-fold cross-validation, 1,000 reiterations were run to obtain a distribution of classification accuracy. Table 2 summarizes these classification results.

To develop a rapid assessment method for future testing, we used data from the 206 samples to develop split points for each of the markers. The split point divides the sample space into two intervals: one for normal and another for cancer. The best split point for each marker was chosen to minimize the number of misclassified individuals. A score of 0 is assigned to an individual if the related analyte value falls in the normal interval; otherwise, a score of 1 is assigned. Table 3 gives the split-point concentration scoring criterion for each analyte.

Individual marker classifications using the split-point system were inadequate to classify cancer from normal when used as single markers. However, by using split-point analysis with four markers in which cancer is predicted by having two or more markers with a score of 1, 96 of 100 EOC patients (96%) were correctly diagnosed with ovarian cancer (including 23 of 24 patients with stage I/II EOC). In the healthy group, 6 of 106 individual were diagnosed incorrectly (5.6%).

Finally, an additional 40 samples were analyzed in a blind fashion by using the split-point classification scheme, and the split points previously determined were used to assign individuals to either normal or EOC groups. Thirty-seven of the 40 samples were correctly identified. The results of the split-point scoring method for all 246 individuals tested in the blind studies are presented in Fig. 3. The overall performance characteristics were as follows: sensi-

Table 3. Split-point scoring for each marker

Marker	Split point	Left interval	Right interval
Leptin	2.5	Cancer (1)	Normal (0)
Prolactin	10	Normal (0)	Cancer (1)
OPN	21	Normal (0)	Cancer (1)
IGF-II	491	Cancer (1)	Normal (0)

The split-point scoring procedure is as follows. For each marker, find the best split point to minimize the number of misclassified individuals. The split point divides the sample space into two intervals: one for normal and another for cancer. A score of 0 is assigned to an individual if the related analyte value falls in the normal interval; otherwise, a score of 1 is assigned. Overall, an individual is assigned a score as the sum of the assigned scores from four different markers, the range of such scores being 0–4.

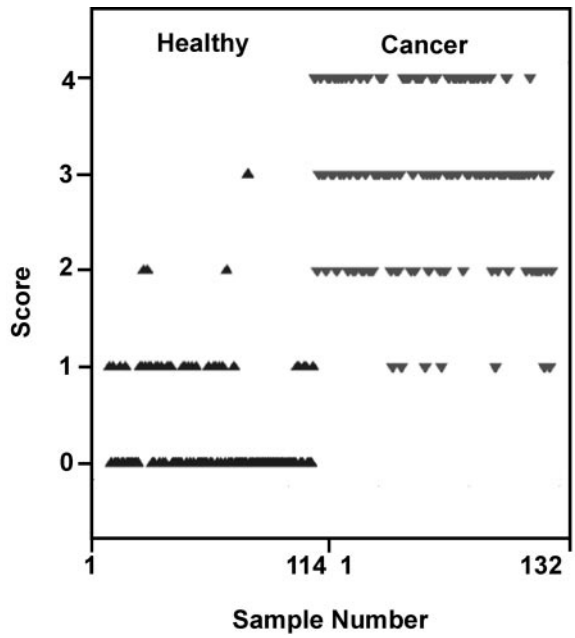

Fig. 3. Four-analyte split-point analysis of serum from healthy women (Healthy) and women with EOC (Cancer). Correctly classified normal serum should have a score of 0 or 1, whereas samples from cancer patients have a score of 2, 3, or 4. False-positive and false-negative samples are readily detected.

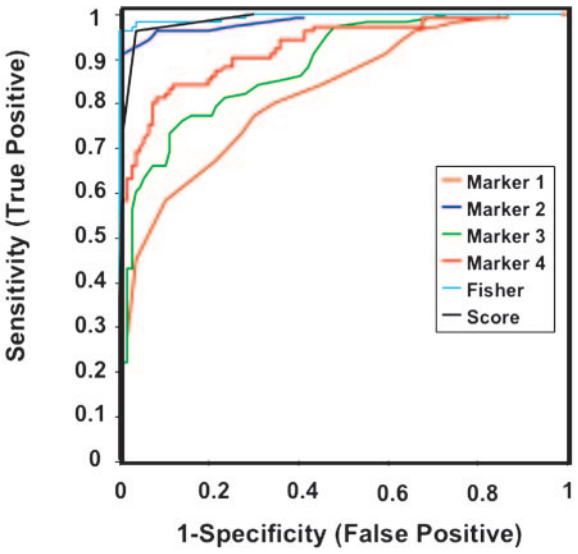

Fig. 4. Receiver operating characteristic (ROC) curves for each individual analyte and a combination of all four analytes. Marker 1, leptin; Marker 2, prolactin; Marker 3, OPN; Marker 4, IGF-II; Fisher, all four analytes using Fisher's linear discriminant analysis; Score, all four analytes using split-point analysis.

tivity 95%, specificity 95%, PPV 95%, and negative predictive value 94%. The predictive performance of each individual marker relative to the combined observations for the four markers was determined by plotting sensitivity (true positive) against 1-specificity (false positive) values. The resultant receiver operating characteristic (ROC) curves (Fig. 4) clearly demonstrate the enhanced predictive performance of combined marker analysis, although prolactin performed quite well as an individual marker.

Discussion

Epithelial ovarian cancer is the leading cause of death from gynecologic malignancies in the U.S., primarily due to the lack of a sensitive screening method. In the present study, we describe development of a blood test, based on four biomarkers, that exhibits a high specificity and sensitivity. This test is able to differentiate healthy individuals from ovarian cancer patients with an overall sensitivity/specificity of ≈95%, including 26/27 patients with stage I and II EOC.

Ovarian cancer is a "relatively silent" disease with intraabdominal inaccessibility that makes the monitoring and early detection of the disease using a noninvasive approach (such as serum tumor markers) an attractive idea (15). This test should improve our ability to accurately detect premalignant change or early stage ovarian cancer in asymptomatic women at increased risk for the development of ovarian cancer. However, it has been suggested that any screening strategy for early detection must achieve a minimum of 99.6% specificity (16). Given the rarity of ovarian cancer, very low levels of false positive classification will result in a large number of women being incorrectly classified as potentially having ovarian cancer. Thus, there is significant need for further improvement of the four-analyte test reported here if the assay is to be used for general population screening. Inclusion of MIF-1, CA125, or other proteins previously implicated in EOC discrimination may further enhance the sensitivity/specificity of such tests. We also assert that initial serum screening of high risk individuals for a combination of such analytes, if confirmed by retesting, should be followed by additional evaluations, such as transvaginal ultrasound. This approach should provide a sufficiently low false-positive rate to justify subsequent laparoscopic surgery on individuals with detectable pelvic masses.

The general strategy for biomarker discovery reported here does not require a large number of initial samples to identify potential biomarkers. Furthermore, these potential biomarkers can then be evaluated, both singly and in combination, by using conventional ELISA assays. It is interesting to note that all of the four biomarkers reported here have been suggested as potential cancer biomarkers by other research groups, although they have never been tested previously as a set. For example, prolactin levels have been reported to be elevated in breast and prostatic cancer (17, 18). Similarly, OPN levels have been reported to be elevated in cancers of the breast (19), prostate (19), lung (19), colon (19), and pancreas (20), multiple myeloma (21), as well as ovarian cancer (22, 23). IGF-II has been reported to be decreased in breast cancer (24). These observations are consistent with the EOC data provided here. Studies on serum leptin present a more complex and paradoxical picture. *In vitro* studies consistently show that leptin has angiogenic and proliferative potential in cancer (25). Several reports document higher leptin levels associated with cancer risk when leptin serum levels are measured prospectively (26, 27). Normal or elevated leptin levels have been documented in analyses of individuals done at time points >1.5 yr before cancer detection for colorectal (ref. 26, elevated) and breast cancer (ref. 27, normal). However, when leptin levels are measured at the time of diagnosis in patients with gastrointestinal and other cancers, serum leptin levels are lower than controls (28, 29). Thus, the levels of leptin found in serum may be significantly affected by the timing of serum collection relative to disease onset.

The markers that discriminate between normal and ovarian cancer reported here may not be specific markers for EOC. Indeed, preliminary studies on a limited number of sera from women with other cancers (breast and uterine) exhibit positive results, in contrast to normal individuals, when assayed by the split-point four-analyte assay, whereas women with benign ovarian and uterine disease score negative. Although the discrimination between potential breast and ovarian cancer may pose a problem with this set of biomarkers, breast cancer is relatively easily detected at early stages by mammography, magnetic resonance, and thermal infrared imaging. The extent to which leptin, OPN, prolactin, and IGF-II can serve as potential biomarkers of cancers other than EOC must be investigated rigorously. Nevertheless, the data presented here support the existence of a highly accurate and distinct multiplex proteomic set that can accurately distinguish between normal and EOC patients, including stage I and II. In summary, our test showed 95% sensitivity, 95% specificity, a PPV of 95%, and a negative predictive value of 94% using known serum protein markers.

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