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Serum biomarker panels for the discrimination of benign from malignant cases in patients with an adnexal mass

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Abstract

Objectives—The diagnosis of an adnexal mass is a prevalent issue among women in the United States while current methods of identifying those at high risk of malignancy remain insufficient. Ineffective triage of women with malignant masses is associated with delayed or inappropriate treatment and a negative effect on disease outcome.

Methods—We performed an evaluation of 65 ovarian cancer-related biomarkers in the circulation of women diagnosed with an adnexal mass. Our subject group consisted of women diagnosed with benign masses and early and late stage ovarian cancer.

Results—Over half of the biomarkers tested were found to differ significantly between benign and malignant cases. As individual markers, HE4 and CA 125 provided the greatest level of discrimination between benign and malignant cases and the combination of these two biomarkers provided a higher level of discriminatory power than either marker considered alone. Multivariate statistical analysis identified several multi-marker panels that could discriminate early stage, late stage, and combined ovarian cancers from benign cases with similar or slightly improved SN/SP levels to the CA 125/HE4 combination, however these larger panels could not outperform the 2-

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biomarker panel in an independent validation set. We also identified a 3-biomarker panel with particular utility in premenopausal women.

Conclusions—Our findings serve to advance the development of blood-based screening methods for the discrimination of benign and malignant ovarian masses by confirming and expanding upon the superior utility of the CA 125/HE4 combination.

Introduction

According to current estimates, 1.4% of women born today, or 1 in 72, will be diagnosed with ovarian cancer at some point in their lifetime. This year in the United States, there will be over 21,000 new cases of ovarian cancer along with over 15,000 deaths. [1] These cases arise from a much larger group of women presenting with adnexal abnormalities. The overall prevalence of adnexal abnormalities is estimated at 7% [2,3] and it is expected that 5-10% of American women will receive prophylactic surgery for suspected ovarian cancer at some point in their lives [2]. A pelvic exam is the primary clinical method by which adnexal masses are diagnosed and it is estimated that for each case of ovarian cancer identified, 10,000 pelvic exams will be performed [3]. A patient's age and menopausal status are important factors to consider upon the identification of an adnexal abnormality as the associated risk of malignancy increases from 13% in premenopausal women to 45% in postmenopausal women [4].

While nearly all women diagnosed with ovarian carcinoma will initially present with an adnexal mass, only a small proportion of all masses detected will be malignant and the expeditious triage of these patients is the most important component of their treatment regimen. The burden of early identification of potential ovarian cancer falls predominantly upon the obstetrician/gynecologist whose training in the management of cancer patients is usually limited. While these practitioners can effectively manage the high percentage of patients diagnosed with functional cysts and benign neoplasms through observation and surgery, respectively [5,6], the clinical outcome for a patient presenting with a malignant mass can be drastically worsened if she is not immediately referred to a gynecological oncologist [7]. A series of diverse studies have demonstrated a decrease in the relative risk of reoperation [8], and increases in disease-free interval [9] and overall survival [10] for women operated on by gynecological oncologists compared to gynecologists and general surgeons. Despite these findings, referral rates remain disappointingly low for patients diagnosed with an adnexal mass [11]. Improvements upon current screening methodologies and the emergence of new techniques should aid general gynecologists in making appropriate referral decisions and thus, improve the effectiveness of ovarian cancer treatment.

While useful in the identification of an adnexal mass, a pelvic examination is ineffective in discriminating benign and malignant lesions. Transvaginal ultrasonography has proven useful as a secondary screening tool, however its utility as a screening tool remains questionable given its demonstrated low positive predictive value and clinically insufficient levels of sensitivity [12]. Advanced imaging techniques such as CT or MRI have proven too expensive for widespread use given their limited SN and SP. In addition to a family history, pelvic examination, and imaging, the CA 125 blood test is a standard component in the complete evaluation of an adnexal mass. Despite its widespread use as a biomarker, CA 125 has demonstrated disappointingly low SP and SN in all evaluated patient cohorts and particularly in pre-menopausal patients [13]. Although CA 125 is associated with ovarian cancer in 80% of tested women over the age of 50, this association drops to less than 25% for women below that age [14]. The development of improved diagnostic screening tests for ovarian cancer is paramount in efforts to effectively triage patients presenting with an adnexal mass. Recently, Richard Moore and collaborators, in an analysis of serum concentrations of CA 125, SMRP, HE4, CA72-4, activin, inhibin, osteopontin, epidermal growth factor receptor (EGFR), and

ErB2 (Her2) from women undergoing surgery for an adnexal mass demonstrated the clinical utility of a CA 125/HE4 combined test for the discrimination of benign and malignant ovarian masses with 76.4% SN at 95% SP [15,16].

We performed an extensive analysis of 63 additional circulating proteins found in the serum of a large group of patients diagnosed with an adnexal mass. Our objective was the identification of a biomarker panel that will surpass the CA 125/HE4 combination for discrimination of benign from malignant disease.

Materials and Methods

Human serum samples

The training and premenopausal sets consisted of serum samples obtained from four sources as outlined in Supplementary Table S1. Cancer patients were women with histologically diagnosed epithelial ovarian cancer, while the benign group consisted of women diagnosed with a spectrum of benign adnexal lesions. Patients diagnosed with pelvic inflammatory disease (PID) were not included. The complete diagnostic breakdown of the study population is presented in Table 1. The training and validation sets consisted of postmenopausal women. A distinct group of premenopausal patients was considered separately and a cutoff age of 48 was utilized to establish menopausal status. FSH serum levels in women age 48-55, obtained during biomarker testing, were used to confirm menopausal status with levels >25mIU/ml indicating postmenopausal. Procedures for serum collection, processing, and storage have been previously described [17]. Written informed consent was received from each subject and protocols were approved by appropriate institutional review boards.

Multiplex bead-based immunoassay

The xMAP™ bead-based technology (Luminex Corp., Austin, TX) permits simultaneous analysis of numerous analytes in a single sample. Sixty-five bead-based xMAP™ immunoassays for a variety of known or potential biomarkers for ovarian and other epithelial cancers were obtained and utilized in the present study (Table 2). The training set was analyzed for the complete set of 65 biomarkers. The premenopausal group was analyzed for a subset of 19 biomarkers chosen based on the results of the training set analysis and other published findings. The validation set was analyzed for the most informative markers identified in the training set. Multiplexed assays, data acquisition, and analysis were performed according to the manufacturer's protocol or as described previously [17].

Statistical Analysis

The Mann-Whitney U test was used to evaluate the significance of differences in serum biomarker levels expressed as observed concentrations between patients diagnosed with benign adnexal masses and ovarian cancer. The minimum level of significance was $p < 0.05$. The multivariate analysis of the biomarker data was performed using the Metropolis algorithm with Monte Carlo simulation (described in detail in Supplementary Materials and previously [18]). All development of multivariate statistical models for distinguishing ovarian cancer cases from benign controls was restricted to the training set. All possible panels consisting of 2, 3 and 4 biomarkers were evaluated for SN at 85% SP. Optimal panels were chosen that offered high cross-validated SN for both early and late stage ovarian cancer and high specificity for benign pelvic disease. These panels and method of combination were evaluated on the validation set to estimate, free from selection bias, the models' SN and SP. Premenopausal cancer patients were considered without stage stratification due to the limited number of late stage cases.

Results

Analysis of individual biomarker levels in benign and malignant adnexal masses

Of the 65 biomarkers tested in the training set, 34 demonstrated significant differences between benign and malignant cases (Table 3). Ovarian malignancy was associated with an increase in circulating levels of 26 tested biomarkers and a decrease in levels of 8 biomarkers. The comparison involving late stage ovarian cancer resulted in 33 biomarkers demonstrating significant differences, compared with 21 biomarkers for early stage cancer and 28 biomarkers for the combined set of cancer patients. CA 125 provided the highest level of discrimination of benign from malignant cases among early stage tumors while HE4 performed best in the late stage disease group (Table 5).

Sera obtained from 76 premenopausal subjects were tested for 19 biomarkers chosen based on the results from the postmenopausal subjects and other published findings. Eleven biomarkers demonstrated significant differences between cancer and benign cases (Table 4). CA 125 provided the highest level of discrimination of benign from malignant cases for any single biomarker evaluated in this group. With the exception of eotaxin-1, all trends in biomarker levels between benign and malignant cases were consistent for the premenopausal and postmenopausal analyses, however the level of significance for many of the tested biomarkers was lower in premenopausal subjects, possibly a result of the smaller sample size. Eotaxin-1 was found to be significantly decreased in the sera of late stage ovarian cancer patients in comparison to benign cases for premenopausal subjects, the opposite of what was found in postmenopausal subjects.

Multivariate analysis of biomarker levels utilizing the MMC algorithm

The classification performance for the best single, 2-, 3-, and 4-biomarker panels identified in our analysis are presented in Table 5. The combination of CA 125 and HE4 was the best performing 2-biomarker combination of all studied 2-biomarker combinations (data for other 2-biomarker combinations are not shown) classifying cancer from benign cases at a SP of 85% with a SN of 74.2% in early cancers, 91.7% in late cancers, and 83% in the combined group. This combination outperformed either CA 125 or HE4, considered individually, in all evaluated disease classes. Our analysis identified three 3-biomarker panels that demonstrated a classification power that was equal to or modestly better than the CA 125/HE4 combination in the training set. Each of these panels contained the CA 125/HE4 combination along with a third biomarker: CEA, Cyfra 21-1, or EGFR. The best 4-biomarker panel consisted of CA 125, HE4, CEA, and Cyfra 21-1 and demonstrated improved SN over the CA 125/HE4 combination in each disease class in the training set. However, when applied to the validation set, each of the identified 3- and 4-biomarker panels performed at a level equal to but did not improve upon the CA 125/HE4 combination. Cut points for the upper 95th percentile of the benign groups were determined for each of the five biomarkers included in the top panels. These values along with the corresponding sensitivities for the combined postmenopausal cancer set are presented in Table S2.

Each of the single and multi-marker panels identified in the analysis of the training set was subsequently applied to the set of premenopausal subjects (Table 5) as the small size of this group precluded any meaningful development of panels based on it alone. In this group, CA 125 alone provided the highest SN and SP at 70.7% and 87.5% respectively. The addition of HE4 to CA 125 did not improve the SN and SP of the test, however a 3-biomarker panel consisting of CA 125, HE4, and EGFR did significantly improve the classification power, demonstrating a SN of 75.9% at 87.5% SP. None of the other identified multi-marker panels provided any appreciable improvement over CA 125 alone in the premenopausal group.

Discussion

With the exception of highly invasive procedures such as biopsy and surgery, the evaluation of circulating biomarkers offers the most definitive means of distinguishing benign from malignant pelvic masses. Several recent studies have evaluated various panels of circulating biomarkers in ovarian cancer patients and benign cases [15,19-22], however our study is the largest and most diverse to date to utilize biomarker profiles to discriminate between the two conditions. Of the 34 descriptive biomarkers identified in our study (Table 3), 24 are in agreement with observations made in the above-referenced studies and the reader is referred to those works for a discussion of the proposed role of each biomarker in the development of ovarian cancer. To the best of our knowledge, we are the first to describe significant differences in circulating levels of CA 15-3, Her2/neu, ACTH, DR5, sFas, sFasL, IGFBP-1, eotaxin-1, TNFRI, and kallikrein 10 in patients diagnosed with benign and malignant adnexal masses. Although most commonly associated with breast cancer, the tumor markers CA 15-3 and Her2/neu have both been previously implicated in the development of ovarian cancer [23-25]. Secretion of the pituitary hormone ACTH has been observed in a number of ovarian tumors and cell lines and has been implicated in the development of Cushing's syndrome among ovarian cancer patients in rare cases [26,27]. The apoptotic mediators DR5, Fas, and FasL have each been previously investigated in ovarian cancer resulting in the observations that elevated DR5 expression correlates with decreased overall survival [28], and the macrophage infiltrate in ovarian cancer expresses high levels of Fas and FasL [29]. Although specific roles for IGFBP-1, TNFRI, and Kallikrein 10 remain to be characterized, these biomarkers have all been shown previously to be associated with the development and progression of ovarian cancer [30-32]. The CC chemokine eotaxin-1 is an emerging biomarker for ovarian cancer with recently described serum level correlates and *in vitro* tumorigenic effects [33].

The biomarker analysis described herein provides a revealing cross-section of the physiological conditions resulting from ovarian malignancy in comparison to benign disease. Further identification of the precise roles and origins of these biomarkers will greatly improve our understanding of ovarian tumorigenesis. While the nature of our analysis does not permit such a complete characterization, we do provide a fairly comprehensive foundation for subsequent targeted biomarker studies. An overview of our findings regarding individual biomarker levels is presented in Figure 1. As shown in the heat map (Figure 1A), HE4 was the most highly elevated biomarker among cancer patients, followed by Cyfra 21-1, CA 125, and CA 19-9. This observation is reflected in the inclusion of several of these markers in our most powerful discriminatory multimarker panels. In the vast majority of cases, the magnitude of biomarker level changes, both up-regulated and down-regulated, was significantly more pronounced in late stage disease and for many biomarkers, the observed differences were significant in only the late stage patients. These findings illustrate the challenges associated with the detection of early stage disease and the need to identify more informative biomarkers. Aside from the preponderance of tumor-associated antigens near the top of the heat map, no appreciable trend in biomarker category distribution is apparent in this analysis. Trends in biomarkers levels according to category are presented in Figure 1B. The distribution reveals a complex network of biological factors mediating inflammation, proliferation, apoptosis, and tissue remodeling at work during ovarian tumorigenesis. Additional work aimed at further characterizing this biomarker network in terms of function and origin is underway in our laboratory and should add valuable insight to diagnostic efforts.

Our analysis reaffirms the superior utility of the CA 125/HE4 combination reported by Moore et al. for the diagnosis of ovarian cancer [15,16] as this combination was able to discriminate cancer patients from benign cases with sensitivities ranging from 74.2% for early stages to 91.7% for late stages at 85% SP and was also included in each of the high performing 3- and 4-biomarker panels we identified. This observation is significant given the much larger array

of biomarkers examined and the more diverse set of subjects with regard to disease stage and menopausal status utilized in our study. The individual and combined sensitivities and specificities of CA 125 and HE4 observed here are very similar to those observed by Moore et al. [15,16], as is the observation that the two biomarkers display diagnostic complementation as each improves upon the discriminatory power of the other. In addition to CA 125 and HE4, we identified 3 other circulating biomarkers in our training analysis that offered at least modest improvement in discriminatory power when added to the 2-biomarker combination. Carcinoembryonic antigen (CEA) has been used to monitor colorectal cancer for decades and is reported to be elevated in 30-65% of ovarian epithelial cancers [34] although as an individual marker several limitations have been noted [35]. A fair amount of work has been devoted to the assessment of the Cyfra21-1 test in a variety of human cancers including lung [36], esophageal [37], head and neck [38], and cervical [39]. Recently, Baron et al. reported an increase in serum levels of Cyfra21-1 in ovarian cancer patients in comparison to benign cases and an association with disease stage [40]. Our findings regarding EGFR reaffirm those of several other groups in that lower serum levels of this marker are associated with ovarian cancer in comparison to benign cases [15,19,41]. Although the results of our validation analysis do not support the inclusion of CEA, Cyfra 21-1, and EGFR in a diagnostic panel at this time, our results further implicate these biomarkers in the clinical development of ovarian cancer. Future investigations utilizing additional refinements in screening methodology may uncover more precise roles for these biomarkers in the diagnostic setting.

In our analysis of premenopausal subjects, we found that CA 125 alone provided the highest SN and SP, 70.7% and 87.5%, respectively, of any individual biomarker tested. This runs counter to most current notions concerning a lack of specificity for CA 125 in premenopausal women. One plausible explanation for this is the enrichment of CA 125 positive women in our limited patient set. All of the women in this set were initially evaluated for an adnexal mass and CA 125 results would be expected to receive priority consideration in patients in this age group, for which malignancy is more uncommon. Our finding that HE4 testing provided a lower SN than CA 125 and resulted in a reduced SN and SP when added to CA 125 also disagrees with the findings of Moore et al [15]. However, such a comparison may not be valid given the considerable discrepancies in experimental design between the two studies and the relatively small number of patients studied. Our premenopausal benign group was considerably smaller, 18 versus 82 subjects, and was compared to an age matched group of cancer cases that contained a high percentage of early stage disease. In the study by Moore et al, the premenopausal benign group was compared to a combined group of cancer patients with a mean age of 65 years and only a small percentage of early stage disease. The biomarker panels identified in our analysis of postmenopausal women were subsequently evaluated in the premenopausal group. It should be noted that this approach may not be optimal given the demonstrated clinical and biochemical differences present in the two populations. We chose this approach based on the small size of the premenopausal group, which prevented independent panel development, and also to evaluate the broader utility of our multimarker panels. We observed that a 3-biomarker panel consisting of CA 125, HE4, and EGFR provided the highest SN and SP of any single biomarker or combination in the premenopausal group. This observation is significant in light of the recent findings of Baron et al. [41]. In that study, the authors conclude that decreased serum levels of EGFR represent a significant risk factor for ovarian cancer with particular relevance to younger, premenopausal women. Thus, our findings expand upon the notion that EGFR may offer subset-specific clinical utility as a biomarker for the early detection of ovarian cancer.

Here we report the identification and evaluation of several novel biomarker panels for the discrimination of benign from malignant cases in women diagnosed with an adnexal mass. Our findings were the result of an extensive analysis of ovarian cancer related biomarkers in the serum of a diverse group of subjects, including a large number of both early and late stage

patients. Our results both corroborate and advance several recent reports regarding the importance of CA 125 and HE4 in this clinical setting and their combined use as a diagnostic test. Continuing efforts to further characterize and implement these developments should lead to improved triage methodologies for women diagnosed with adnexal masses and a positive impact on overall disease outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Summary of biomarkers changes observed in the sera of women diagnosed with benign and malignant adnexal masses

A. Biomarker Heat Map. Values represent the percentage change over observed biomarkers levels in the benign group for the training set: 141 women with benign masses and 264 women diagnosed with ovarian cancer. Values are based on results presented in Table 3. Red indicates an increase in cancer levels over benign while green indicates a decrease. The minimum level of significance in differences was $p < 0.05$ by Mann-Whitney U test (values in italics were non-significant). **B. Biological Trends.** Trends in biomarker level changes observed in malignant versus benign masses are organized according to each biological category of biomarkers evaluated.

Table 1

Clinical characteristics of study population

	Training		Validation		Premenopausal	
	N (%)	Age Range (Median)	N (%)	Age Range (Median)	N (%)	Age Range (Median)
Benign	141 (100)	48-88 (63)	140 (100)	48-84 (65)	18 (100)	15-47 (42)
Histology						
Mucinous	24 (17)		18 (13)		6 (33)	
Serous	51 (36)		57 (41)		7 (39)	
Other/Unknown	66 (47)		65 (46)		5 (28)	
Ovarian Cancer	264 (100)	48-87 (63)	169 (100)	48-86 (62)	58 (100)	27-47 (41)
Stage						
Stage I-II	132 (50)		63 (37)		31 (53)	
Stage III-IV	132 (50)		106 (63)		27 (47)	
Histology						
Clear Cell	26 (10)		11 (7)		4 (7)	
Endometrioid	58 (22)		26 (15)		9 (16)	
Mucinous	11 (4)		4 (2)		14 (24)	
Serous	119 (45)		113 (67)		20 (34)	
Other/Unknown	50 (19)		15 (9)		11 (19)	

Table 2

Complete list of analyzed biomarkers

Biological groups	Proteins	Plex No.	Source
<u>Inflammatory Mediators</u>	IP-10, TNFR I, II, IL-1R α , IL-2R, IL-6R	1	1
	Eotaxin-1, interleukins 1b, 2, 4, 5, 6, 7, 8, 10, 12p70, 13, GM-CSF, IFN- γ TNF- α , MIP-1 β , MIF, CD40L, fractalkine	2	2
	MPO	8	2
<u>Growth/angiogenic factors</u>	EGFR, Her2/neu	3	3
	IGFBP-1	4	3
	TGF α	2	2
<u>Tumor-associated antigens</u>	CA 125, CA 15-3, CEA, CA 19-9, CA 72-4	3	3
	AFP	4	3
	HE4	12	3
<u>Apoptotic proteins</u>	Cyfra 21-1	3	3
	DR5	1	1
	sFas, sFasL	5	2
<u>Proteases/Inhibitors</u>	Kallikrein 10	4	3
	MMP 2, 3, 9	6	4
	TIMPS 1-4	7	4
	tPAI-1	8	2
<u>Adhesion molecules</u>	sICAM, sVCAM	8	2
<u>Hormones</u>	prolactin, TSH, LH, ACTH, FSH, GH	9	2
<u>Adipokines</u>	Adiponectin	8	2
Other markers	Mesothelin	4	3
	SMRP	10	3
	Osteopontin, tissue transglutaminase	11	3
	apolipoprotein A1	13	2
	TTR, SCC	14	3

Source No: 1 - Invitrogen/Biosource, Camarillo, CA; 2 - Millipore/Linco, St. Louis, MO; 3 – Luminex Core Facility, University of Pittsburgh, Pittsburgh, PA; 4 – R&D Systems, Minneapolis, MN

Plex No. indicates multiplexed panel, i.e. biomarkers that were analyzed simultaneously

Table 3

Serum Biomarker Levels Across Adnexal Mass Diagnoses for Subjects in Training Set

Biomarker	Mean Biomarker Level (pg/ml p-value ^d)						
	Benign	All Cancer		Stage I-II		Stage III-IV	
ACTH	36.4	28.1	0.05	32.0	NS	22.1	0.01
ApoA1 ³	2213	2088	0.01	2120	0.05	1858	0.01
CA 19-9 ^l	0.130	0.347	0.001	0.315	0.001	0.471	0.001
CA 125 ^l	3.6	34.5	0.001	23.7	0.001	72.4	0.001
CA-153 ^l	0.49	0.83	0.001	0.66	0.01	1.06	0.001
CA72-4 ^l	8016	11724	0.001	9874	0.001	14748	NS
Cyfra 21-1	669	1421	0.001	1355	0.001	1840	0.001
DR5	120	173	0.001	135	NS	294	0.001
EGFR	19592	16902	0.001	18212	0.001	13713	0.001
EOTAXIN	169.5	185.5	NS	161.5	NS	218.0	0.001
ErbB2	1854	1948	NS	1791	NS	2412	0.001
Fas	4152	4248	NS	4118	NS	4890	0.001
HE4	2645	9621	0.001	5612	0.001	29779	0.001
IGFBP-1	48784	40957	NS	58629	0.01	24078	0.001
IL-10	11.25	18.50	0.001	17.10	0.001	21.85	0.001
IL-1Ra	1193	1288	NS	1035	NS	1751	0.01
IL-2R	578	803	0.001	645	0.05	1401	0.001
IL-6	15.85	24.05	0.001	21.25	0.01	31.95	0.001
IL-7	8.01	10.50	0.001	10.20	0.001	11.65	0.001
IL-8	8.41	12.45	0.001	11.20	0.01	14.95	0.001
IP-10	78.4	79.7	NS	66.6	NS	106	0.001
Kallikrein 10	47082	50163	0.001	48504	0.05	53054	0.001
MMP-2	172917	152645	0.01	154843	0.05	150823	0.001
MMP-9	184359	249059	0.01	241996	0.05	256590	0.01
MPO	38480	59049	0.05	50142	NS	74169	0.01

Biomarker	Mean Biomarker Level (pg/ml p-value ⁴)				
	Benign	All Cancer	Stage I-II	Stage III-IV	
sFasL	43.75	29.60	0.01	36.05	NS
sVCAM-1	821776	716309	0.05	721565	NS
TG II ²	61.80	82.25	0.001		NA
TIMP-1	104826	121653	0.001		NA
TNF-α	6.69	7.23	0.001	6.88	NS
TNF-RI	3090	4132	0.001	3410	0.01
TNF-RII	2240	2806	0.001	2590	0.01
tPAI 1	33723	42974	0.001	41068	0.01
Transferrin	2273	1708	0.001	1921	0.01
				1362	0.001

NS – not significant, NT – not tested, NA – not applicable

¹ U/ml

² mU/ml

³ ng/ml

⁴ minimum level of significance determined by Mann-Whitney U test for comparison of cancer vs. benign biomarker levels

Table 4
Serum Biomarker Levels Across Adnexal Mass Diagnoses for Premenopausal Subjects

	Mean Biomarker Levels (pg/ml p-value ³)				
	Benign	All Cancer	Stage I-II	Stage III-IV	
CA 125 ¹	3.37	43.75	0.001	37.40	0.001
CA72-4 ¹	6.28	17.10	0.001	17.20	0.001
Cyfra 21-1	0.71	59.90	NS	0.70	643
EGFR	16802	14841	0.001	15080	0.01
EOTAXIN	143	101.5	0.05	107	NS
FSH ²	8129	5155	NS	4143	0.05
HE4	2180	5768	0.01	5904	0.05
IL-2R	478	699	0.01	692	0.05
sV-CAM	688369	601087	NS	611459	NS
TNF-RI	2512	3216	0.01	3177	0.01
tPAI 1	38178	51939	0.01	52048	0.01

NS – not significant

¹ U/ml

² μIU/ml

³ minimum level of significance determined by Mann-Whitney U test for comparison of cancer vs. benign biomarker levels

Table 5

Biomarker panels that discriminate benign from malignant cases

Panel	Training					Validation				Premenopausal	
	SP	All	Early	Late	AUC	ROC _I	SP	All	Early	Late	SN
CA 125	85	79.5	72.0	87.1	0.860	63.5	82.1	76.3	61.9	84.9	87.5
HE4	85	70.5	50.8	90.2	0.835	64.3	84.3	83.4	69.8	91.5	93.8
CA 125	85	83.0	74.2	91.7	0.868	68.6	77.9	89.4	79.4	95.3	87.5
CA 125	85	83.0	73.5	92.4	0.872	69.0	77.1	90.5	82.5	95.3	87.5
CA 125	85	84.1	76.5	91.7	0.875	70.7	79.3	85.8	71.4	94.3	81.3
CA 125	85	83.3	75.0	91.7	0.889	70.4	74.3	89.3	81.0	94.3	87.5
CA 125	85	86.4	78.0	94.7	0.878	71.6	75.0	90.0	81.0	95.3	87.5

All values (with exception of AUC) represent percentages (%)

I ROC Area Under Curve (AUC) and Sensitivity (SN) calculated for 90% specificity, Benign vs. Ovarian Cancer