A biomarker panel for distinguishing between malignant and benign ovarian tumors

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Introduction

According to The American College of Obstetricians and Gynecologists (ACOG), 5-10% of women in the US will undergo a surgical procedure for a suspected ovarian neoplasm during their lifetime. A significant proportion (~20%) of women with pelvic masses have malignant disease, and the proportion becomes even higher among post-menopausal patients. It has been demonstrated in several studies that early referral to gynecologic oncologist for laparotomy and appropriate surgical staging of cancer patients improves survival. We have used proteomics approaches to identify novel biomarkers that can potentially distinguish between ovarian cancer and benign pelvic disease as well as healthy individuals, and several biomarkers have been independently verified. In order to characterize these markers, we performed a prospective clinical trial including women with a suspicious pelvic mass. The design of this clinical trial is outlined in figure 1. Pre-operative samples were analyzed for five markers (transthyretin, apolipoprotein A1, transferrin, beta-2 microglobulin, and CA125) using standard immunoassays. Two sets of samples were used to train and test the OvaCalc algorithm, and the algorithm was then applied to an independent validation set. The primary aim of the study was to determine the ability of the OvaCalc algorithm to estimate the risk of malignancy in pre and postmenopausal women who are scheduled for surgery with an ovarian mass.

Materials and Methods

Sample collection. Blood samples from two patient cohorts were utilized in developing and validating the multi-marker test. A training cohort was prospectively collected at the University of Kentucky (UK) from women scheduled for surgery for a known ovarian tumor. Two hundred eighty four patients with ovarian tumors had blood specimens obtained preoperatively and stored in the UK Biospecimens Core. A second patient cohort was collected from a prospective trial (OVA-001-C01) utilizing 27 demographically diverse collection sites, representative of those who would utilize the test. Study inclusion criteria were as follows: female age 18 years or older; signed informed consent; agreeable to venipuncture; no diagnosis of malignancy in previous 5 years (except non-melanoma skin cancer); and a documented ovarian mass with planned surgical intervention. Subject demographic and clinicopathological information was collected and recorded on standardized forms.

Sample measurements. CA125-II was measured on the Elecsys 2010 (Roche) and the other four markers (beta 2 microglobulin, transthyretin, apolipoprotein A1, and transferrin) were measured on the BNII (Siemens).

Training of algorithm. Two training sample sets were used to derive the algorithm. Set 1: 284 pre-operative serum samples were obtained from University of Kentucky, including 175 benign diseases, 29 LMPs, 64 epithelial ovarian cancers, three other primary ovarian malignancies, and 13 other malignancies. Training Set 1 had 274 samples with complete laboratory data, of which 109 were malignant cases and 175 were benign controls for algorithm development. Training Set 2 was a randomly selected subset of 125 pre-operative serum samples from the multi-center prospective collection (OVA-001-C01). The protocol for algorithm validation (OVA-002) mirrored the subject enrollment and collection in OVA-001-CO1. Training set 2 consisted of 89 benign diseases, 10 LMPs, 19 epithelial ovarian cancers, one primary and three non-primary ovarian cancers, and three other malignancies.

Application to prospective set. OvaCalc software was used to import the values for TT, Apo A-1, β2M, Tfr and CA 125 II, reconcile, and numerically combine the values from the five biomarker assays, and use the OVA1 algorithm to generate an ovarian malignancy risk index score for each individual specimen. The output of the OVA1 algorithm is a numeric index between 0.0 and 10.0. Cut-off values at 5.0 for pre-menopausal women and at 4.4 for post-menopausal women were determined based on the training data. The cutoff value classifies a patient based on her OVA1 test score into one of two ovarian malignancy risk zones: low or high.

Results

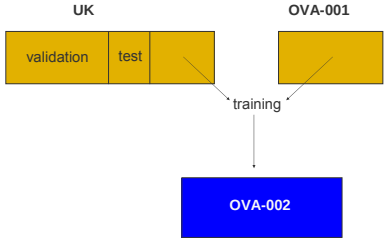

Figure 1. Algorithm development. Parameters for both model and learning algorithms were iteratively varied. Note the 60/40 random division was re-performed for each training session. Models with area-under-curve in ROC analysis > 0.75 for all three data sets were kept for further consideration. Among the retained models, the one with the highest AUC for validation data formed the core classifier of the OVA1 algorithm. The model and cutoff values were fixed and applied to the OVA1 prospective study (the OVA-002 prospective study).

Analyte	Correlation OVA1 with MinusOne	% disagreement OVA1 vs MinusOne
CA125	0.17	32.3
Transthyretin	0.94	13.9
B2m	0.97	8.2
Transferrin	0.97	7.3
Apo A1	0.98	5.2

Table 1. Assessment of contribution of individual markers to OVA1 algorithm. Since the nonlinear OvaCalc algorithm makes it difficult to estimate marker contribution directly, we performed the following analysis. For each marker, for all evaluable subjects (N=524), we substituted test results of one of the five analytes with a single constant, which is the analyte's population mean (estimated over all evaluable subjects). We then computed a "MinusOne (analyte) OVA1" value for all evaluable subjects using the OVA1 software system with this constant and the real test results from the other four analytes. We estimated the correlation between the true OVA1 results and the MinusOne results and calculated the percentage of times MinusOne calls disagreed with OVA1 calls. The higher the disagreement rate, the greater the contribution to the OVA1 algorithm.

Characteristic	All Evaluable Subjects (N= 524)
Age, years	
Mean (SD)	52.1 (13.90)
Range (min to max)	18 to 92
Menopausal status*, n (%)	
Pre	239 (45.6)
Post	285 (54.4)
Pathology Diagnosis, n (%)	
Benign ovarian conditions	363 (69.3)
Epithelial ovarian cancer	96 (18.3)
Other primary ovarian malignancies (not EOC)	9 (1.7)
Low malignant potential (Borderline)	28 (5.3)
Non-primary ovarian malignancies with involvement of the ovaries	18 (3.4)
Non-primary ovarian malignancies with no involvement of the ovaries	10 (1.9)

Table 2. Demographic characteristics and pathology result for all evaluable subjects. *The menopausal status was not specified for 44 patients and has been imputed on the basis of age. Subjects aged more than 50 years are considered post-menopausal, aged 50 years or less as pre-menopausal.

OVA1 test Score	All Evaluable Subjects (N= 524)	Pre-Menopausal Subjects* (N= 239)	Post-Menopausal Subjects* (N= 285)
Sensitivity, %	92.5	86.7	94.8
95% CI	87.4 to 95.7	73.8 to 93.7	89.2 to 97.6
Specificity, %	43.0	52.1	32.5
95% CI	38.0 to 48.1	45.1 to 59.0	25.9 to 39.9
Positive predictive value, %	41.9	29.5	49.1
95% CI	36.8 to 47.0	22.4 to 37.8	42.6 to 55.6
Negative predictive value, %	92.9	94.4	90.2
95% CI	87.9 to 95.9	88.3 to 97.4	80.2 to 95.4

Table 3. Clinical Statistics.

	Epithelial	Non-epithelial	Low malignant potential	Metastatic	Non-ovarian malignancy
No. of Subjects	96	9	28	18	10
OVA1 test Score, n (%)					
High risk	95 (99.0)	7 (77.8)	21 (75.0)	17 (94.4)	9 (90.0)
Low risk	1 (1.0)	2 (22.2)	7 (25.0)	1 (5.6)	1 (10.0)
Sensitivity, %	99.0	77.8	75.0	94.4	90.0

Table 4. Distribution of OVA1 test scores by histologic subtype. OVA1 detected all stage 2-4 EOC's and 90% (28/31) of stage I EOC's.

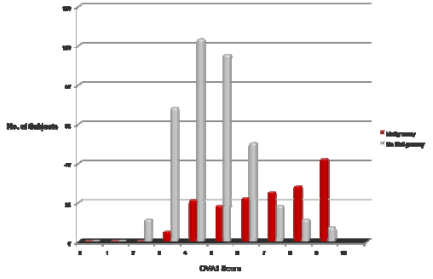

Figure 2. Histogram of OVA1 scores by pathology result. Higher OVA1 scores indicate a higher likelihood of malignancy.

Conclusions

- In this cohort of women undergoing surgery for an ovarian tumor, the OvaCalc algorithm effectively identifies women with a higher likelihood of malignancy.
- Within the study population, the OvaCalc algorithm detected 90% of stage I epithelial ovarian cancer cases and 100% of stage 2-4 cancers while maintaining a greater than 90% negative predictive value among both premenopausal and postmenopausal patients.