A Combinatorial Proteomic Biomarker Assay to Detect Ovarian Cancer in Women

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ABSTRACT: Ovarian cancer is often fatal and incidence in the general population is low, underscoring the necessity (and the challenges) for advancements in screening and early detection. The goal of this study was to design a serum-based biomarker panel and corresponding multivariate algorithm that can be used to accurately detect ovarian cancer. A combinatorial protein biomarker assay (CPBA) that uses CA125, HE4, and 3 tumor-associated autoantibodies resulted in an area under the curve of 0.98. The CPBA Ov algorithm was trained using subjects who were suspected to have gynecological cancer and were scheduled for surgery. As a surgical rule-out test, the clinical performance achieves 100% sensitivity and 83.7% specificity. Although sample size (n = 60) is a limiting factor, the CPBA Ov algorithm performed better than either CA-125 alone or the Risk of Ovarian Malignancy Algorithm.

KEYWORDS: Ovarian cancer, biomarker, proteomics, serum proteins, tumor-associated autoantibodies, multivariate prediction modeling

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Background

Although the incidence of ovarian cancer (OvCa) is relatively rare in the general population, it is often lethal because most women (60%) are diagnosed with advanced-stage (III or IV) disease, wherein 5-year survival is only 17% to 39%.[1] If diagnosed early (stage I or II), survival is 70% to 92%.[1] These numbers underscore the imminent need for improvement in OvCa diagnostics.

The field of OvCa screening faces a unique paradoxical hurdle. Low disease prevalence means general population screening trials have difficulty achieving the required high specificity (low false-positive rate), which necessitates the use of an enriched population (such as women with adnexal pelvic masses).[2,3] However, serous adenocarcinoma, which accounts for up to 70% of epithelial OvCas, often originates in the fallopian tubes and presents as advanced disease without a clear "pelvic mass" as a diagnostic point.[4,5] When OvCa is suspected, a woman undergoes invasive surgery that often results in sterilization. Therefore, a noninvasive OvCa diagnostic must be highly accurate if the goal is to prevent unnecessary invasive surgeries while not overlooking true invasive cancers.

In women with a pelvic mass, transvaginal ultrasound and/or serum CA-125 is often used to determine the likelihood of malignancy. CA-125 is elevated in 85% of advanced epithelial OvCa but is normal in up to 50% of early-stage cancers.[6] CA-125 lacks clinical specificity (resulting in a high false-positive rate), which limits its utility as a diagnostic.[7,8] More recently, the combination of CA-125 and HE4 using the Risk of Ovarian Malignancy Algorithm (ROMA) in women with pelvic masses planned for surgery imparted better performance characteristics compared with CA-125 alone.[9,10] In addition, OVA1, a panel of 5 OvCa serum biomarkers was shown to improve clinical sensitivity beyond ROMA but clinical specificity was lower than ROMA.[11]

Outside of serum proteins, tumor-associated autoantibodies (TAAb) have shown promise in detecting OvCa.[12,13] However, the biggest drawback to using TAAb in a diagnostic setting is the low prevalence for any single TAAb; not all subjects with cancer will produce an autoantibody response. Even among patients with a p53 mutation, most will not produce detectable levels of circulating p53 TAAb.[14,15] Despite this caveat, TAAb may still impart utility as serum biomarkers due to their high specificity. Although p53 autoantibodies are rare within a given population, it is almost guaranteed that a patient with p53 TAAb does have cancer (although it may not be OvCa, specifically).[14] Because of redundancy (meaning that an individual TAAb can be found in multiple cancer types) and low sensitivity, TAAb should be combined into future diagnostic panels, along with other serum biomarkers, to impart optimal clinical performance characteristics.[16,17]

In the effort to diagnose OvCa at the earliest possible stage, additional advances are necessary to create a serum biomarker diagnostic with high sensitivity and high specificity. Serum protein biomarker (SPB) panels most often result in assays with high sensitivity, but low specificity. Conversely, serum autoantibody panels most often result in assays with low sensitivity, but high specificity. We have demonstrated previously that the combination of SPB and TAAb results in high sensitivity and specificity in the detection of breast cancer.[18,19] In this study, we sought to determine whether SPB could be combined with TAAb to create a novel algorithm that can accurately detect OvCa.

Methods

Serum samples

Plasma and serum samples used for assay and biomarker development were purchased from multiple biorepositories (Indivumed, Asterand, Oregon Health & Science University, and The University of Arizona). A total of n = 122 samples were used to develop and refine the SPB and TAAb assays (Table 1). Sample numbers were skewed, with an abundance of OvCa samples, due to the expectation of low TAAb prevalence. The data obtained from these analyses were used in feature selection during algorithm and model development (see "Model development and statistical analysis" section). Samples used for biomarker development were independent of those used for algorithm development.

The serum specimens used to design a training algorithm were collected from women who presented for pelvic surgery at the Catholic Health Initiatives Center for Translational Research (CHI-CTR) and whose physician has indicated a clinical suspicion of gynecological cancer. A total of 196 serum specimens were collected from CHI-CTR for this study. Of these, 17 were collected presurgery from subjects diagnosed with OvCa and 43 were collected presurgery from subjects diagnosed with a benign gynecological condition (eg, ovarian cysts, endometriosis) (Supplementary Table 1). Samples categorized by the site as fallopian tube cancer were recategorized as OvCa due to evidence linking fallopian tubes to OvCa origins.[4,5] The remaining 136 samples were excluded from training due to postsurgical collection, prior gynecological cancer diagnosis (ie, recurrence monitoring), borderline tumor status (as defined by pathology), and/or cancer origin other than ovarian or fallopian tube. All specimens were de-identified by the collection site, thus personal information was not identifiable to the investigator nor any other individual associated with this investigation. The study design was granted institutional review board (IRB) exemption under 45 CFR 46.101(b)(4).

Measurement of SPB and TAAb

Protein and hormone biomarkers (Supplementary Table 2) were chosen for analysis based on published literature.[20–23] Serum was evaluated for the concentrations of 9 SPB using Abbott Architect i1000SR immunoassays, following manufacturer's specifications. The Architect assays use chemiluminescent microparticles to determine analyte concentrations. Calibrator and control samples were run for each assay as recommended by the manufacturer. Samples resulting in an upper limit of quantification error flag (analyte concentration above the reportable range) were diluted appropriately and rerun to obtain a valid measurement.

Autoantibody biomarkers were chosen for analysis based on published literature.[12,18,24–26] Samples were processed in duplicate and evaluated for the relative presence/absence of 47 TAAb (protein targets listed in Supplementary Table 2), as described previously.[18] Associated autoantibodies were detected using an indirect enzyme-linked immunosorbent assay (ELISA), which involves coating standard-bind plates (MSD, Rockville, MD, USA) with recombinant protein. Proteins were diluted in 1× phosphate-buffered saline and coated onto blank plates at a final concentration of 20 ng/well. All recombinant proteins, certified as >80% pure (sodium dodecyl sulfate polyacrylamide gel electrophoresis), were purchased from OriGene (Rockville, MD, USA) or Abnova (Taipei City, Taiwan). OriGene proteins were myc/DDK peptide tagged and produced in HEK-293 cells. Abnova proteins were GST tagged and produced in wheat germ cells. Appropriate controls (TAAb-negative serum spiked with anti-myc/DDK or anti-GST) were included on each plate to monitor assay performance. Electrochemiluminescent signal was detected using a Meso Sector S600 plate reader and MSD Workbench 4.0 software. The TAAb ratio values were determined using the following calculation:

$$\frac{(\text{Target MFI} - \text{True Target MFI})}{\text{Median Sample Background MFI}}$$

where Target MFI = mean fluorescence intensity (MFI) of sample plus target and True Target MFI = MFI of corresponding target protein without sample (protein background).

Sample run order was randomized and laboratory staff was blinded to subject disease status.

ROMA calculation

The ROMA was calculated for samples included in the training group as described previously,[27] with a cutoff of 12.5% used for premenopausal subjects and a cutoff of 14.4% used for postmenopausal subjects. Menopause data were not collected for CHI-CTR samples; therefore, the follicle-stimulating hormone (FSH) value was used to approximate menopause status

Table 1. Subjects used for biomarker analytical development and algorithm training.

	NO EVIDENCE OF DISEASE	BENIGN GYNECOLOGICAL CONDITION	OVARIAN CANCER
Biomarker development	n = 22 (48, 30-72)	n = 20 (52, 28-73)	n = 80 (60, 35-90)
Algorithm training	NA	n = 43 (53, 30-86)	n = 17 (57, 41-79)

Abbreviation: NA, not applicable.
Median age, along with minimum and maximum age, for each group is shown in parentheses. Subjects with no evidence of gynecological disease were not included in algorithm training.

(with >30 mIU/mL corresponding to postmenopause). When a valid FSH measurement was not available (n = 2), ROMA was calculated for both pre- and postmenopause. For these samples, the outcome (high or low) was the same when using both calculations.

Model development and statistical analysis

Feature selection was used to select biomarkers that are either biologically relevant to OvCa or statistically significant in the biomarker development and/or training sample set. Multiple approaches were used, including literature review, logistic modeling (all biomarkers were individually used as a predictor in a logistic model with cancer as a response; biomarkers that were significant at the .1 level were recorded), and univariate 2-sample *t* tests for association (biomarkers that were significant at the .1 level were recorded). In addition, 2 different bootstrap methods were used for feature selection. Elastic net (ELNET) and generalized boosted models (GBMs) were applied to 200 bootstrap samples; biomarkers that were selected at least 50% of samples for ELNET or 60% of samples for GBM were recorded. Three models (SPB alone, TAAb alone, and SPB + TAAb) were originally built using a logistic boost approach that used a Synthetic Minority Over-sampling Technique (SMOTE) to increase the number of cancer cases.

Receiver operator characteristic (ROC) and area under the curve (AUC) metrics were used to determine algorithm performance regarding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Model cut points were optimized for sensitivity (rule-out malignancy) and specificity (rule-in malignancy). Confidence intervals (CI) were reported as 2-sided binomial 95% CIs. Logit boost models were created using R (version 3.0.3, March 6, 2014). All analyses were conducted using SAS (version 9.4) and GraphPad Prism (version 6.03).

Results

Biomarker assay development: univariate analysis of serum protein biomarkers

Nine OvCa-specific SPB were analyzed using Abbott Architect assays, as described in the "Methods" section. Individual patient health characteristics (including but not limited to age and menopausal status) and clinical outcomes were extracted from each de-identified patient record where available. Univariate analyses were completed to determine whether individual SPB differed between women diagnosed with OvCa and women with no evidence of ovarian disease (Table 1, biomarker development population). These patient populations were chosen for assay development to assess the limits of detection based on expected population concentration ranges. Samples that did not have adequate volume for SPB analysis were excluded (n = 43). A total of 5/9 SPB (CA-125, CA 15.3, CA19-9, HE4, and Prolactin) were found to be differentially expressed at statistically significant levels ($P < .05$; Figure 1) between the 2 groups.

Biomarker assay development: univariate analysis of tumor-associated autoantibodies

A total of 47 TAAb were analyzed by MSD-indirect ELISA, as described in the "Methods" section. The TAAb ratios indicate the relative presence or absence of target-specific autoantibodies. Based on published literature,[12,13] prevalence was expected to be low for each individual TAAb, even in OvCa samples. Because of the expected low prevalence and because prevalence in the healthy/non-disease (ND) population is expected to be at or close to zero, OvCa samples were compared with benign gynecological disease samples (Table 1, biomarker development population). Samples that did not have adequate volume for TAAb analysis were excluded (n = 3). Although individual TAAb were not significantly different between the 2 groups, differences in overall prevalence were noted (Figure 2) and some trends may achieve significance in a larger sample set. Full TAAb comparison data are shown in Supplementary Figure 1.

A blood-based multimarker panel detects OvCa

A multivariate algorithm using prospectively collected (presurgery) samples from CHI-CTR was developed to differentiate subjects with benign gynecological conditions from those with OvCa (as described in the "Methods" section). A total of 60 samples were selected for model development; all samples were drawn from subjects prior to undergoing surgery. Feature selection and logistic modeling were conducted as described in the "Methods" section; the final model (combinatorial protein biomarker assay [CPBA] Ov) includes 2 SPB (CA-125 and HE4) and 3 TAAb (ACSBG1, CTAG1B, and DHFR). Clinical performance was evaluated for other biomarker models (CA-125 alone, HE4 alone, and ROMA) using the same sample data. The CPBA Ov ROC is shown in Figure 3, with the CA-125, HE4, and ROMA ROC curves shown for comparison. Although CA-125 and ROMA each performed well individually, the AUC was highest for the CPBA Ov algorithm (0.98).

Two model cut points were chosen to represent a surgery rule-in model (optimized for specificity) and a surgery rule-out model (optimized for sensitivity). The training set clinical performance metrics are shown in Table 2 with CA-125 and ROMA metrics shown for comparison. The rule-in model resulted in high specificity (97.7%), which is necessary to appropriately rule-in subjects for surgery while maintaining a low number of false positives. However, as the population evaluated in this study consisted of women identified for surgery, a rule-out model would more closely match an intended-use population wherein surgery may be ruled out.

Although ROMA (combined results for pre- and postmenopausal subjects) missed only 1/17 cancer subjects, all were

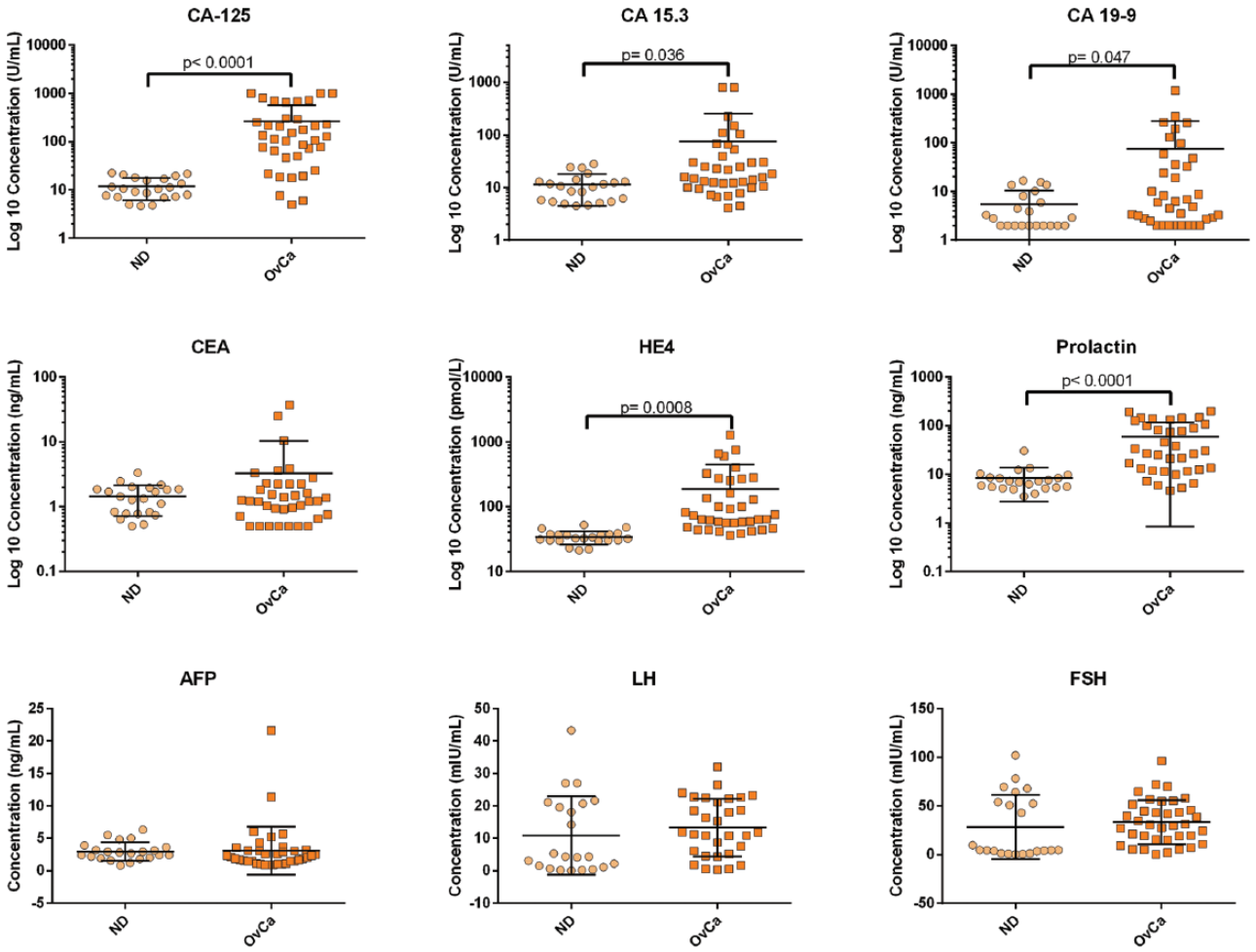

Figure 1. Scatter plot distributions of SPB and LH/FSH in subjects with ovarian cancer (OvCa, n=37) and no evidence of ovarian disease (ND, n=22). Analyte mean and standard deviations are shown for each population. Log-10 scales are used where appropriate to better illustrate distributions. Statistically significant *P* values (as determined by unpaired *t* test with Welch correction, $P < .05$) are noted. FSH indicates follicle-stimulating hormone; LH, luteinizing hormone; OvCa, ovarian cancer; SPB, serum protein biomarkers.

correctly identified with the CPBA Ov algorithm (rule-out model) (Table 2). High clinical sensitivity is essential for a physician to recommend against invasive surgery when OvCa is suspected by standard clinical follow-up. For the subjects within this study, 60 underwent surgery, 43 of which were benign conditions (a 71.7% false-positive rate). In contrast, inclusion of the CPBA Ov would have resulted in a false-positive rate of only 11.7%. ROMA and CA-125 are not intended to rule-out surgery and further studies are necessary to determine whether ROMA can be integrated successfully into CPBA Ov to create a screening test that can be optimized for both rule-in and rule-out applications.

To compare CPBA Ov algorithm performance with samples that were collected postsurgery, 36 postsurgical OvCa serum samples were evaluated for clinical performance. This cohort comprised 4 CHI-CTR samples and 32 biomarker development samples. Subjects whose blood was collected postsurgery did not perform as well as subjects whose blood was collected presurgery, with the CPBA Ov algorithm correctly predicting 31/36 postsurgery OvCa subjects (sensitivity, 86.1%; NPV, 87.5%) (Supplementary Table 3). This is not unexpected because OvCa surgery frequently involves tumor removal/debulking, which might result in changes in circulating tumor biomarkers.[28,29] These results underscore the fundamental requirement for presurgical samples when evaluating OvCa diagnostic biomarkers. Clinical performance was also poor for subjects with recurrent OvCa (Supplementary Table 3). As such, additional studies would be necessary to develop an algorithm that can adequately detect OvCa recurrence.

Discussion

The CPBA Ov algorithm was developed using 60 prospective samples from women scheduled to undergo surgery due to the suspicion of gynecological cancer. Although sample size is limited, the final algorithm is highly accurate, with 100% sensitivity and 83.7% specificity (Table 2). The negative predictive value of the CPBA Ov algorithm (100%) was greater than either CA-125 alone (91.7% NPV) or ROMA (97.2% NPV). This implies improved clinical utility, as high NPV is necessary to ensure subjects are correctly being ruled out for

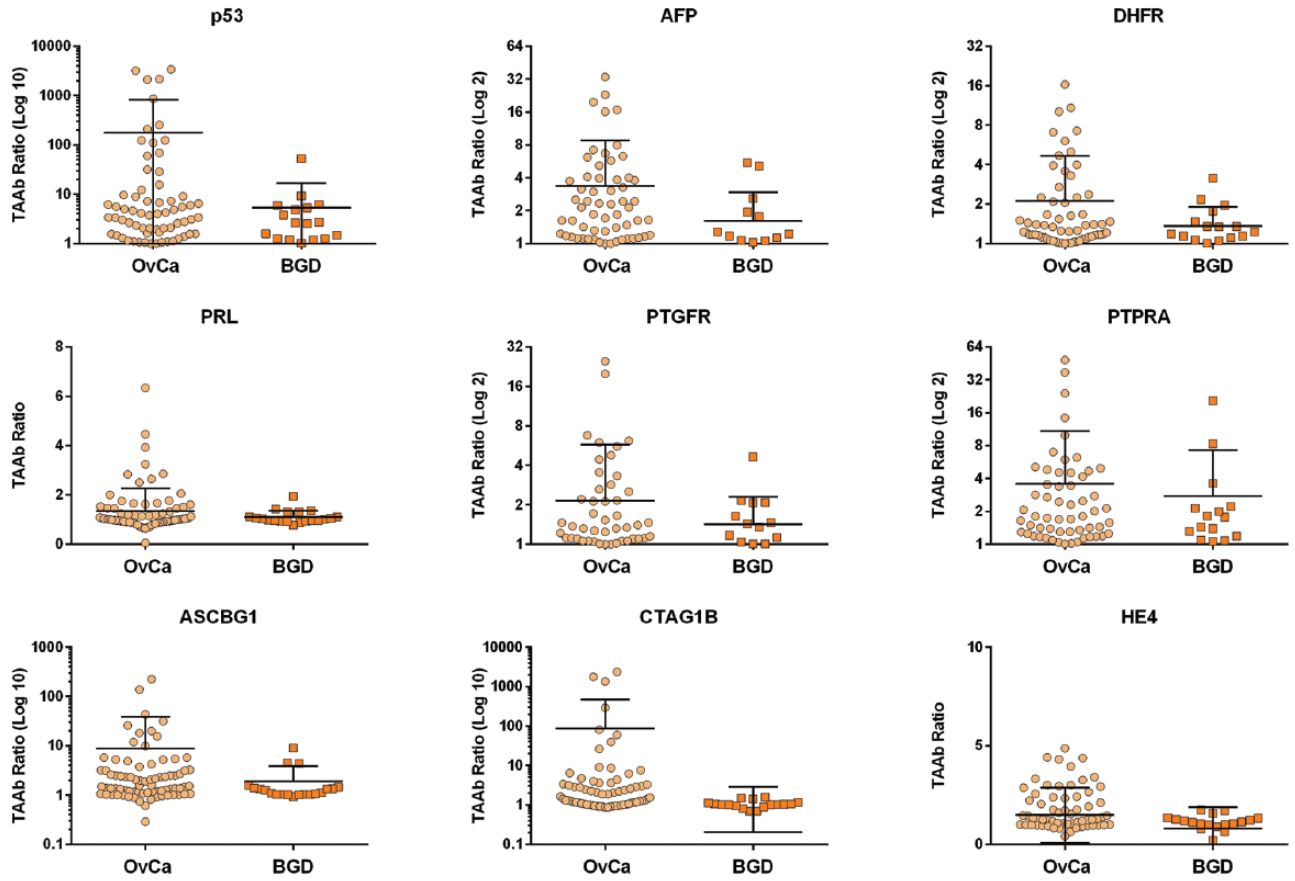

Figure 2. Representative distributions of select ovarian cancer-specific TAAb in subjects with ovarian cancer (OvCa, n=77) and benign gynecological disease (BGD, n=20). Analyte mean and standard deviations are shown for each population. Log-2 or Log-10 scales are used where appropriate to better illustrate TAAb ratio distributions. BGD indicates benign gynecological disease; OvCa, ovarian cancer; TAAb, tumor-associated autoantibodies.

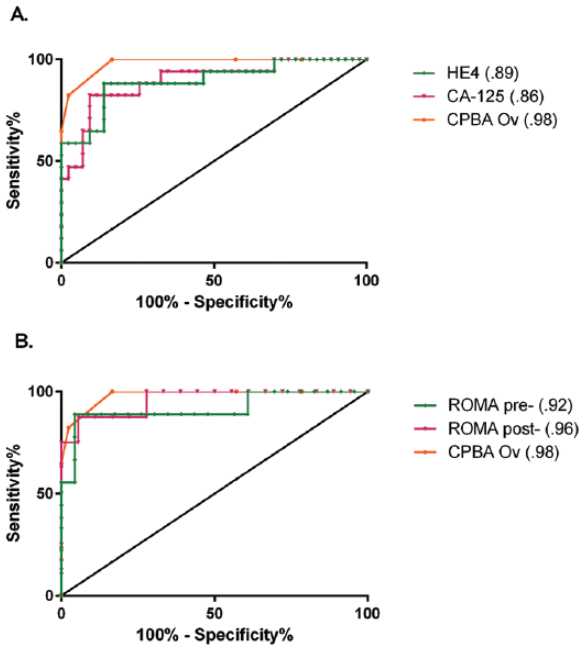

Figure 3. Receiver operator characteristic (ROC) curve of ovarian cancer algorithm, developed on n=60 serum samples. Curves are also shown for the same cohort using (A) CA-125 alone and HE4 alone as well as (B) ROMA premenopausal and ROMA postmenopausal. Area under the curve (AUC) is shown for each test in parentheses. ROMA indicates Risk of Ovarian Malignancy Algorithm.

surgery. False negatives are most worrisome in a rule-out test. ROMA performance (which is intended to determine risk of malignancy, not surgery rule out) was high, with only 1/17 subjects being a false negative. The CPBA Ov algorithm, however, detected all OvCa cases (0/17 false negatives) while also improving specificity above the comprehensive ROMA (83.7% vs 81.4%, respectively).

Although ROMA has generally shown good performance, clinical utility is somewhat limited and at least one study reported no improvement over CA-125 alone.[30] Moore et al[9] had reported higher ROMA specificity in premenopausal as opposed to postmenopausal women, which we found to be true of our sample cohort as well (premenopausal specificity, 91.3% vs postmenopausal specificity, 72.2%). In contrast, the CPBA Ov algorithm resulted in higher specificity in postmenopausal as opposed to premenopausal subjects (94.4% vs 73.9%, respectively). Because most OvCa subjects are postmenopausal, performance metrics are particularly important in this population. These data suggest that the inclusion of additional biomarkers into ROMA (or combination with an independent rule-out model) might result in higher overall specificity and, thus, improved clinical utility. The CPBA Ov algorithm already includes CA-125 and HE4 (the 2 biomarkers included in ROMA) so integrating the 2 models without overfitting is

Table 2. CPBA Ov algorithm results with clinical performance metrics.

	CPBA OV		OTHER OVCA BIOMARKER TESTS		
	RULE-IN	RULE-OUT	CA-125	ROMA[a] (PRE)	ROMA[a] (POST)
TN	42	36	33	21	13
FP	1	7	10	2	5
TP	14	17	14	8	8
FN	3	0	3	1	0
Sensitivity, %	82.4 (56.6-96.2)	100 (80.5-100)	82.4 (56.6-96.2)	88.9 (51.8-99.7)	100 (63.1-100)
Specificity, %	97.7 (87.4-99.9)	83.7 (68.6-93.0)	76.7 (61.4-88.2)	91.3 (72.0-98.9)	72.2 (46.5-90.3)
NPV, %	93.3 (81.7-98.6)	100.0 (90.3-100)	91.7 (77.5-98.2)	95.5 (77.2-99.9)	100.0 (75.3-100)
PPV, %	93.3 (68.1-99.8)	70.8 (48.9-87.4)	58.3 (36.6-77.9)	80.0 (44.4-97.5)	61.5 (31.6-86.1)

Abbreviations: CPBA, combinatorial protein biomarker assay; FN, false negative; FP, false positive; NPV, negative predictive value; OvCa, ovarian cancer; PPV, positive predictive value; ROMA, Risk of Ovarian Malignancy Algorithm; TN, true negative; TP, true positive.
Assay cut points were optimized to enhance sensitivity in a rule-out model and specificity in a rule-in model. Clinical performance is also shown for CA-125 alone and ROMA (pre- and postmenopausal). Confidence intervals are shown for all calculations.
[a]A total of n = 2 subjects could not be assessed for ROMA due to lack of a valid FSH value to determine menopause status.

difficult. Sample size is a limiting factor in these analyses, assessment of additional subjects will be necessary to confirm these conclusions.

In considering further development of a blood test to detect OvCa, we must acknowledge the difficulty faced in developing such a test and the best population of women on which to evaluate its safety and efficacy. Many women with serous OvCa present with advanced disease with peritoneal carcinomatosis and without a dominant pelvic mass.[4,5] In addition, prior large screening trials (PLCO and UKCTOCS) using serial CA-125 in a general population of women have yet to find efficacy in improving survival in OvCa.[31,32] However, some promise has been shown recently in women at high risk for developing OvCa because of genetic factors and or family history, using ROMA in a subset evaluation of the UKCTOCS trial.[33,34]

In our study, CPBA Ov performance was not as strong when applied to samples collected postsurgery (Supplementary Table 3). Tumor debulking often results in a sizable change in circulating biomarkers[28,29] so these results are not unexpected. However, they do underscore the necessity for analyzing presurgery samples when developing a liquid biopsy test for OvCa detection. This is inherently difficult due to low prevalence—the middle ground in addressing this issue has been to use enriched samples (such as high-risk subjects or women with pelvic masses on imaging). Some research studies choose to use postsurgical samples but the resulting models will likely suffer in terms of clinical performance and may end up being trained more on noise than on signal. Regardless, such models will have to demonstrate diagnostic utility in a presurgery population and such samples are rare in collection banks. Given the low disease prevalence and high barrier-to-entry for OvCa liquid biopsy tests, sample banks may be better served to collect samples with these considerations in mind.

For these reasons, the greatest limitation in developing new OvCa diagnostics is sample size and availability. Although many were not statistically significant, the trends noted in biomarker distributions may achieve statistical significance in a larger sample set. Also, it will be necessary to test the CPBA Ov algorithm in an independent cohort to assess whether clinical validation performance is consistent with the results obtained from the training cohort.

Conclusions

The CPBA Ov algorithm is a novel liquid biopsy test that accurately detects OvCa in a presurgical population. With 100% sensitivity and 83.7% specificity, the test would provide assurance that a subject may avoid invasive surgery without the concern that invasive OvCa would be missed (false negative). Although these results are impressive, the study size is small (n = 60). It will be necessary to analyze additional samples obtained from an independent, presurgery population before true clinical performance can be reported. Additional studies are being conducted to establish the clinical validity of CPBA Ov in an independent sample cohort.

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Author Contributions

MCH, MS, and JKW conceived and designed the experiments. MCH, MS, and EL analyzed the data. MCH wrote the first

draft of the manuscript. MCH, SB, QT, RM, JKW, and DER contributed to the writing of the manuscript. MCH, RM, and DER jointly developed the structure and arguments for the paper. MCH, JKW, and DER made critical revisions and approved final version. All authors agree with manuscript results and conclusions, reviewed, and approved the final manuscript.

Disclosures and Ethics

Institutional review board exemption was granted under 45 CFR 46.101(b)(4) by Western IRB. As a requirement of publication, authors have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality, and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

Supplementary Table 1. Clinical and demographic information for training samples.

SAMPLE NO.	RACE	AGE AT COLLECTION	DX	CANCER	SUBTYPE	AJCC/UICC STAGE GROUP	TUMOR GRADE	TNM	BRCA STATUS
701.175	White	51	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
701.201	Asian	65	Benign Uterus	0	n/a	n/a	n/a	n/a	Pos
701.207	White	37	Benign Cervix	0	n/a	n/a	n/a	n/a	Unk
701.208	White	35	Benign Fallopian	0	n/a	n/a	n/a	n/a	Unk
701.212	White	53	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
701.213	White	57	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
701.215	White	42	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
701.255	White	53	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
701.393	White	75	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
701.398	White	53	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
701.400	White	30	Benign Cervix	0	n/a	n/a	n/a	n/a	Unk
701.401	White	55	Benign Cervix	0	n/a	n/a	n/a	n/a	Unk
702.512	White	58	Benign Ovary	0	n/a	n/a	n/a	n/a	Pos
702.518	White	44	Benign Ovary	0	n/a	n/a	n/a	n/a	Pos
702.522	White	66	Benign Ovary	0	n/a	n/a	n/a	n/a	Pos
702.528	White	54	Benign Ovary	0	n/a	n/a	n/a	n/a	Pos
702.529	White	53	Benign Ovary	0	n/a	n/a	n/a	n/a	Pos
702.531	White	42	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.580	White	42	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.582	White	58	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
702.586	White	42	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
702.587	White	61	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
702.589	White	50	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.600	White	53	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
702.601	White	48	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk

Supplementary Table 1. (Continued)

SAMPLE NO.	RACE	AGE AT COLLECTION	DX	CANCER	SUBTYPE	AJCC/UICC STAGE GROUP	TUMOR GRADE	TNM	BRCA STATUS
702.602	White	49	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.619	White	57	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.620	White	40	Benign Cervix	0	n/a	n/a	n/a	n/a	Unk
702.621	White	45	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.631	Black	42	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.666	White	35	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.667	White	65	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.675	White	64	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
702.698	White	86	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.710	White	48	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.717	White	68	Benign Uterus	0	n/a	n/a	n/a	n/a	Unk
702.730	White	67	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.750	White	71	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
702.751	White	53	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
703.526	White	31	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
703.543	White	71	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
703.571	White	45	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
703.608	White	49	Benign Ovary	0	n/a	n/a	n/a	n/a	Unk
701.211	White	44	Ovarian Cancer	1	Serous	III	1	T2c, NX	Unk
701.386	White	50	Ovarian Cancer	1	Stromal (Granulosa)	1A	INA	pT1a, pN0	Unk
701.387	White	68	Ovarian Cancer	1	Serous	IIIB	3	pT3b, pNX	Unk
701.391	White	62	Ovarian Cancer	1	Mixed	IIIB	3	pT3b, pNX	Unk
701.407	White	67	Ovarian Cancer	1	Mixed Epithelial	IIIC	3	pT3c, pN0	Unk
702.507	White	54	Ovarian Cancer	1	Serous	II	3	pT2c, pN0	Unk
702.536	White	52	Ovarian Cancer	1	Serous	IIIC	3	T3c, N1	Unk
702.584	White	79	Ovarian Cancer	1	Serous	II	3	pT2, pN0	Unk
702.625	White	72	Ovarian Cancer	1	Serous	INA	3	INA	Unk
702.672	Caucasian	63	Fallopian Tube Cancer	1	Serous	IIIC	3	pT3c, pN1	Unk
702.699	Caucasian	65	Fallopian Tube Cancer	1	Serous	IIIC	3	pT3c, pN0	Unk
702.702	Black	41	Ovarian Cancer	1	Germ Cell (Strumal)	1A	1	pT1a, pNX	Unk
702.716	Other	58	Ovarian Cancer	1	Endometrioid	IIB	2	pT2b, pN0	Unk

Supplementary Table 1. (Continued)

SAMPLE NO.	RACE	AGE AT COLLECTION	DX	CANCER	SUBTYPE	AJCC/UICC STAGE GROUP	TUMOR GRADE	TNM	BRCA STATUS
702.725	White	51	Ovarian Cancer	1	Serous	IIIC	3	pT3c, pN0, pM1	Unk
702.728	White	55	Ovarian Cancer	1	Clear Cell	IB	3	pT1b, pN0	Unk
703.534	Other	53	Ovarian Cancer	1	Serous Papillary	3C	3	pT3b, pN1, MX	Unk
703.570	White	57	Ovarian Cancer	1	Serous	IIIC	2	pT3c, pN1, pM1	Unk

Description: CHI-CTR training subjects clinical and demographic information, including race, age, diagnosis, tumor stage/grade, TNM, and BRCA status. INA, information not available.

Supplementary Table 2. Serum protein biomarkers (SPB) and tumor-associated autoantibodies (TAAb) evaluated during this study.

PROTEIN CLASS	PROTEIN	UNIPROT ID	FULL NAME FROM UNIPROT	UNIPROT LINK
SPB	CA-125	Q8WXI7	Mucin-16	http://www.uniprot.org/uniprot/Q8WXI7
SPB	CA19-9	Q969X2	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	http://www.uniprot.org/uniprot/Q969X2
SPB	CEA	P06731	Carcinoembryonic antigen-related cell adhesion molecule 5	http://www.uniprot.org/uniprot/P06731
SPB (hormone)	FSH	N/A	Follicle stimulating hormone	N/A
SPB (hormone)	LH	N/A	Luteinizing hormone	N/A
TAAb	ACSBG1	Q96GR2	Long-chain-fatty-acid–CoA ligase ACSBG1	http://www.uniprot.org/uniprot/Q96GR2
TAAb	ATF3	P18847	Cyclic AMP-dependent transcription factor ATF-3	http://www.uniprot.org/uniprot/P18847
TAAb	ATP6AP1	Q15904	V-type proton ATPase subunit S1	http://www.uniprot.org/uniprot/Q15904
TAAb	BAT4 (GPANK1)	O95872	G patch domain and ankyrin repeat-containing protein 1	http://www.uniprot.org/uniprot/O95872
TAAb	BDNF	P23560	Brain-derived neurotrophic factor	http://www.uniprot.org/uniprot/P23560
TAAb	BMX	P51813	Cytoplasmic tyrosine-protein kinase BMX	http://www.uniprot.org/uniprot/P51813
TAAb	CSNK1A1L	Q8N752	Casein kinase I isoform alpha-like	http://www.uniprot.org/uniprot/Q8N752
TAAb	CSNK1E	P49674	Casein kinase I isoform epsilon	http://www.uniprot.org/uniprot/P49674
TAAb	CTAG1A	P78358	Cancer/testis antigen 1	http://www.uniprot.org/uniprot/P78358
TAAb	CTAG1B	P78358	Cancer/testis antigen 1	http://www.uniprot.org/uniprot/P78358
TAAb	CTBP1	Q13363	C-terminal-binding protein 1	http://www.uniprot.org/uniprot/Q13363

Supplementary Table 2. (Continued)

PROTEIN CLASS	PROTEIN	UNIPROT ID	FULL NAME FROM UNIPROT	UNIPROT LINK
TAAb	DBT	P11182	Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial	http://www.uniprot.org/uniprot/P11182
TAAb	DHFR	P00374	Dihydrofolate reductase	http://www.uniprot.org/uniprot/P00374
TAAb	EIF3E	P60228	Eukaryotic translation initiation factor 3 subunit E	http://www.uniprot.org/uniprot/P60228
TAAb	ErbB2	P04626	Receptor tyrosine-protein kinase erbB-2	http://www.uniprot.org/uniprot/P04626
TAAb	FRS3	O43559	Fibroblast growth factor receptor substrate 3	http://www.uniprot.org/uniprot/O43559
TAAb	GPR157	Q5UAW9	Probable G-protein coupled receptor 157	http://www.uniprot.org/uniprot/Q5UAW9
TAAb	HOXD1	Q9GZZ0	Homeobox protein Hox-D1	http://www.uniprot.org/uniprot/Q9GZZ0
TAAb	IGFBP2	P18065	Insulin-like growth factor binding protein 2	http://www.uniprot.org/uniprot/P18065
TAAb	MYOZ2	Q9NPC6	Myozenin-2	http://www.uniprot.org/uniprot/Q9NPC6
TAAb	p53	P04637	Cellular tumor antigen p53	http://www.uniprot.org/uniprot/P04637
TAAb	PDCD6IP	Q8WUM4	Programmed cell death 6-interacting protein	http://www.uniprot.org/uniprot/Q8WUM4
TAAb	PSMC1	P62191	26S protease regulatory subunit 4	http://www.uniprot.org/uniprot/P62191
TAAb	PTGFR	P43088	Prostaglandin F2-alpha receptor	http://www.uniprot.org/uniprot/P43088
TAAb	PTPRA	P18433	Receptor-type tyrosine-protein phosphatase alpha	http://www.uniprot.org/uniprot/P18433
TAAb	RAB5A	P20339	Ras-related protein Rab-5A	http://www.uniprot.org/uniprot/P20339
TAAb	RAB7L1	O14966	Ras-related protein Rab-7L1	http://www.uniprot.org/uniprot/O14966
TAAb	RAC3	P60763	Ras-related C3 botulinum toxin substrate 3	http://www.uniprot.org/uniprot/P60763
TAAb	SCYL3	Q8IZE3	Protein-associating with the carboxyl-terminal domain of ezrin	http://www.uniprot.org/uniprot/Q8IZE3
TAAb	SELL	P14151	L-selectin	http://www.uniprot.org/uniprot/P14151
TAAb	SERPINH1	P50454	Serpin H1	http://www.uniprot.org/uniprot/P50454
TAAb	SF3A1	Q15459	Splicing factor 3A subunit 1	http://www.uniprot.org/uniprot/Q15459
TAAb	SLC33A1	O00400	Acetyl-coenzyme A transporter 1	http://www.uniprot.org/uniprot/O00400
TAAb	SOX2	P48431	Transcription factor SOX-2	http://www.uniprot.org/uniprot/P48431
TAAb	TFCP2	Q12800	Alpha-globin transcription factor CP2	http://www.uniprot.org/uniprot/Q12800

Supplementary Table 2. (Continued)

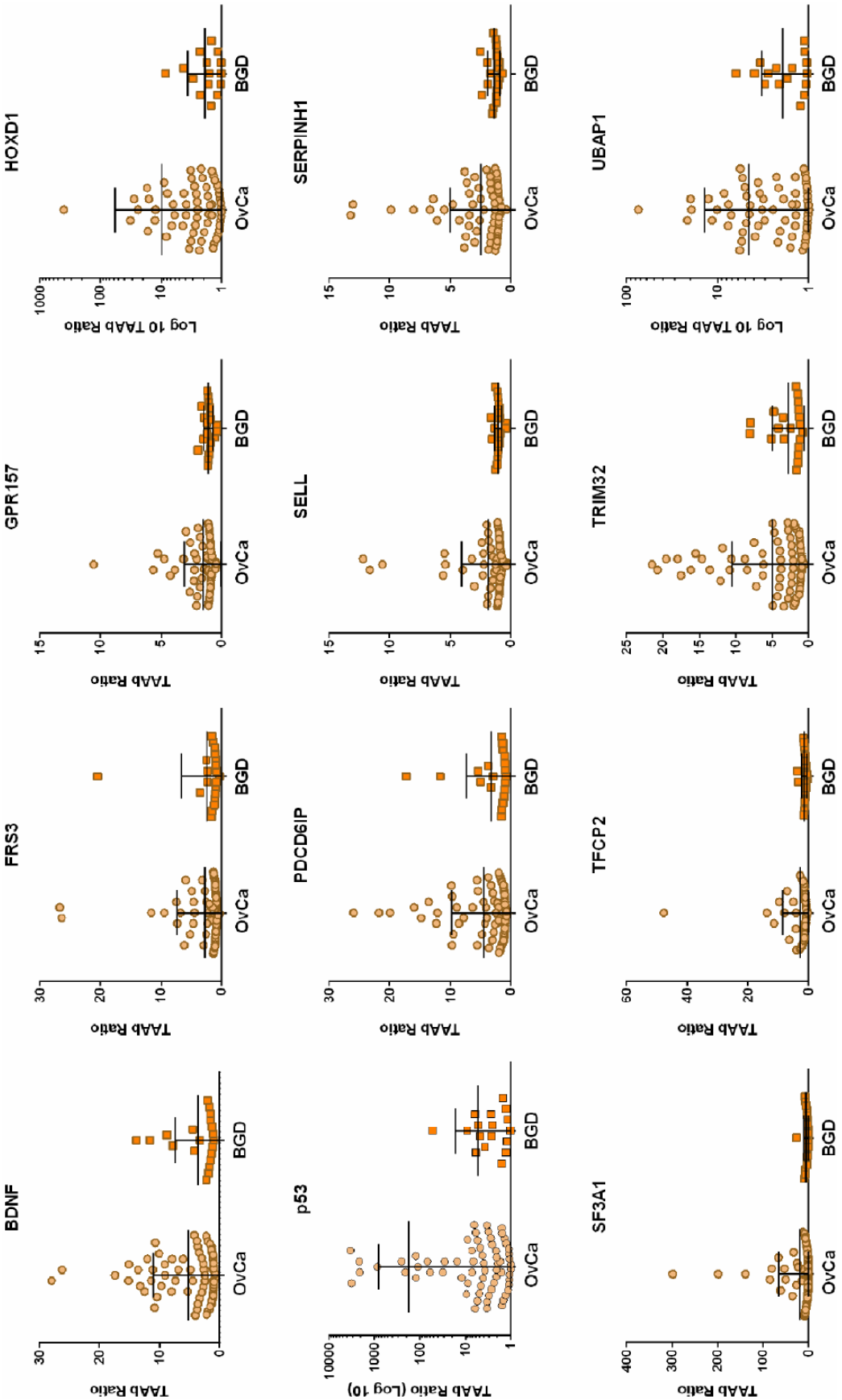
PROTEIN CLASS	PROTEIN	UNIPROT ID	FULL NAME FROM UNIPROT	UNIPROT LINK
TAAb	TRIM32	Q13049	E3 ubiquitin-protein ligase TRIM32	http://www.uniprot.org/uniprot/Q13049
TAAb	UBAP1	Q9NZ09	Ubiquitin-associated protein 1	http://www.uniprot.org/uniprot/Q9NZ09
TAAb	ZMYM6	O95789	Zinc finger MYM-type protein 6	http://www.uniprot.org/uniprot/O95789
TAAb	ZNF510	Q9Y2H8	Zinc finger protein 510	http://www.uniprot.org/uniprot/Q9Y2H8
TAAb	MBNL1	Q9NR56	Muscleblind-like protein 1	http://www.uniprot.org/uniprot/Q9NR56
TAAb, SPB	CA15.3/MUC1	P15941	Mucin-1	http://www.uniprot.org/uniprot/P15941
TAAb, SPB	HE4	Q14508	WAP four-disulfide core domain protein 2	http://www.uniprot.org/uniprot/Q14508
TAAb, SPB	PRL	P01236	Prolactin	http://www.uniprot.org/uniprot/P01236
TAAb,SPB	AFP	P02771	Alpha-fetoprotein	http://www.uniprot.org/uniprot/P02771

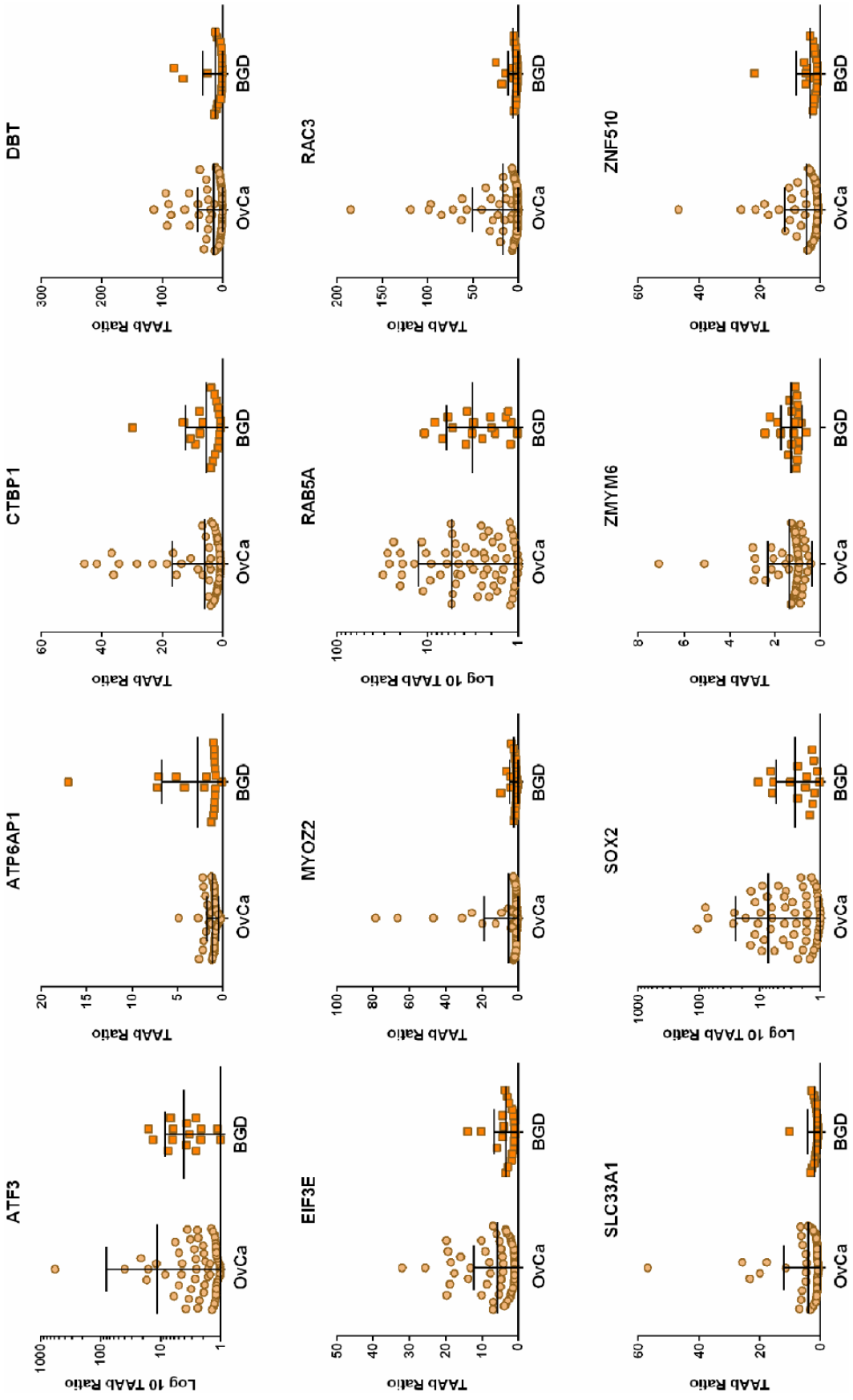
Description: Hormone biomarkers are noted as SPB, with no corresponding Uniprot information. Four biomarkers were assessed as both SPB and TAAb. All SPB were evaluated using Abbott Architect assays and all TAAb were evaluated using indirect ELISA.
Note. Some targets (e.g. MUC1) were assessed for multiple TAAb variants.

Supplementary Table 3. CPBA Ov clinical performance metrics for subjects where sample was collected postsurgery or from subjects with recurrent OvCa.

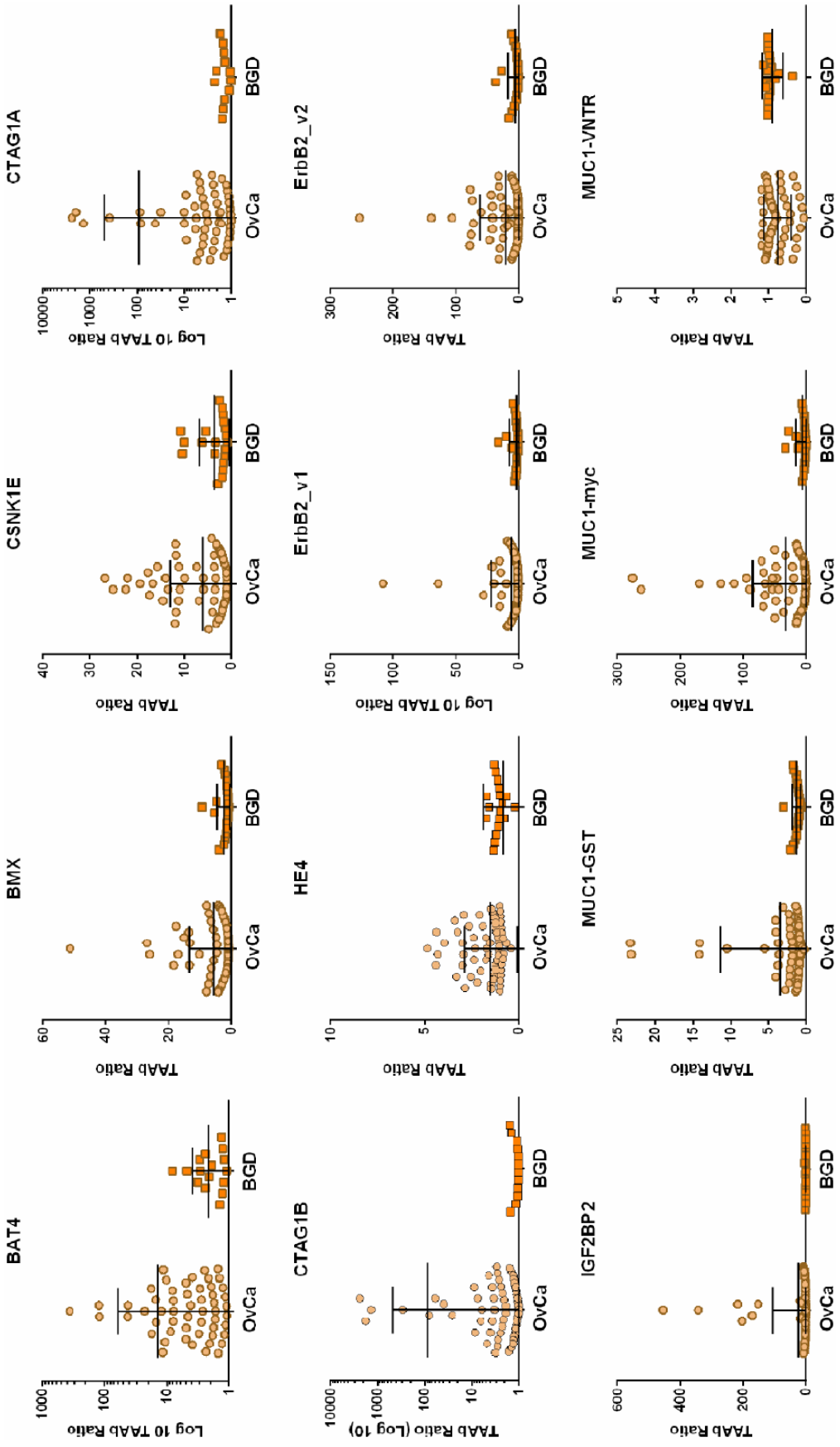
	OVCA- POST SURGERY		OVCA- RECURRENCE	
	RULE-IN	RULE-OUT	RULE-IN	RULE-OUT
TN	41	35	41	35
FP	1	7	1	7
TP	27	31	7	10
FN	9	5	6	3
Sensitivity	75.0% (57.4 – 87.2%)	86.1% (69.7 – 94.8%)	53.8% (26.1 – 79.6%)	76.9% (46.0 – 93.8%)
Specificity	97.6% (85.9 – 99.9%)	83.3% (68.0 – 92.5%)	97.6% (85.9 – 99.9%)	83.3% (68.0 – 92.5%)
NPV	82.0% (68.1 – 91.0%)	87.5% (72.4 – 95.3%)	87.2% (73.6 – 94.7%)	92.1% (77.5 – 97.9%)
PPV	96.4% (79.8 – 99.8%)	81.6% (65.1 – 91.7%)	87.5% (46.7 – 99.3%)	58.8% (33.5 – 80.6%)

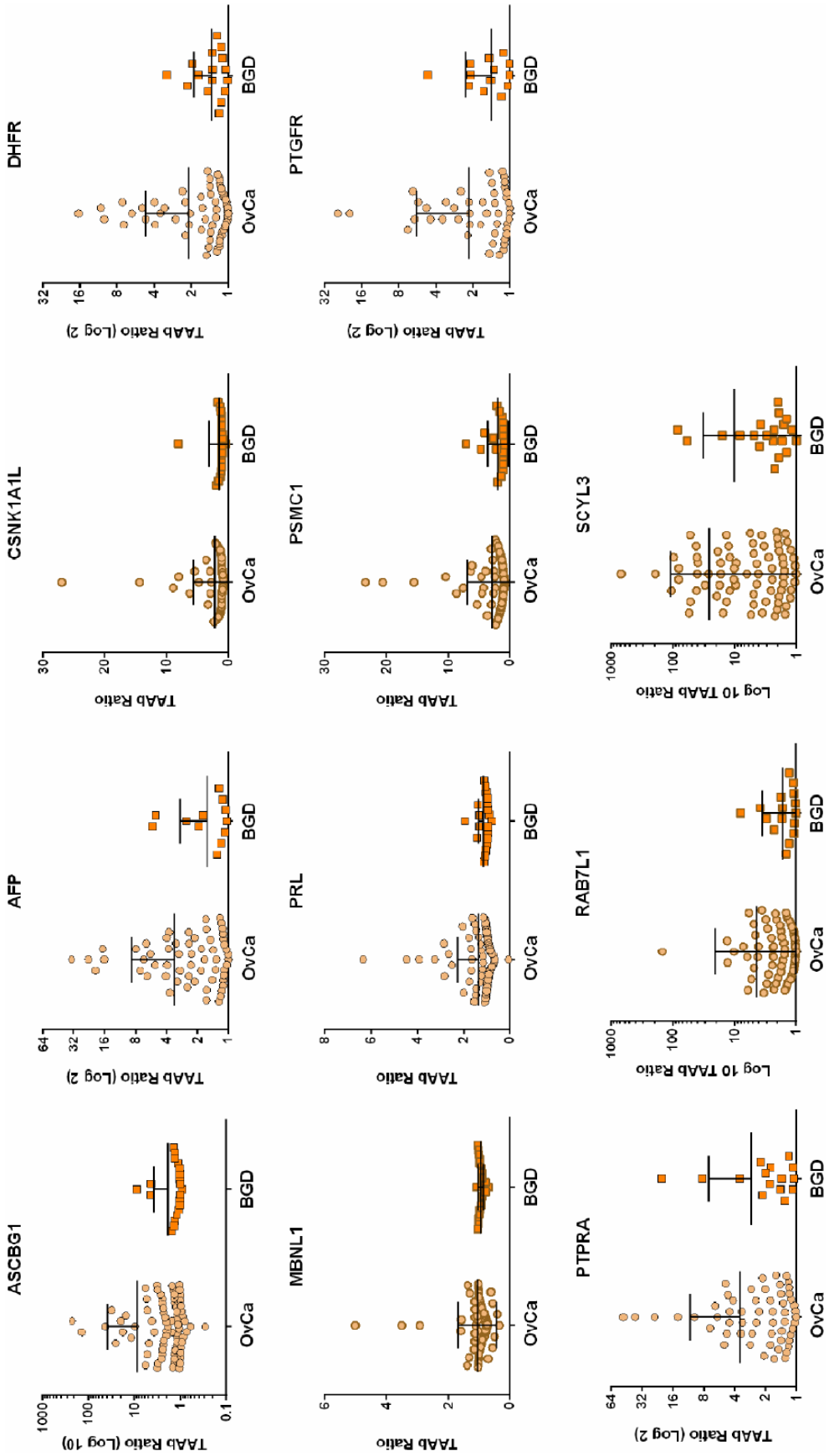
Description: A total of 36 OvCa subjects had samples drawn postsurgery and a total of 13 subjects were diagnosed with recurrent OvCa. Benign training samples (n=42) included due to a lack of post-biopsy benign samples. For sensitivity, specificity, negative predictive value (NPV), and positive predictive values (PPV), 95% confidence intervals (CIs) are shown in parentheses.


HOXD1
Log 10 TAAb Ratio
GPR157
TAAb Ratio
SERPINH1
TAAb Ratio
UBAP1
Log 10 TAAb Ratio
FRS3
TAAb Ratio
SELL
TAAb Ratio
TRIM32
TAAb Ratio
PDCD6IP
TAAb Ratio
TFCP2
TAAb Ratio
BDNF
TAAb Ratio
p53
TAAb Ratio (Log 10)
SF3A1
TAAb Ratio
OvCa
BGD


DBT
CTBP1
RAC3
ATP6AP1
RAB5A
ZNF510
ZMYM6
ATF3
MYOZ2
EIF3E
SOX2
SLC33A1
TAAb Ratio
Log 10 TAAb Ratio
OvCa
BGD

(Continued)


CTAG1A
Log 10 TAAb Ratio
10000
1000
100
10
1
OvCa
BGD
ErbB2_v2
TAAb Ratio
300
200
100
0
MUC1-VNTR
5
4
3
2
1
CSNK1E
40
30
20
10
ErbB2_v1
150
100
50
MUC1-myc
BMX
60
HE4
MUC1-GST
25
20
15
BAT4
CTAG1B
TAAb Ratio (Log 10)
IGF2BP2
600
400



Supplementary Figure 1. Scatter plots of breast- and OvCa-specific TAAb.
Description: Subjects with OvCa (OvCa, n=77) and benign gynecological disease (BGD, n=20) were assessed for the relative presence or absence of TAAb (expressed as ratio of target signal normalized to sample and protein backgrounds). Analyte mean and standard deviations are shown for each population. Log-2 or Log-10 scales are used where appropriate to better illustrate TAAb ratio distributions.

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