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A novel multiple biomarker panel for the early detection of high-grade serous ovarian carcinoma

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HIGHLIGHTS

- CA 125/HE4/E-CAD/IL-6 may represent a novel biomarker panel.
- CA 125/HE4/E-CAD/IL-6 may outperform CA125, HE4 or their combination.
- Microfluidic immunoassay platform may be useful in assessing multiple biomarkers.

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ABSTRACT

Introduction. Since the majority of patients are diagnosed at an advanced stage, ovarian cancer remains the most lethal gynecologic malignancy. There is no single biomarker with the sensitivity and specificity required for effective cancer screening; therefore, we investigated a panel of novel biomarkers for the early detection of high-grade serous ovarian carcinoma.

Methods. Twelve serum biomarkers with high differential gene expression and validated antibodies were selected: IL-1Ra, IL-6, Dkk-1, uPA, E-CAD, ErbB2, SLPI, HE4, CA125, LCN2, MSLN, and OPN. They were tested using Simple Plex™, a multi-analyte immunoassay platform, in samples collected from 172 patients who were either healthy, had benign gynecologic pathologies, or had high-grade serous ovarian adenocarcinomas. The receiver operating characteristic (ROC) curve, ROC area under the curve (AUC), and standard error (SE) of the AUC were obtained. Univariate ROC analyses and multivariate ROC analyses with the combination of multiple biomarkers were performed.

Results. The 4-marker panel consisting of CA125, HE4, E-CAD, and IL-6 had the highest ROC AUC. When evaluated for the ability to distinguish early stage ovarian cancer from a non-cancer control, not only did this 4-marker panel (AUC = 0.961) performed better than CA 125 alone (AUC = 0.851; $P = 0.0150$) and HE4 alone (AUC = 0.870; $P = 0.0220$), but also performed significantly better than the 2- marker combination of CA125 + HE4 (AUC = 0.922; $P = 0.0278$). The 4-marker panel had the highest average sensitivity under the region of its ROC curve corresponding to specificity ranging from 100% down to ~95%.

Conclusion. The four-marker panel, CA125, HE4, E-CAD, and IL-6, shows potential in detecting serous ovarian cancer at earlier stages. Additional validation studies using the biomarker combination in ovarian cancer patients are warranted.

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1. Introduction

The American Cancer Society estimates that 22,440 new ovarian cancer cases and 14,080 ovarian cancer-related deaths will occur in the United States in 2017 [1]. Ovarian cancer is identified at an advanced stage (e.g. stage III or IV) in over two-thirds of patients, and a diagnosis at advanced stages is responsible for the 5-year survival rate of only 36% and 17% for stage III and stage IV, respectively. In contrast, the 5-year survival rate is significantly improved when ovarian cancer is detected at an early stage, especially when confined to the ovary; in these cases, the 5-year survival rate improves to 89%, and the 10-year survival rate to 84% [2].

Given the clinical significance of early diagnosis, over the last three decades, multiple prediction models have been developed to detect ovarian cancer at early stages [3–6]. In this regard, the cancer antigen 125 (CA125) serum marker, which was initially utilized to monitor patients with ovarian cancer and not for screening, was extensively studied either alone or in combination with transvaginal sonography as a tool for early cancer detection in large population studies [5–7]. Unfortunately, multiple reports have demonstrated that the sensitivity and specificity of CA125 is insufficient for early detection of ovarian cancer because the biomarker can be falsely elevated in many common benign gynecologic and non-gynecologic conditions [8,9]. Furthermore, only about 50% of early-stage ovarian cancers demonstrate elevated CA125 [9].

Human epididymis protein 4 (HE4), a protease inhibitor encoded by the *WFDC2* gene, is a biomarker overexpressed by epithelial ovarian tumors, especially in serous and endometrioid histologies [10,11]. Since HE4 is measurable in the serum of women with ovarian cancer and overexpressed when compared to healthy controls, several studies have investigated whether the inclusion of HE4 analysis may improve the early diagnosis of ovarian cancer [12–15]. Because HE4 is not falsely elevated in many other benign gynecologic and medical conditions and is elevated in >50% of ovarian cancers that do not express CA 125, HE4 has also been used in combination with CA 125 in pre-operative triaging of women affected by pelvic masses [10,16]. However, though HE4 seems promising in assessing the malignant potential of pelvic masses, it is not a "perfect" biomarker. Indeed, there are many variables affecting HE4 serum level - such as age, smoking, renal function, and non-gynecologic cancers [17,18].

In order to achieve the goal of early ovarian cancer detection, it is essential to identify additional biomarkers with high sensitivity and specificity for the disease that may also distinguish malignant pelvic masses from benign ones. Consistent with this view, in the last few years several groups including ours, have used gene expression-profiling assays to identify novel biomarkers highly differentially expressed in ovarian cancer patients [19–21]. The use of a panel of biomarkers using a multiplex approach may indeed be rapid and highly reproducible with potentially higher sensitivity and specificity than single biomarkers, such as CA125 or HE4 for the early detection of ovarian cancer [22–24].

In this study we have used Simple Plex™, a newly developed immunoassay platform, and identified a panel of novel biomarkers for early detection of high grade serous ovarian cancer by simultaneously evaluating 12 biomarkers that are measurable in the serum of women and highly elevated in ovarian cancer compared to benign conditions.

2. Methods and materials

2.1. Biomarker selection

Initially, we pooled genes that were known to have at least 5-fold higher expression in high-grade serous ovarian cancer compared to those of non-cancer controls [19–21]. Among these genes, we identified 12 biomarkers by selecting genes that encode for secreted proteins and for which validated antibodies for ELISA testing were available. These genes are: interleukin 1 receptor antagonist (IL-1Ra), interleukin 6 (IL-6), Dickkopf-related protein-1 (Dkk-1), urokinase plasminogen activator (uPA), E-CAD, epidermal growth factor receptor-2 (ErbB2), secretory leukocyte protease inhibitors (SLPI), HE4, CA125, lipocalin-2 (LCN2), mesothelin (MSLN), and osteopontin (OPN).

2.2. Study population and serum sample collection

Our study included 47 women in a control group (17 healthy women and 30 women with benign ovarian masses) and 125 women with newly diagnosed ovarian cancer in a case group. All pathologic diagnoses were determined by expert gynecological pathologist. Ten milliliter of peripheral blood was collected from study subjects after written informed consent was obtained. Within 2 to 4 h after collection, the blood sample was centrifuged, and the serum was collected, dispensed into cryotubes, and stored at −80 °C. Twenty-three of these samples were obtained through the Tina Brozman Ovarian Consortium and 149 samples were from Yale University. All blood samples were obtained within 1 week prior to a surgery for patients who underwent surgery.

2.3. Simple Plex™ assay procedure

Simple Plex™ (Protein Simple, San Jose, CA) is a multi-analyte immunoassay platform, which uses a single disposable microfluidic cartridge. The cartridges are self-contained with all reagents consisting of capture antibodies, biotinylated detection antibodies, and streptavidin-dye conjugate. As a result, inconsistency by multiple sample handlings is eliminated. Each microfluidic channel has three glass nanoreactors which require very low sample volumes for the assay. Reagents for the Simple Plex™ were custom developed by the manufacturer, and divided into 3 panels. Panel 1 included biomarkers at a 1:2 dilution: IL-1Ra, IL-6, Dkk-1, and uPA. Panel 2 included biomarkers at a 1:10 dilution: E-CAD, ErbB2, SLPI, and HE4. Panel 3 included biomarkers at a 1:100 dilution: CA125, LCN2, MSLN, and OPN. The serum samples were diluted in the diluent provided by the manufacturer. Then, 50 μL of diluted samples were loaded into the analyzer cartridge, followed by placement into the Simple Plex™ instrument. The results were calculated using Simple Plex™ Explorer software. The serum samples were evaluated in a blinded manner; the technical personnel performing the testing were not aware of the patients' disease status.

2.4. Statistical analysis

Statistical analyses were performed using SAS v9.4 (The SAS institute Inc., Cary, NC). The Kruskal-Wallis test was used to compare the age distributions of patients with ovarian cancer versus non-cancer controls, whereas Fisher's exact test was used to compare the race distribution of ovarian cancer patients and non-cancer controls. Both types of tests utilized 2-sided $\alpha = 0.05$ significance levels. Prior to receiver operating characteristics (ROC) analysis, the serum concentrations of the biomarkers were transformed to their natural logs, both to reduce right-skewing and to follow the practice of Moore et al. in developing their Risk of Ovarian Malignancy Algorithm (ROMA) risk-prediction equations [25]. ROC analysis was performed in SAS using its Logistic Procedure, and was initially performed on all ovarian cancers versus all non-cancer controls. To evaluate the diagnostic power of each biomarker alone, we performed univariate ROC analysis on each biomarker in order to obtain its ROC curve, ROC area under the curve (AUC), and standard error (SE) of the AUC. After univariate ROC analysis on each of the 12 markers, we assembled all 165 possible 4-marker panels that included CA125. We performed multivariate ROC analysis on each 4-marker panel in order to obtain that panel's ROC curve and AUC. Our goal was to select the panel with the highest ROC AUC. In case of a tie for highest AUC, we would break the tie by choosing the panel with the lowest SE (AUC).

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Once we found the "best" 4-marker panel with the highest AUC, we evaluated further its performance at distinguishing cancers from controls, as follows: First, we generated the ROC curve and ROC AUC for the 4-marker panel, for CA125 alone, for HE4 alone, and for HE4 + CA125. Second, we compared the ROC AUCs of the last three panels (CA125 alone, HE4 alone, and HE4 + CA125) to the ROC AUC of the 4-marker panel using the method of Delong et al. [26] with 1-sided tests at unadjusted $\alpha = 0.05$ significance levels. Third, we found the cut point on each panel's ROC curve that maximized its discriminative index (i.e., its Youden index, which is calculated as sensitivity minus false-positive rate), and calculated the panel's sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at that cut point. Fourth and finally, we used the trapezoid method to calculate the normalized partial AUC (npAUC) of each panel's ROC curve in the region of the curve where false-positive rates range from 0% to a target value of 5% (i.e., where specificity ranges from 95% to 100%). The divisor for normalizing a ROC curve's partial AUC was the highest observed false-positive rate (FPR) below its target value (FPR < Target). These four steps were first performed with the "best" 4-marker panel applied to all ovarian cancers versus all non-cancer controls. The same 4 steps then were repeated using the same 4-marker panel applied to the following reduced patient denominators: Early-stage cancers (FIGO stages I and II) versus all non-cancer controls; all ovarian cancers versus subjects with benign disease only; and all ovarian cancers versus only "healthy" subjects free of gynecological pathology. When controls consisted only of "healthy" subjects, the small number of such subjects, N = 17, forced us to raise the target FPR to ≥5.88% (≥1/17) in order to be able to calculate ROC npAUCs. We follow Pepe [27] and interpret npAUC as the panel's average sensitivity when FPR ranges from 0 to the highest observed FPR < Target.

3. Results

Table 1 shows the study subjects' demographic characteristics. All ovarian cancers were high-grade serous ovarian carcinoma. Serous cystadenoma was the most common benign pathologies (57%), followed by ovarian teratomas (10%) and mucinous cystadenomas (7%). There was only one case of ovarian endometriosis in the benign pathology group. The mean ± SD of age in years was 58.4 ± 13.3 among non-cancer controls (52.5 ± 10.7 among healthy volunteers, 63.1 ± 13.6 among women with benign ovarian masses) and 62.7 ± 11.9 among those with ovarian cancer ($P = 0.048$). There was no significant distribution difference in race between case and control groups ($P = 1.00$).

The results of univariate ROC analysis are shown in Table 2. The ROC AUCs and standard errors were based on complete data from 172 samples, except for IL-6 (missing in 1 benign sample), OPN (missing in 1 Stage IIIC sample), and SLPI (missing in 1 Stage IIIC sample). Except for uPA (AUC = 0.490), the AUC of each studied biomarker was >0.5, as expected. Multivariate ROC analyses of the combinations of four biomarkers were performed. Among all the 165 possible four-marker panels that included CA125 (Table S1), one panel had the highest ROC AUC, despite being based on complete data from 171 instead of 172 samples. This panel consisted of CA125, HE4, E-CAD, and IL-6, and will be referred to below as "the 4-marker panel". It was further evaluated for its performance at distinguishing cancer samples from non-cancer samples.

3.1. All ovarian cancers versus all non-cancer controls

Fig. 1A shows the ROC curves for CA125 alone, HE4 alone, CA125 + HE4, and the 4-marker panel when these four panels are applied to all 171 samples with complete data (125 cancers and 46 controls). The AUC under each panel's ROC curve is shown in Table 3A along with results comparing the other three panels to the 4-marker panel for AUC differences. The 4-marker panel had the highest ROC AUC at 0.971, followed by HE4 + CA125 (AUC = 0.959), then by HE4 alone (AUC = 0.940), and lastly by CA125 alone (AUC = 0.935). The last two panels had statistically significant decreases in their ROC AUCs compared to the 4-marker panel (Table 3A). Each panel was evaluated for sensitivity, specificity, PPV, and NPV at the ROC curve's cut point that maximized the panel's discriminative index on the entire data set; Table 4A shows the results. Among the four panels, CA125 alone had the highest sensitivity (90.4%), but at the cost of having lowest specificity (87.0%) and thus the highest FPR (100%–87.0% = 13.0%). In contrast, the 4-marker panel had the highest specificity (100%) and the highest PPV (100%) among the four panels, but had only 86.4% sensitivity. Table 4A also shows the npAUCs for the ROC curves within the region defined by target FPR < 5% (specificity > 95%). The highest observed FPR < target was 4.3%. Within the FPR region from 0% to 4.3%, the 4-marker panel had the highest average sensitivity (npAUC = 86.8%), CA125 alone had the lowest average sensitivity (npAUC = 64.4%), and the other two panels had intermediate average sensitivities (npAUCs of 65.6% for HE4 alone and 78.0% for HE4 + CA125; Table 4A).

3.2. Early-stage ovarian cancers versus all non-cancer controls

Fig. 1B shows the ROC curves for CA125 alone, HE4 alone, CA125 + HE4, and the 4-marker panel when these four panels are applied to 65 samples with complete data (19 early-stage cancers and 46 controls) after excluding 106 late-stage cancers from the analysis. The AUC under each panel's ROC curve is shown in Table 3B along with results comparing the other three panels to the 4-marker panel for AUC differences. The four panels retained their previous ranking by AUC (4-marker panel > HE4 + CA125 > HE4 alone > CA125 alone), and this time, the 4-marker panel had a significantly higher AUC than all 3 of the other panels (Table 3B). Each panel's sensitivity, specificity, PPV, and NPV

Table 1
Characteristics of study subjects.

	Number of patients (*n*)	Age (years, mean ± SD, range)	Race (AA/EA/O)
Control	47	58.4 ± 13.3 (33–83)	5/41/1
Healthy subjects	17	52.5 ± 10.7 (35–71)	3/14/0
Benign disease	30	61.7 ± 13.6 (33–83)	2/27/1
Case	125	62.7 ± 11.9 (27–87)	13/107/5
Early stage ovarian cancer[a]	19	68.9 ± 13.0 (42–87)	1/17/1
Late stage ovarian cancer[b]	106	61.6 ± 11.3 (27–87)	12/90/4
P-value		0.048 ‡	1.00§

SD, standard deviation; AA, African American; EA, European American; O, Other (Hispanic, Asian, or N/A).
P-value for the comparison of Controls and Cases via ‡Kruskal-Wallis test and §Fisher's exact test.
[a] Stage I and II ovarian cancer.
[b] Stage III and IV ovarian cancer.

Table 2
Univariate ROC analysis of biomarkers.

Marker name	ROC AUC ± SE (AUC)
HE4	0.941 ± 0.017
CA125	0.935 ± 0.019
IL-6	0.855 ± 0.029§
MSLN	0.832 ± 0.034
OPN	0.813 ± 0.034[a]
SLPI	0.705 ± 0.042[a]
IL-1ra	0.579 ± 0.046
LCN2	0.572 ± 0.051
Dkk-1	0.564 ± 0.049
E-CAD	0.558 ± 0.046
ErbB2	0.556 ± 0.050
uPA	0.490 ± 0.053

ROC AUC, Receiver Operating Characteristics Area Under Curve; SE (AUC), standard error of ROC AUC.
[a] One missing value.

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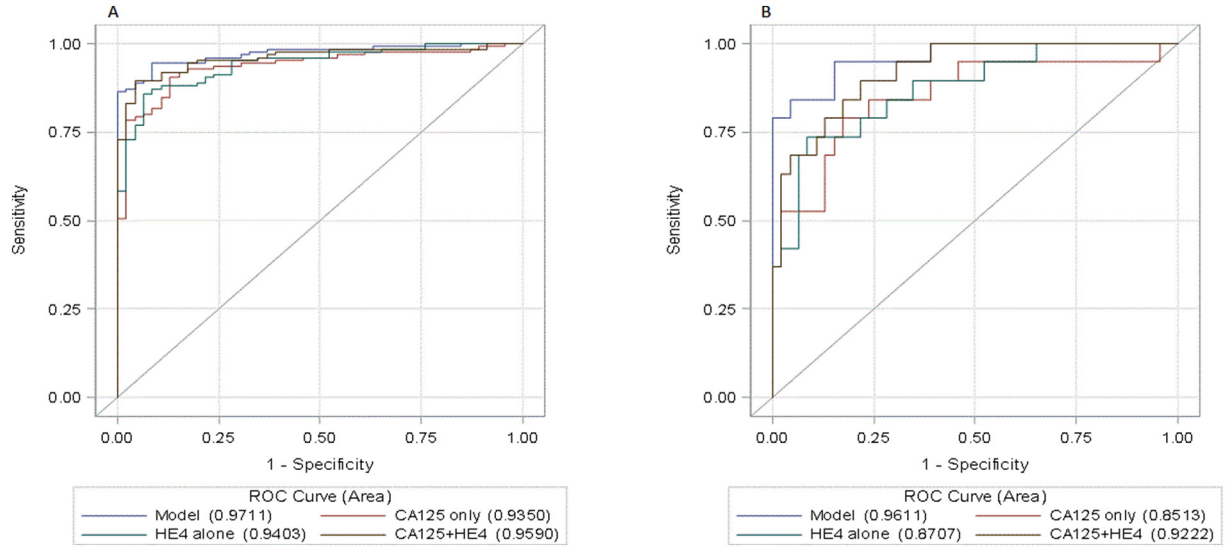

Fig. 1. A. ROC Curves distinguishing cancers (all stages) from non-cancer controls. The ROC curves show the classification behavior of four different biomarker panels used to distinguish 125 cancer samples (all stages) from 46 non-cancer controls. "Model" refers to the 4-marker panel consisting of CA125, E-CAD, HE4, and IL-6. Comparisons of classification performance among the four different biomarker panels appear in Tables 3A and 4A. B. ROC Curves distinguishing early-stage cancers from non-cancer controls. The ROC curves show the classification behavior of four different biomarker panels used to distinguish 19 early-stage cancer samples (FIGO stages I and II) from 46 non-cancer controls. "Model" refers to the 4-marker panel consisting of CA125, E-CAD, HE4, and IL-6. Comparisons of classification performance among the four different biomarker panels appear in Tables 3B and 4B.

was calculated at the cut point on its ROC curve where its discriminative index was maximized; Table 4B shows the results. This time, HE4 + CA125 had the highest sensitivity (89.5%), but at the cost of having lowest specificity (78.3%) along with lowest PPV (63.0%). On the other hand, the 4-marker panel had the highest specificity (95.7%) and highest PPV (88.9%) while managing to attain 84.2% sensitivity. Table 4B also shows the npAUCs for the ROC curves within the region defined by target FPR < 5%. The highest observed FPR < target was 4.3%. Within the FPR region from 0% to 4.3%, the 4-marker panel had highest average sensitivity (npAUC = 79.0%), followed by HE4 + CA125 (npAUC = 50.0%), CA125 alone (npAUc = 44.7%), and HE4 alone (npAUC = 39.5%) (Table 4B).

Table 3
Performance of classification panels assessed using ROC AUCs.

Classification panel	ROC AUC	SE[a] of AUC	Difference in AUCs[b]	SE[a] of difference	1-sided *P*-value
3A. Ovarian cancers (all stages) versus all non-cancer controls.					
All 4 markers[c]	0.9711	0.0110	–	–	–
CA125 only	0.9350	0.0193	−0.0362	0.0135	0.0036
HE4 alone	0.9403	0.0174	−0.0308	0.0128	0.0083
HE4 + CA125	0.9590	0.0142	−0.0122	0.0080	0.0644
3B. Early-stage ovarian cancers versus all non-cancer controls.					
All 4 markers[c]	0.9611	0.0243	–	–	–
CA125 only	0.8513	0.0592	−0.1098	0.0506	0.0150
HE4 alone	0.8707	0.0497	−0.0904	0.0449	0.0220
HE4 + CA125	0.9222	0.0332	−0.0389	0.0203	0.0278
3C. Ovarian cancers (all stages) versus subjects with benign gynecological conditions					
All 4 markers[c]	0.9608	0.0139	–	–	–
CA125 only	0.9178	0.0247	−0.0430	0.0175	0.0069
HE4 alone	0.9192	0.0233	−0.0417	0.0179	0.0099
HE4 + CA125	0.9473	0.0173	−0.0135	0.0091	0.0679
3D. Ovarian cancers (all stages) versus "healthy" subjects with no gynecological pathology					
All 4 markers[c]	0.9896	0.0075	–	–	–
CA125 only	0.9642	0.0145	−0.0254	0.0128	0.0234
HE4 alone	0.9765	0.0112	−0.0132	0.0096	0.0846
HE4 + CA125	0.9807	0.0107	−0.0089	0.0084	0.1441

[a] Standard error.
[b] Calculated as AUC of indicated panel minus AUC of All 4 markers.
[c] The 4 markers are: CA125, E-CAD, HE4, and IL-6.

3.3. All ovarian cancers versus subjects with benign pathologies only

Fig. 2A shows the ROC curves for CA125 alone, HE4 alone, CA125 + HE4, and the 4-marker panel when these four panels are applied to 154 samples with complete data (125 cancers and 29 controls with benign pathologies) after excluding 17 "healthy" subjects with no gynecological pathologies from the analysis. The AUC under each panel's ROC curve is shown in Table 3C along with results comparing the other three panels to the 4-marker panel for AUC differences. The four panels retained their original ranking by AUC, with the 4-marker panel placing first (AUC = 0.961), HE4 + CA125 placing second (AUC = 0.947), and with the other two markers almost tied for last (AUCs of 0.919 for HE4 alone and 0.918 for CA125 alone). The last two panels had statistically significant decreases in their ROC AUCs compared to the 4-marker panel (Table 3C). Each panel was evaluated for sensitivity, specificity, PPV, and NPV at the ROC curve's cut point that maximized the panel's discriminative index on this reduced patient denominator; Table 4C shows the results. HE4 + CA125 attained the highest sensitivity (89.6%), but it was combined with 2nd-lowest specificity (93.1%). The 4-marker panel, on the other hand, achieved 100% specificity and 100% PPV while maintaining 87.2% sensitivity. Table 4C also shows the npAUCs for the ROC curves within the region defined by target FPR < 5%. This time, the highest observed FPR < target was only 3.4% (=1/29) because of the smaller number of controls. Within the FPR region from 0% to 3.4%, the 4-marker panel had highest average sensitivity (npAUC = 87.2%), followed by HE4 + CA125 (npAUC = 74.4%), HE4 alone (58.4%), and CA125 only (50.4%) (Table 4C).

3.4. All ovarian cancers versus only "healthy" subjects with no gynecological pathologies

Fig. 2B shows the ROC curves for CA125 alone, HE4 alone, CA125 + HE4, and the 4-marker panel when these four panels are applied to 142 samples (125 cancers and 17 control subjects with no gynecological pathologies) after excluding the 29 controls with benign pathologies from the analysis. The AUC under each panel's ROC curve is shown in Table 3D along with results comparing the other three panels to the 4-marker panel for AUC differences. The four panels continued to keep

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Table 4
Reporting of operating characteristic of the classification panels; operating characteristics are sensitivity, specificity, positive predictive value, and negative predictive value. Also included are partial AUCs for false-positive rates less than their target values.

Classification panel	Sensitivity[a]	Specificity[a]	Positive predictive value[a]	Negative predictive value[a]	Highest FPR < Target[b]	Normalized partial AUC[c]
4A. Ovarian cancers (all stages) versus all non-cancer controls.						
All 4 markers[d]	86.4%	100.0%	100.0%	73.0%	4.3%	86.8%
CA125 only	90.4%	87.0%	95.0%	76.9%	4.3%	64.4%
HE4 alone	85.6%	93.5%	97.3%	70.5%	4.3%	65.6%
HE4 + CA125	89.6%	95.7%	98.3%	77.2%	4.3%	78.0%
4B. Early-stage ovarian cancers versus all non-cancer controls.						
All 4 markers[d]	84.2%	95.7%	88.9%	93.6%	4.3%	79.0%
CA125 only	79.0%	82.6%	65.2%	90.5%	4.3%	44.7%
HE4 alone	73.7%	91.3%	77.8%	89.4%	4.3%	39.5%
HE4 + CA125	89.5%	78.3%	63.0%	94.7%	4.3%	50.0%
4C. Ovarian cancers (all stages) versus subjects with benign gynecological conditions						
All 4 markers[d]	87.2%	100.0%	100.0%	64.4%	3.4%	87.2%
CA125 only	78.4%	96.6%	99.0%	50.9%	3.4%	50.4%
HE4 alone	85.6%	89.7%	97.3%	59.1%	3.4%	58.4%
HE4 + CA125	89.6%	93.1%	98.3%	67.5%	3.4%	74.4%
4D. Ovarian cancers (all stages) versus "healthy" subjects with no gynecological pathology						
All 4 markers[d]	98.4%	100.0%	100.0%	89.5%	5.9%	98.4%
CA125 only	90.4%	100.0%	100.0%	58.6%	5.9%	90.4%
HE4 alone	95.2%	94.1%	99.2%	72.7%	5.9%	88.8%
HE4 + CA125	95.2%	100.0%	100.0%	73.9%	5.9%	95.2%

[a] Calculated at the classification panel's cut point along its ROC curve that maximizes the panel's discriminative index (calculated as Sensitivity minus False-Positive Rate at each cut point).
[b] Highest False-Positive Rate (FPR) that is less than the target value. This is used to calculate the Normalized Partial AUC. Target FPR values are 5%, 5%, 5%, and 6%, respectively, for the patient denominators in 4A, 4B, 4C, and 4D.
[c] Normalized Partial Area under the ROC curve, calculated under the ROC curve for FPR values restricted to the range from 0 to Highest FPR < Target.
[d] The 4 markers are: CA125, E-CAD, HE4, and IL-6.

their original ordering by AUC (4-marker panel > HE4 + CA125 > HE4 alone > CA125 alone), but only the AUC difference between CA125 alone and the 4-marker panel attained statistical significance (Table 3D), possibly because of the small number of controls. Table 4D shows the sensitivity, specificity, PPV, and NPV of each panel when its discriminative index was maximized. This time, three of the four panels achieved 100% specificity and 100% PPV, but almost certainly because of the small number of control samples. Among these three, the 4-marker panel had highest sensitivity (98.4%), followed by HE4 + CA125 (95.2%) and CA125 alone (90.4%). HE4 alone had 95.2% sensitivity and 94.1% specificity when its discriminative index was maximized. Table 4D also shows the npAUCs for the panels' ROC curves within the region defined by the target FPR. Because of the small number of "healthy" controls, the target FPR was raised to 6%, yielding a highest observed FPR < target of 5.9%. Within the FPR region from 0% to 5.9%, the 4-marker panel had highest average sensitivity (npAUC = 98.4%), followed by HE4 + CA125 (npAUC = 95.2%), CA125 alone (npAUC = 90.4%), and HE4 alone (npAUC = 88.8%).

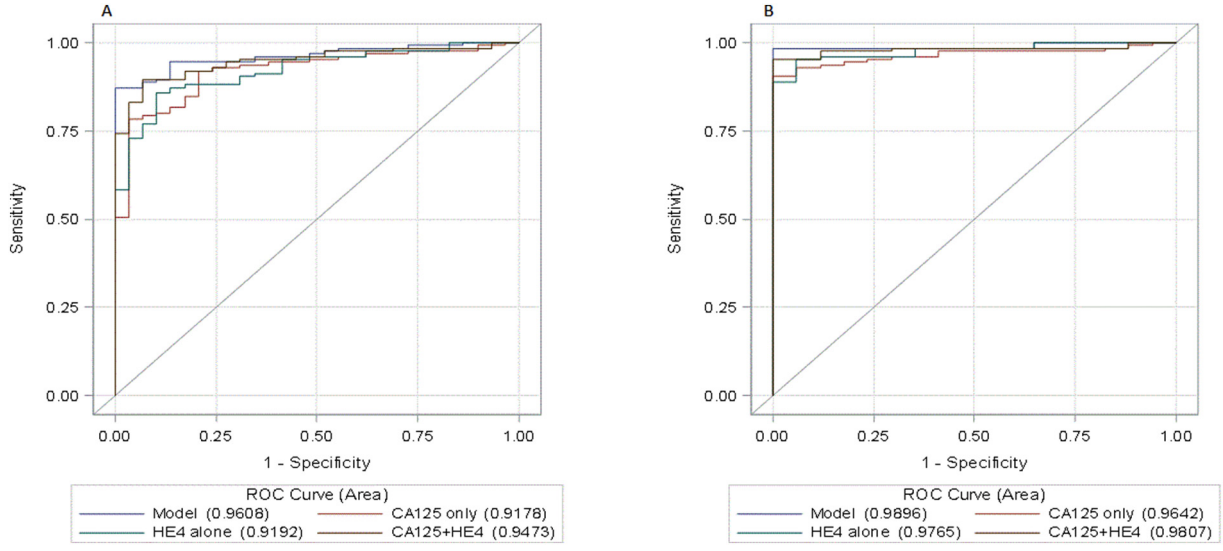

Fig. 2. A. ROC Curves distinguishing cancers (all stages) from subjects with Benign Disease. The ROC curves show the classification behavior of four different biomarker panels used to distinguish 125 cancer samples (all stages) from 29 control subjects with benign disease. "Model" refers to the 4-marker panel consisting of CA125, E-CAD, HE4, and IL-6. Comparisons of classification performance among the four different biomarker panels appear in Tables 3C and 4C. B. ROC Curves distinguishing cancers (all stages) from subjects with no gynecological pathology. The ROC curves show the classification behavior of four different biomarker panels used to distinguish 125 cancer samples (all stages) from 17 "healthy" subjects with no gynecological pathologic condition. "Model" refers to the 4-marker panel consisting of CA125, E-CAD, HE4, and IL-6. Comparisons of classification performance among the four different biomarker panels appear in Tables 3D and 4D.

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3.5. Summary of results

In all four of the above analyses, the 4-marker panel had the highest ROC AUC, with HE4 + CA125 having the 2nd-highest, HE4 alone having the 3rd-highest, and CA125 alone having the lowest ROC AUC. Importantly, when evaluated for the ability to distinguish early stage ovarian cancer from a non-cancer control, the 4-marker panel had a significantly higher AUC than all 3 of the other panels. Perhaps more importantly for translation to a clinical setting, all four analyses also found that the 4-marker panel had the highest normalized partial AUC (i.e., highest average sensitivity) under the region of its ROC curve corresponding to specificity ranging from 100% down to ~95%.

4. Discussion

Detecting ovarian cancer at an early stage and attaining optimal cytoreduction are two major factors affecting an ovarian cancer patient's prognosis. This highlights the importance of sensitive and reliable strategies for the early detection of ovarian cancer as well as the triage of patients to tertiary care institutions for cytoreductive surgery. There have been major efforts to develop strategies to detect ovarian cancer at an early stage and to distinguish benign pelvic masses from ovarian cancer. Along these lines, circulating serum biomarkers have been a major focus in these efforts. Our study was initiated with the goal of developing a panel of serum biomarkers that is operator-independent, easily reproducible, and outperforms the biomarkers that have been proposed in the past.

The Risk of Malignancy Index (RMI), proposed by Jacobs et al. assessed the risk of ovarian cancer by combining the value of CA125 with ultrasound findings and menopausal status [3]. This algorithm showed 85% sensitivity and 97% specificity using an RMI cut-off level of 200. Patients with an RMI score >200 had a 42 times increased risk of cancer compared to background risk. Although RMI was more accurate in assessing malignancy risk in women with pelvic masses, high false-positive rate of ultrasound along with poor sensitivity and specificity of CA125, especially in premenopausal women, was the main limitation of RMI.

The Risk of Ovarian Cancer Algorithm (ROCA), a velocity-based algorithm in cancer screening strategies, has been developed to overcome the limitation of a single-value cut-off of CA125 and been shown to improve both sensitivity and specificity [4,28]. Jacobs et al. recently reported the impact of ovarian cancer screening using the ROCA from the UK Collaborative Trial of Ovarian Cancer Screening. In their analysis, there was a reduction in cancer deaths on 14 years' follow up [29]. Although longer follow-up is definitely needed before firm conclusions can be made, this finding caught the attention of many investigators. However, it is premature to use this technique to screen the general population. In fact, after the ROCA study results, the American College of Obstetrics and Gynecology, the Society of Gynecologic Oncology, and the Food and Drug Administration (FDA) made an announcement stating that currently available screening tests such as ROCA are neither sufficiently accurate nor reliable to screen asymptomatic women for early ovarian cancer, and expressed concerns that ROCA might lead to unnecessary surgical procedures. Both RMI and ROCA studies involve ultrasound in their algorithms. Although ultrasound is useful and effective to evaluate an adnexal mass, it is examiner-dependent [30]. Thus, well-trained and experienced ultrasonographers are required in order to attain consistent results. This suggests that a pure biomarker screening strategy would be more objective and reproducible in certain settings such as a primary-care office where the initial evaluation for a pelvic mass likely begins.

In the past few years, several novel algorithms using multiple biomarkers have been validated to distinguish benign pelvic masses from ovarian cancer. These are not true diagnostic tests, but rather triage tools which heavily rely on CA125 and HE4. The OVA-1 test, a five-biomarker assay was the first test cleared by the U.S. FDA, based on high sensitivity and negative predictive value, for estimating the preoperative cancer risk of an adnexal mass. The ROMA is another multiple-biomarker algorithm which utilizes CA125, HE4, and menopausal status [16]. The ROMA algorithm successfully classified patients into high and low risk groups, effectively triaging women who need referral to a tertiary center for surgery. In 2016, the FDA approved Overa, the next generation of OVA-1, which includes CA125, HE4, follicle-stimulating hormone, apolipoprotein A-1, and transferrin. This newest panel of multiple biomarkers demonstrated 91.3% sensitivity and 69.1% specificity [31,32]. With these multiple-biomarker algorithms, sensitivity has improved, but specificity and positive predictive value continue to be challenging. We note that 69.1% specificity means a 30.9% false positive rate, which is probably acceptable for a triage test, but probably too high for a true diagnostic test. Also, these tests appeared to have somewhat limited performance in premenopausal women compared to postmenopausal women [33].

As seen in the evolution of ovarian cancer biomarkers, significant research effort has been placed into the development of minimally invasive, sensitive, and specific tests that combine multiple serum biomarkers with or without imaging. Our study is one such effort to identify a panel of multiple serum biomarkers which is not just sensitive and specific, but also time-efficient and cost-effective. Simple Plex™ is a new multi-analyte immunoassay platform, which has been proven to have significant advantages over traditional approaches in terms of low volume requirements (2.5 to 25 μL), sensitivity, and reproducibility [34,35]. Simple Plex™ eliminates inconsistency in sample handling and produces results in a short amount of time, thus providing results with minimal error and in timely fashion. The cost for each analyte is estimated to be $12 in our study, thus it would cost $48 for a 4-analytes analysis. Compared to the OVA1 test (approximately at $600) and ROMA test (approximately $100), Simplex Plex™ costs much less and would be more cost-efficient to analyze multiple biomarkers. In our study, using the Simple Plex™ platform, we simultaneously evaluated the performance of 12 serum biomarkers originally identified by gene expression profiling assays as highly differentially expressed ovarian cancer genes that encode for circulating proteins [19–21]. We found that the combination of four serum biomarkers consisting of CA125, HE4, E-CAD and IL-6 displayed a significantly higher ROC AUC than CA125 and HE4. More importantly, we saw a statistically significant improvement in the use of the 4-marker panel in distinguishing early stage ovarian cancer from a non-cancer control group, including both healthy individuals as well as patients harboring pelvic masses, than CA125, HE4, and the combination of CA125 + HE4.

E-CAD is a cell-surface glycoprotein involved in epithelial cell-to-cell adhesion. E-CAD is required for epithelial histogenesis, tissue stabilization, and differentiated functions, and the protein is expressed in the majority of primary ovarian carcinoma [36]. Down regulation or mutation of E-CAD is associated with ovarian cancer progression and invasiveness, and of interest, several previous studies demonstrated a positive relationship between E-CAD and CA125. For example, Auersperg et al. reported that E-CAD induced CA125 production in ovarian cancer [37]. Similarly, Rosso et al. demonstrated the association of higher E-CAD mRNA level with increased CA125 level [38]. Our study results are in agreement with these data demonstrating the increased ability to detect early ovarian cancer using a panel of proteins including CA125, HE4, IL-6 and E-CAD.

Interleukin-6 is a cytokine associated with a variety of pathological conditions and previous studies in ovarian cancer demonstrated that IL-6 enhances tumor cell survival, regulates immune cell infiltration in tumor tissue and ascites, and increases resistance to chemotherapy [39]. Importantly, Gopinathan et al. reported direct effects of IL- 6 on endothelial cell proliferation, migration, and angiogenesis which is one of the initial critical events in cancer development [40], supporting our study results for a role of IL-6 as an important biomarker in early detection of ovarian cancer.

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Our study's finding that the 4-marker panel shows better performance in early ovarian cancer detection is of importance, and carries the potential for clinical application. However, this study has limitations. Our study cohort was limited to high-grade serous cancer, so our findings might not be applicable to other subtypes of ovarian cancer. However, considering that serous histology is the most prevalent and one of the most lethal subtypes of ovarian cancer, that it is almost always detected in an advanced stage, and that it is the most common histologic cancer type detected in high-risk populations that require a sensitive and effective screening test, our study results may have important implications for this group of ovarian cancer patients. Another limitation is the relatively small number of patients, which could have affected the statistical analysis. While we acknowledge that larger studies with independent cohorts of patients will be necessary to validate our findings, we believe our results using a novel multiplex biomarker platform may have value to inform further investigations which seek to improve screening and triage algorithms for the detection of high-grade serous ovarian carcinoma.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2018.03.050.

Conflict of interest statement

We declare no conflict of interest associated with this manuscript.

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References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2017, CA Cancer J. Clin. 67 (2017) 7–30.
[2] L.A. Baldwin, B. Huang, R.W. Miller, T. Tucker, S.T. Goodrich, I. Podzielinski, et al., Ten-year relative survival for epithelial ovarian cancer, Obstet. Gynecol. 120 (2012) 612–618.
[3] I. Jacobs, D. Oram, J. Fairbanks, J. Turner, C. Frost, J.G. Grudzinskas, A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer, Br. J. Obstet. Gynaecol. 97 (1990) 922–929.
[4] U. Menon, A. Ryan, J. Kalsi, A. Gentry-Maharaj, A. Dawnay, M. Habib, et al., Risk algorithm using serial biomarker measurements doubles the number of screen-detected cancers compared with a single-threshold rule in the United Kingdom collaborative trial of ovarian cancer screening, J. Clin. Oncol. 33 (2015) 2062–2071.
[5] I.J. Jacobs, S.J. Skates, N. MacDonald, U. Menon, A.N. Rosenthal, A.P. Davies, et al., Screening for ovarian cancer: a pilot randomised controlled trial, Lancet 353 (1999) 1207–1210.
[6] E. Partridge, A.R. Kreimer, R.T. Greenlee, C. Williams, J.L. Xu, T.R. Church, et al., Results from four rounds of ovarian cancer screening in a randomized trial, Obstet. Gynecol. 113 (2009) 775–782.
[7] U. Menon, A. Talaat, A.N. Rosenthal, N.D. Macdonald, A.R. Jeyerajah, S.J. Skates, et al., Performance of ultrasound as a second line test to serum CA125 in ovarian cancer screening, BJOG 107 (2000) 165–169.
[8] S.S. Buys, E. Partridge, M.H. Greene, P.C. Prorok, D. Reding, T.L. Riley, et al., Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial, Am. J. Obstet. Gynecol. 193 (2005) 1630–1639.
[9] G. Soletormos, M.J. Duffy, S. Othman Abu Hassan, R.H. Verheijen, B. Tholander, R.C. Bast Jr., et al., Clinical use of cancer biomarkers in epithelial ovarian cancer: updated guidelines from the European Group on Tumor Markers, Int. J. Gynecol. Cancer 26 (2016) 43–51.
[10] I. Hellstrom, J. Raycraft, M. Hayden-Ledbetter, J.A. Ledbetter, M. Schummer, M. McIntosh, et al., The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma, Cancer Res. 63 (2003) 3695–3700.
[11] R. Drapkin, H.H. von Horsten, Y. Lin, S.C. Mok, C.P. Crum, W.R. Welch, et al., Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas, Cancer Res. 65 (2005) 2162–2169.
[12] M.A. Karlsen, N. Sandhu, C. Hogdall, I.J. Christensen, L. Nedergaard, L. Lundvall, et al., Evaluation of HE4, CA125, risk of ovarian malignancy algorithm (ROMA) and risk of malignancy index (RMI) as diagnostic tools of epithelial ovarian cancer in patients with a pelvic mass, Gynecol. Oncol. 127 (2012) 379–383.
[13] T. Granato, M.G. Porpora, F. Longo, A. Angeloni, L. Manganaro, E. Anastasi, HE4 in the differential diagnosis of ovarian masses, Clin. Chim. Acta 446 (2015) 147–155.
[14] M. Montagnana, E. Danese, O. Ruzzenente, V. Bresciani, T. Nuzzo, M. Gelati, et al., The ROMA (Risk of Ovarian Malignancy Algorithm) for estimating the risk of epithelial ovarian cancer in women presenting with pelvic mass: is it really useful? Clin. Chem. Lab. Med. 49 (2011) 521–525.
[15] T. Van Gorp, I. Cadron, E. Despierre, A. Daemen, K. Leunen, F. Amant, et al., HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm, Br. J. Cancer 104 (2011) 863–870.
[16] R.G. Moore, D.S. McMeekin, A.K. Brown, P. DiSilvestro, M.C. Miller, W.J. Allard, et al., A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass, Gynecol. Oncol. 112 (2009) 40–46.
[17] S. Ferraro, D. Schiumarini, M. Panteghini, Human epididymis protein 4: factors of variation, Clin. Chim. Acta 438 (2015) 171–177.
[18] Q. Zeng, M. Liu, N. Zhou, L. Liu, X. Song, Serum human epididymis protein 4 (HE4) may be a better tumor marker in early lung cancer, Clin. Chim. Acta 455 (2016) 102–106.
[19] A.D. Santin, F. Zhan, S. Bellone, M. Palmieri, S. Cane, E. Bignotti, et al., Gene expression profiles in primary ovarian serous papillary tumors and normal ovarian epithelium: identification of candidate molecular markers for ovarian cancer diagnosis and therapy, Int. J. Cancer 112 (2004) 14–25.
[20] E. Bignotti, R.A. Tassi, S. Calza, A. Ravaggi, C. Romani, E. Rossi, et al., Differential gene expression profiles between tumor biopsies and short-term primary cultures of ovarian serous carcinomas: identification of novel molecular biomarkers for early diagnosis and therapy, Gynecol. Oncol. 103 (2006) 405–416.
[21] E. Bignotti, R.A. Tassi, S. Calza, A. Ravaggi, E. Bandiera, E. Rossi, et al., Gene expression profile of ovarian serous papillary carcinomas: identification of metastasis-associated genes, Am. J. Obstet. Gynecol. 196 (2007) (245 e1–11).
[22] S.D. Amonkar, G.P. Bertenshaw, T.H. Chen, K.J. Bergstrom, J. Zhao, P. Seshaiah, et al., Development and preliminary evaluation of a multivariate index assay for ovarian cancer, PLoS One 4 (2009), e4599. .
[23] T. Edgell, G. Martin-Roussety, G. Barker, D.J. Autelitano, D. Allen, P. Grant, et al., Phase II biomarker trial of a multimarker diagnostic for ovarian cancer, J. Cancer Res. Clin. Oncol. 136 (2010) 1079–1088.
[24] Z. Yurkovetsky, S. Skates, A. Lomakin, B. Nolen, T. Pulsipher, F. Modugno, et al., Development of a multimarker assay for early detection of ovarian cancer, J. Clin. Oncol. 28 (2010) 2159–2166.
[25] R.G. Moore, A.K. Brown, M.C. Miller, S. Skates, W.J. Allard, T. Verch, et al., The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass, Gynecol. Oncol. 108 (2008) 402–408.
[26] E.R. DeLong, D.M. DeLong, D.L. Clarke-Pearson, Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach, Biometrics 44 (1988) 837–845.
[27] M.S. Pepe, The Statistical Evaluation of Medical Tests for Classification and Prediction, Oxford University Press, Inc., New York, 2003.
[28] K.H. Lu, S. Skates, M.A. Hernandez, D. Bedi, T. Bevers, L. Leeds, et al., A 2-stage ovarian cancer screening strategy using the Risk of Ovarian Cancer Algorithm (ROCA) identifies early-stage incident cancers and demonstrates high positive predictive value, Cancer 119 (2013) 3454–3461.
[29] I.J. Jacobs, U. Menon, A. Ryan, A. Gentry-Maharaj, M. Burnell, J.K. Kalsi, et al., Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial, Lancet 387 (2016) 945–956.
[30] C. Van Holsbeke, A. Daemen, J. Yazbek, T.K. Holland, T. Bourne, T. Mesens, et al., Ultrasound methods to distinguish between malignant and benign adnexal masses in the hands of examiners with different levels of experience, Ultrasound Obstet. Gynecol. 34 (2009) 454–461.
[31] R.L. Coleman, T.J. Herzog, D.W. Chan, D.G. Munroe, T.C. Pappas, A. Smith, et al., Validation of a second-generation multivariate index assay for malignancy risk of adnexal masses, Am. J. Obstet. Gynecol. 215 (2016) (82 e1–e11).
[32] F.R. Ueland, A perspective on ovarian cancer biomarkers: past, present and yet-to-come, Diagnostics (Basel) 7 (2017).
[33] F. Dayyani, S. Uhlig, B. Colson, K. Simon, V. Rolny, D. Morgenstern, et al., Diagnostic performance of risk of ovarian malignancy algorithm against CA125 and HE4 in connection with ovarian cancer: a meta-analysis, Int. J. Gynecol. Cancer 26 (2016) 1586–1593.
[34] P. Aldo, G. Marusov, D. Svancara, J. David, G. Mor, Simple Plex(™): a novel multi-analyte, automated microfluidic immunoassay platform for the detection of human and mouse cytokines and chemokines, Am. J. Reprod. Immunol. 75 (2016) 678–693.
[35] V. Gupta, T. Davancaze, J. Good, N. Kalia, M. Anderson, J.J. Wallin, et al., Bioanalytical qualification of clinical biomarker assays in plasma using a novel multi-analyte Simple Plex platform, Bioanalysis 8 (2016) 2415–2428.
[36] J. Fujimoto, S. Ichigo, R. Hirose, H. Sakaguchi, T. Tamaya, Expression of E-cadherin and alpha- and beta-catenin mRNAs in ovarian cancers, Cancer Lett. 115 (1997) 207–212.
[37] N. Auersperg, J. Pan, B.D. Grove, T. Peterson, J. Fisher, S. Maines-Bandiera, et al., E-cadherin induces mesenchymal-to-epithelial transition in human ovarian surface epithelium, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6249–6254.
[38] M. Rosso, B. Majem, L. Devis, L. Lapyckyj, M.J. Besso, M. Llaurado, et al., E-cadherin: a determinant molecule associated with ovarian cancer progression, dissemination and aggressiveness, PLoS One 12 (2017), e0184439. .
[39] C.W. Lo, M.W. Chen, M. Hsiao, S. Wang, C.A. Chen, S.M. Hsiao, et al., IL-6 trans-signaling in formation and progression of malignant ascites in ovarian cancer, Cancer Res. 71 (2011) 424–434.
[40] G. Gopinathan, C. Milagre, O.M. Pearce, L.E. Reynolds, K. Hodivala-Dilke, D.A. Leinster, et al., Interleukin-6 stimulates defective angiogenesis, Cancer Res. 75 (2015) 3098–3107.

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