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Multianalyte assay systems in the differential diagnosis of ovarian cancer

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Abstract

Introduction—The efficient triage of women diagnosed with a pelvic mass presents a current area of unmet need. Unnecessary surgical intervention performed on patients at a decreased risk of malignancy represents a significant source of preventable morbidity, anxiety and cost. Likewise, delayed or overlooked referral of patients harboring malignant tumors is strongly associated with diminished outcomes. Current tools including imaging modalities and the CA 125 blood test are of insufficient accuracy to overcome these challenges. The use of multianalyte assays systems which include additional biomarkers capable of complementing the performance of CA 125 may offer the best hope of improvement.

Areas Covered—Recent findings regarding the use of multianalyte biomarker panels for the differential diagnosis of a pelvic mass are reviewed and discussed. Particular attention is paid to the FDA approved ROMA and OVA1 tests. The development, validation, recent evaluation and comparative performances of these two tests are reviewed in detail.

Expert Opinion—The performances achieved by the ROMA and OVA1 diagnostic tests represent significant milestones in the application of multianalyte assay systems into standard clinical practice. The overall impact and cost-effectiveness of widespread clinical use of these tools remains to be evaluated.

Keywords

pelvic mass; multianalyte; biomarker; ovarian cancer; ROMA; OVA1

1. Introduction

The use of biomarker panels for the differential diagnosis of pelvic masses represents a clinical setting within the management of ovarian cancer where significant improvements could be achieved in the near future. According to current estimates, 1.4% of women born today, or 1 in 72, will be diagnosed with ovarian cancer at some point in their lifetime. This

year in the United States, there will be over 21,000 new cases of ovarian cancer along with nearly 14,000 deaths.¹ These cases arise from a much larger group of women presenting with pelvic masses. The overall prevalence of pelvic abnormalities is estimated at 7% and it is expected that 5-10% of American women will receive prophylactic surgery for suspected ovarian cancer at some point in their lives.² Effective preoperative risk assessment of the pelvic mass is currently an area of great unmet need. At present, a patient's age and menopausal status are the most important factors to consider upon the identification of an pelvic abnormality as the associated risk of malignancy increases from 13% in premenopausal women to 45% in postmenopausal women.³

Although the vast majority of women diagnosed with ovarian carcinoma will initially present with a pelvic mass, only a small proportion of all masses detected will prove to be malignant. The expeditious triage of these patients often represents the most important component of their clinical management. The burden of early identification of potential ovarian cancer falls predominantly upon the obstetrician/gynecologist whose training in the management of cancer patients is usually limited. While these practitioners can effectively manage the high percentage of patients diagnosed with functional cysts and benign neoplasms through observation and surgery, respectively, the clinical outcome for a patient presenting with a malignant mass can be drastically worsened if she is not immediately referred to a gynecologic oncologist⁴⁻⁶. A series of diverse studies have demonstrated a decrease in the relative risk of reoperation, and increases in disease-free interval and overall survival for women operated on by gynecological oncologists compared to gynecologists and general surgeons⁷⁻⁹. The overall prognosis for patients diagnosed with ovarian cancer has improved significantly over the past 30 years, demonstrated by an increase in 5-year survival from 20% to 50%^{10,11}. This improvement can be largely attributed to the development and application of cytoreductive surgical techniques and the optimization of chemotherapeutic regimens. Despite these findings and advancements, referral rates remain disappointingly low for patients diagnosed with a pelvic mass¹². Improvements upon current screening methodologies and the emergence of new techniques should aid gynecologists and general practitioners in making appropriate referral decisions leading to improvements in the overall clinical management of ovarian cancer.

A pelvic examination is the method most widely utilized in the identification of a pelvic mass, however a physical exam alone cannot discriminate between benign from malignant lesions. Transvaginal ultrasonography has proven useful as a secondary diagnostic tool, however its utility as a first line screening tool remains questionable given its demonstrated low positive predictive value (3-5%) and clinically insufficient levels of sensitivity (75%)¹³. Advanced imaging techniques such as CT or MRI have proven too expensive for widespread use given their limited sensitivity (SN) and specificity (SP). PET/CT has proven useful in the evaluation of a pelvic mass¹⁴ (SN/SP of 100%/92.5%; n=97) however the high cost of this technique also limits its routine use. In addition to family history, pelvic examination, ascites and evidence of local or distant metastases, the CA 125 blood test is included in the standard criteria espoused by The Society of Gynecologic Oncology and the American College of Obstetrics and Gynecology regarding referral of a patient with a pelvic mass to a gynecological oncologist. This set of criteria has produced disappointing results in prospective studies, particularly those evaluating premenopausal women with early stage disease, with SN/SP levels as low as 47%/77%.¹⁵ This disappointing performance reflects the well noted limitations of CA 125, including poor SN for early stage disease, frequent elevation in a number of benign peritoneal conditions, and poor performance in premenopausal women. Much of the research into improved diagnostic tools is now focused on the identification of additional biomarkers capable of complementing the performance of CA 125. The potential clinical impact of a biomarker-based diagnostic test is considerable, given the reduced performance requirements necessary for implementation in comparison to

general screening. In this setting, a diagnostic test would be used as a risk assessment tool to aid physicians in the decision to refer a patient presenting with a suspicious pelvic mass for evaluation by a gynecologic oncologist and/or laparoscopy. Sensitivity is paramount within this strategy and should meet or approach 100% in order to ensure proper referral of all malignant cases. Specificity should be sufficient to produce a meaningful reduction in unnecessary laporoscopies in order to be cost-effective and beneficial to the target population.

2. Recent progress in multianalyte diagnostic tool development

The Risk of Malignancy Index (RMI), first described by Jacobs et al. in 1990¹⁶, is the most widely used and accepted diagnostic index for the assessment of a pelvic mass. The RMI combines ultrasound findings with the measurement of CA 125 and menopausal status. Risk assessment using the RMI is achieved through direct multiplication of scores representing each parameter, rendering this model relatively simple to use and cost-effective. In an initial analysis the RMI achieved a SN of 85% at a SP of 97% for the prediction of malignancy in women diagnosed with a pelvic mass. The model has been modified in several subsequent studies and its performance has been validated in multiple trials with SN and SP values ranging between 71-88.5% and 74.3-97%, respectively¹⁷⁻²⁰. The RMI is used routinely in the United Kingdom and several other European countries. A recent evaluation of the use of the RMI in a tertiary care setting in Denmark which included 1159 women diagnosed with a pelvic mass, reported SN and SP values of 92% and 82%, respectively with a positive predictive value (PPV) of 62% and a negative predictive value (NPV) of 97%²¹.

In light of the well described limitations exhibited by CA 125, investigators have adopted a number of distinct approaches aimed at improved risk of malignancy assessment for women presenting with a pelvic mass. Several models of this type are compared systematically in a recent review by Geomini et al²². In that review, the authors concluded that the RMI performed optimally with respect to all other models for the preoperative assessment of a pelvic mass with a pooled SN of 78% at a SP of 87%. Timmerman et al.²³ recently described a diagnostic index composed of over 50 discrete clinical variables evaluated in over 1000 patients enrolled by the International Ovarian Tumor Analysis (IOTA) group. A subset of 12 of these variables incorporated into a logistic regression model provided a SN of 93% and a SP of 77% in the IOTA cohort. The descriptive factors comprising this model included: age, personal history of ovarian cancer, maximum diameter of the lesion, maximum diameter of the solid component, presence of ascites, Doppler blood flow, presence of a purely solid lesion, irregular cyst wall, increased color score, hormone therapy, and pain with ultrasound. This model and several related IOTA models were found to outperform the RMI in an external prospective validation study²⁴. The performance of these models does appear encouraging and may represent a cost-effective method of risk assessment, however the complex nature of the algorithms involved may present a barrier to broad clinical implementation.

A number of groups have reported encouraging results through the use of multimarker panels which attempt to improve upon the performance of CA 125 by combining it with various additional biomarkers found to be associated with ovarian cancer (Table 1). The most recent findings related to this endeavor are reviewed here. Hogdall et al. recently described the development of a pelvic mass risk index, termed the DK index, comprised of CA 125, age, and three additional serum biomarkers identified through SELDI-TOF-MS proteomic analysis (beta-2-microglobulin (B2M), transthyretin (TT), transferrin (TF))²⁵. In a large group of women diagnosed with pelvic masses, the DK index provided a SN of 95% at a SP of 81%. Additional work by this group suggests that the incorporation of urine biomarkers identified through proteomic techniques into biomarker-based tools may result

in improved diagnostic performance²⁶. In a separate study, a panel of CA 125, midkine (MDK), and anterior gradient 2 protein (AGR2) performed very well in the discrimination of benign and malignant pelvic masses with a SN/SP of 95.2%/97.7%²⁷. This study was limited by a relatively small sample size and a lack of validation, however further analysis of this panel appears warranted. Elsewhere, several groups have examined the role of cytokines in ovarian cancer diagnosis in an attempt to utilize the network of local and systemic signaling involved in ovarian tumorigenesis. A panel consisting of CA 125, G-CSF, IL-6, EGF, and VEGF provided a SN/SP of 86.5%/93% in a small preliminary study²⁸ while the combination of CA 125 and IL-7 provided a SN of 69% at 100% SP in a somewhat larger patient group²⁹. While the high level of SP achieved by the latter study is certainly meritorious, this model should be further examined to determine if it is capable of providing the high SN required of a diagnostic test in this setting. Yip et al. conducted an analysis of 175 circulating biomarkers in 499 women scheduled for surgery for pelvic abnormalities, including 149 cases of ovarian cancer³⁰. A nine biomarker combination was identified through logistic regression that provided a SN of 90% at a SP of 88.9% for the discrimination of benign vs. malignant patients. Notably, this combination outperformed the five biomarker panel comprising the OVA1 diagnostic test (discussed below), however the performance of this panel has not been independently validated.

3. The CA 125/HE4 combination and ROMA

HE4, or human epididymal secretory protein 4, is a secreted glycoprotein product of the *WFDC2* gene. Gene expression profiling indicates that HE4 is expressed in several tissues outside of the male reproductive system and is highly overexpressed in epithelial ovarian cancers³¹. Studies focusing on the potential use of HE4 as a biomarker of ovarian cancer suggest that it is elevated in over 50% of ovarian cancer patients whose tumors do not express CA 125³². HE4 has also demonstrated greater SN than CA 125 among early stage ovarian cancer patients and greater SP in comparison with benign ovarian lesions^{32,33}. A diagnostic assay for HE4 has been developed and commercialized by Fujirebio Diagnostics Inc. (Malvern, PA) and the use of HE4 for ovarian cancer monitoring has recently been approved by the United States Food and Drug Administration (FDA)³⁴. Investigations into the use of HE4 as an ovarian cancer biomarker have proceeded both in the area of population based screening and in the differential diagnosis of a pelvic mass. Despite a number of promising reports, it has become apparent that HE4 is not sufficiently sensitive or specific to function effectively as a standalone test. However the combined use of CA 125 and HE4 in the differential diagnosis of pelvic masses has received a considerable amount of attention (Table 2).

The diagnostic potential of the CA 125/HE4 combination was first recognized by Moore et al. in an investigation of circulating levels of 9 biomarkers (CA 125, SMRP, HE4, CA 72-4, activin, inhibin, osteopontin, EGFR, ErbB2) in sera obtained from 233 women diagnosed with a pelvic mass³². HE4 was identified as the most sensitive marker in that study, and this was especially true among early stage ovarian cancer patients. The combination of CA 125 and HE4 provided a greater overall classification accuracy than either biomarker used alone and provided a SN of 76.4% at a SP of 95%. Moore et al. then utilized this combination in a prospective multicenter study involving 531 patients³³. Measurements of CA 125 and HE4 were used to categorize patients into high or low risk of ovarian cancer with 93.8% of ovarian cancer patients correctly classified into the high risk group³³. Elsewhere, Huhtinen et al. demonstrated the efficacy of this combination in the discrimination of malignant ovarian masses from benign ovarian endometriotic cysts with a SN of 92.9% at 95% SP³⁵. In a separate and independent study involving 790 patients separated into distinct training and validation sets, Nolen et al. evaluated circulating levels of 65 biomarkers and determined that the combination of CA 125 and HE4 performed optimally with respect to all

other possible panels, resulting in a SN of 83% at a SP of 85%³⁶. Another recent study by Holcomb et al. reported that the combination of CA 125 and HE4 provided a SN of 91.2% at a SP of 55.4% with a PPV of 26.3% and a NPV of 97.3%³⁷. Although SP in this study is somewhat diminished, the high NPV is encouraging given the paucity of ovarian cancers 16/229 (8%) included in the cohort of premenopausal women.

Recently, Steven Skates and colleagues developed a scoring model termed the Risk of Ovarian Malignancy Algorithm (ROMA) which incorporates measurements of CA 125 and HE4 along with menopausal status in order to assign high or low risk of malignancy to a woman presenting with a pelvic mass. In a comparison of ROMA and RMI in 467 patients, the ROMA provided a higher SN (94.3% vs. 84.6%) at a SP of 75%³⁸. This was particularly evident among stage I and II cancer, where ROMA detected 85% and RMI 65%. The comparative performance of ROMA vs RMI remains in question, however as a subsequent evaluation by a separate group found that RMI outperformed ROMA among both pre- and postmenopausal women diagnosed with a pelvic mass (n=432)³⁹. In the latter study, both ROMA and RMI were outperformed by subjective assessment by ultrasound. Most recently, ROMA was evaluated in a prospective, multicenter, blinded clinical trial involving 472 patients diagnosed with a pelvic mass, 89 of which were found to have ovarian cancer⁴⁰. In that trial, ROMA provided an overall SN of 93.8% at a SP of 74.9% with a negative predictive value of 98%. ROMA performed particularly well in the premenopausal patient subset, achieving a SN of 100% at a SP of 74.2%. Based upon the results of this clinical trial, ROMA was recently approved by the FDA for use in determining the risk of ovarian cancer in pre- and post-menopausal women with a pelvic mass.

Recent evaluations of HE4 and ROMA have produced mixed results. A number of studies have reported results which reaffirm the complementary performance of HE4 to CA 125 and the superior diagnostic abilities of the HE4/CA 125 combination or ROMA over CA 125 alone^{35,41-46}. However, several groups have reported contrary results. Van Gorp et al.⁴⁷ concluded that the addition of HE4 or the use of ROMA does not offer improvement upon CA 125 based on a large prospective study of women diagnosed with a pelvic mass. Several notable differences in the composition of the patient cohorts exist between this study and the previous study by Moore et al.³³. These differences include an increased proportion in the number of overall cancers, mucinous tumors, borderline tumors, metastatic tumors, and postmenopausal women in the later study. Montagnana et al.⁴⁸ found that ROMA was effective in ovarian cancer diagnosis in postmenopausal women but not in premenopausal women; however HE4 alone outperformed ROMA in either group. The study design here also differed from Moore et al. regarding the incidence of ovarian cancer in the pre- and postmenopausal groups. A third study, which included a large proportion of borderline and extra-ovarian tumors found that HE4 offered several advantages over CA 125 for ovarian cancer diagnosis, however no diagnostic benefit was derived from combining them⁴⁹. Thus, variability in the composition of the target population appears to impact the performance of the CA 125/HE4 combination. Going forward, this issue may be addressed through a reevaluation of the specific threshold values employed in the test to better respond to this variability or through the incorporation of additional useful biomarkers which make the test more robust.

4. The OVA1 test

A biomarker-based diagnostic test for the evaluation of patients with a pelvic mass was approved by the United States FDA on September 11, 2009, and is currently available under the trade name OVA1 (Vermillion, Inc.)⁵⁰. The test utilizes a five biomarker combination (CA 125, transthyretin, ApoA1, β -2 microglobulin, transferrin) identified through serum

proteomics using SELDI⁵¹. Following validation of SELDI derived markers in retrospective samples, the final combination was assembled based on the successful development of immunoassays. The test is currently approved for use as an adjunct to physical examination and imaging and produces a risk assessment score within the range of 0-10. Separate cutoff values are employed for premenopausal (5.0) and postmenopausal women (4.4). Although the full impact of clinical implementation of the OVA1 test remains to be evaluated, the advancement of the test thus far is testament to the beneficial use of systemic biomarkers and a marked divergence from other efforts which rely heavily on tumor-derived factors such as CA 125 and HE4.

The panel was evaluated in a clinical trial which utilized immunoassays targeting each of the five markers in a set of 524 women diagnosed with a pelvic mass and recommended for surgery^{52,53}. At the time of surgery there were 363 benign tumors and 161 malignancies of which 151 were ovarian cancers. In a pair of reports, the developers of the kit evaluate its efficacy in several clinical settings (Table 3). When the OVA1 panel was substituted for CA 125 within the American College of Obstetricians and Gynecologists (ACOG) ovarian tumor referral guidelines, it provided a SN of 94% at a SP of 35% with a PPV of 40% and a NPV of 93%. This represented an increase in SN and NPV in comparison to CA 125, but also a decrease in SP and PPV. When the OVA1 test was added to a normal physician assessment, it provided a SN of 96% at a SP of 35% with a PPV of 40% and a NPV of 95%. Among gynecological oncologists, SN and NPV were higher at 99% and 98%, respectively, however SP was lower at 26%. In comparison to physician assessment alone, the incorporation of the OVA1 test again resulted in improvements in SN and NPV along with decreased levels of SP and PPV. When the OVA1 test was directly compared to CA 125, similar trends in performance were observed. A recent evaluation of the OVA1 markers, which included all 7 proteins originally identified by SELDI-TOF-MS, suggested that these markers do not improve upon the performance of CA 125 in prediagnostic samples⁵⁴. A similar finding by Cramer et al. in prediagnostic samples collected as part of the large Prostate, Lung, Colorectal, Ovarian (PLCO) trial may indicate a potential limitation in the usefulness of the OVA1 test⁵⁵.

5. Conclusions

Investigation into the use of multianalyte systems for the differential diagnosis of a pelvic mass has yielded a number of promising findings. The results presented in this review almost exclusively describe the development of biomarker panels built upon the foundation of CA 125. Several groups have achieved noteworthy performances using diagnostic indices which do not include biomarker measurements, however the practicality associated with widespread implementation of these models has been questioned. The RMI, which incorporates CA 125, ultrasound, and menopausal status, remains a viable risk assessment tool. Biomarkers observed to complement the performance of CA 125 and thus offer improvements in the classification of benign and malignant pelvic masses include tumor antigens, cytokines, acute phase proteins, and growth factors. Multianalyte panels assembled from these biomarkers demonstrate the potential for advancement within the clinical management of pelvic mass diagnoses. Two multianalyte diagnostic systems have emerged from the assembled findings and have received FDA approval for use in the assessment of malignant potential of an identified pelvic mass. ROMA, a relatively simple risk assessment algorithm, categorizes women diagnosed with a pelvic mass into high or low risk of malignancy based on measurements of CA 125 and HE4 along with menopausal status. ROMA has performed well, demonstrating relatively high SN, SP and NPV in two clinical trials, however its performance in comparison to RMI has been mixed. The OVA1 test, based on the measurement of 5 serum biomarkers using immunoassays, has been approved

for use as an adjunct to clinical examination and imaging based on strong performance in a clinical trial.

The ROMA and OVA1 test have not been compared directly in a single trial, and such an evaluation would be eagerly anticipated. Based on the separate results of the clinical trials involving each test, the two are likely to perform with similar levels of SN and NPV. The most notable difference between the two tests is SP, wherein ROMA appears to be substantially more specific (75% vs. 43%). While such a difference in SP should not affect patient outcomes, it could produce an impact on cost-effectiveness and the distribution of medical resources. Some comparative information may also be gleaned from several studies which evaluate the biomarkers included in each panel individually. In the report by Yip et al., CA-125, HE4 and each of the OVA1 markers were evaluated in 499 women scheduled for pelvic mass surgery³⁰. HE4 was the highest performing individual marker in that study, while none of the OVA1 markers were among the top nine analytes selected for panel development. Similarly, when each of the markers were evaluated in pre-diagnostic sample collected during the PLCO trial, HE4 was found to outperform all other biomarkers with the exception of CA 125⁵⁵. Although transthyretin ranked third among all markers evaluated in that study, its performance in samples drawn 6 months prior to ovarian cancer diagnosis was significantly less than HE4 (SN of 2% vs. 73% at 95% SP).

In conclusion, the successful development of the ROMA and OVA1 test represents a significant milestone in the clinical management of women diagnosed with a pelvic mass. The limited amount of clinical data available suggest that ROMA may be advantageous to OVA1 in terms of SP and this advantage may stem from the inclusion of HE4, a marker which has demonstrated superior performance on an individual basis. However, additional clinical validation will be necessary to fully evaluate the impact of either test on the overall burden of ovarian cancer.

Expert Opinion

Recent reports describing the performance of several multianalyte and multimodal diagnostic models in large, prospective, multicenter clinical trials indicate that the widespread implementation of this type of testing into clinical practice is near at hand. The FDA approved ROMA and OVA1 tests are the clearest examples of advancement within the field. Both developments represent variations upon a common theme, which is the inclusion of biomarker alternatives to CA 125 which complement its diagnostic performance. The underlying goal of such a strategy is to detect a wider range of ovarian cancers, including early stage disease and disease among premenopausal women, while providing a beneficial level of specificity with respect to benign masses. Ongoing efforts must now focus on determining whether or not the improvements noted for these multianalyte tests are of sufficient magnitude to produce a meaningful impact on the clinical management of the pelvic mass and a measurable reduction in ovarian cancer related disease burden. Measurements of success in this clinical setting include a reduction in morbidity and cost resulting from unnecessary surgical procedures and improved outcome for ovarian cancer patients achieved by efficient referral to clinical centers of excellence.

Cost-effectiveness is likely to be the most critical parameter in the ongoing evaluation of the ROMA and OVA1 tests. The OVA1 test is currently priced at \$600-650 (\$540 Medicare reimbursement) while the developers of ROMA estimated the cost of their test to be in the range of \$60-130. This price difference alone is likely to sway a fair number of physicians and patients, particularly those forced to pay for the test out-of-pocket. Coupled with the diminished specificity demonstrated by the OVA1 test, the high relative value of ROMA becomes apparent. The lower specificity of OVA1 has been cited as a concern by the kit's

developers and also by gynecologists who fear a loss of revenue due to unnecessary referrals of patients with benign lesions and false-positive test results. Thus the preliminary clinical and economic assessment of the two tests appears to favor ROMA.

Despite its apparent advantages, ROMA has yet to demonstrate a clear benefit to ovarian cancer patients in terms of mortality and morbidity. Widespread clinical evaluation of both ROMA and OVA1 appears warranted based on their performance thus far and such an evaluation will be necessary in order to definitively assess their impact. Indeed, RMI remains a valuable risk-assessment tool and neither newly developed test has demonstrated a clear advantage over this established standard. The current challenge is the wider implementation of each of these tests in the United States. In course of this implementation, it is important to note that each of these tests is indicated for the assessment of the malignant potential of a pelvic mass and not for the general screening for ovarian cancer among asymptomatic women. Off-label use of these kits for that purpose should be conducted with considerable caution. Indeed, patient and physician education regarding the intended use and interpretation of these newly approved tests will be a critical component of expanded adoption.

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Article Highlights

- Efforts aimed at the development of multianalyte systems for the assessment of malignancy in women diagnosed with a pelvic mass have yielded two FDA approved tests: ROMA and OVA1
- The ROMA algorithm is based on serum measurements of CA 125 and HE4 in addition to the menopausal status of the patient. The OVA1 test is based on the measurement of five serum biomarkers: CA 125, ApoA1, transthyretin, β 2-microglobulin, and transferrin.
- Each test has demonstrated similarly high levels of sensitivity and negative predictive value in separate clinical trials, however ROMA appears to be significantly more specific.
- Both tests demonstrate marked improvement over CA 125 alone, however a clear advantage over the currently used RMI algorithm has not been conclusively established.
- ROMA appears to be the more cost-effective of the two tests based on its lower estimated base cost and higher specificity.

Table 1

Multimarker panels which discriminate benign from malignant pelvic masses.

Panel	Cases	Controls	SN	SP	Reference
CA 125, β 2-microglobulin, transthyretin, transferrin	144	509	95	81	Hogdall et al. ²⁵
CA 125, midkine, anterior gradient 2 protein	46	61	95.2	97.7	Rice et al. ²⁷
CA 125, G-CSF, IL-6, EGF, VEGF	44	37	86.5	93	Gorelik et al. ²⁸
CA 125, IL-7	187	45	69	100	Lambeck et al. ²⁹
CA 125, HE4, IL-2Ra, α 1-antitrypsin, CRP, YKL-40, cellular fibronectin, CA 72-4, prostasin	149	350	90	89.9	Yip et al. ³⁰

Table 2

The CA 125/HE4 combination in the differential diagnosis of a pelvic mass

Panel	Cases	Controls	SN	SP	Reference
CA 125, HE4	129	352	92.3	75	Moore et al. ³³
CA 125, HE4	14	129 [‡]	92.9	95	Huhtinen et al. ³⁵
CA 125, HE4	491 [‡]	299 [‡]	83	85	Nolen et al. ³⁶
CA 125, HE4	34	195	91.2	55.4	Holcomb et al. ³⁷
ROMA [*]	89	383	93.8	74.9	Moore et al. ⁴⁰

[‡] ovarian endometriotic cysts;
[‡] includes independent validation set;
^{*} negative predictive value: 98%

Table 3Clinical evaluation of the OVA1 test (reported in ^{52,53})

	SN	SP	PPV	NPV
ACOG + CA 125	77	68	52	87
ACOG + OVA1	94	35	40	93
PA alone	75	79	62	88
PA + OVA1	96	35	40	95
CA 125 alone	69-77	73-84	56-65	86-88
OVA1 alone	93	43	42	93

ACOG: American College of Obstetricians and Gynecologists' ovarian tumor referral guidelines

PA: physician assessment