Published in final edited form as:
Mol Diagn Ther. 2013 June ; 17(3): 139–146. doi:10.1007/s40291-013-0027-6.

Biomarker Testing for Ovarian Cancer: Clinical Utility of Multiplex Assays

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Abstract

The improved detection of ovarian cancer at the earliest stages of development would confer a significant benefit in the therapeutic efficacy and overall survival associated with this devastating disease. The inadequate performance of currently used imaging modalities and the CA 125 biomarker test have precluded the establishment of screening programs and hindered the development of diagnostic tests for ovarian cancer. Two recently completed large clinical trials of ovarian cancer screening have reported findings of mixed impact, further clouding the issue. Considerable effort has been applied to the development of multiplexed biomarker-based tests and the most recent advances are discussed here. Within the clinical setting of pelvic mass differential diagnosis and triage, several significant advancements have been achieved recently, including the FDA approved ROMA and OVA1 tests. The development and evaluation of those tests are described in this review. Thus while effective routine screening for ovarian cancer remains a lofty goal, advancement within the clinical management of pelvic mass diagnoses appears to be near at hand.

Keywords

Ovarian cancer; screening; diagnosis; CA 125; HE4; ROMA; OVA1

1. Introduction

Ovarian cancer represents the eighth most common cancer among women and the second most frequently diagnosed gynecological malignancy in the United States and Europe. [1] The overall mortality attributed to ovarian cancer exceeds that of any other gynecological cancer with over 50% of the more than 200,000 women newly diagnosed each year worldwide expected to perish from the disease [2]. A critical factor in the elevated mortality associated with ovarian cancer is the lack of disease-specific symptoms. Compounding the problem of

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The authors declare that they have no conflicts of interest.

ubiquitous clinical presentation is the observation that the majority of early-stage cancers are asymptomatic resulting in over three-quarters of all diagnoses being made at a time when the disease has already established regional or distant metastases [3]. Despite aggressive cytoreductive surgery and platinum-based chemotherapy, the 5-year survival rate for patients with clinically advanced ovarian cancer is only 15-20%, although the cure rate for stage I disease is usually greater than 90% [3,4]. This strongly suggests that finding and removing tumors that remain confined to the ovary should confer a substantial improvement in survival. Several strategies have been employed in an effort to produce a stage shift in ovarian cancer diagnoses leading to improved clinical outcomes. These efforts are proceeding on two distinct but equally crucial fronts: i) screening for ovarian cancer among asymptomatic women; and ii) the improved triage of women presenting with a potentially malignant pelvic mass. Recent advances along both of these fronts will be reviewed and discussed here.

2. Multiplexed biomarker assays as screening tools

2.1 The PLCO and UKCTOCS prospective screening trials

The best currently available protocol for early detection of ovarian cancer, a combination of screening for elevated CA-125 and transvaginal ultrasound (TVS) in the presence of elevated CA-125 [5,6], does not meet the stringent criteria for cost-effectiveness espoused by the U.S. Preventive Services Task Force. [7] As a result, no professional group currently recommends screening for ovarian cancer in the general population. [8-10] Two large, randomized trials that were designed to evaluate the survival benefit of ovarian cancer screening based on CA-125 and TVS have reported final and preliminary results.

In the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, 78,216 healthy women between the ages of 55 and 74 were randomly assigned to undergo either annual CA 125 testing plus TVS or to receive “usual care”. [11] The positive predictive value of a positive screening test was 1.0 to 1.3% during the 4 years of screening. In the PLCO, 72% of screen-detected cases were stage III or IV, indicating that screening has not resulted in stage shift. The PLCO project team recently released its report in which they conclude that the CA 125/TVS screening approach does not reduce disease-specific mortality in comparison to usual care, but does result in an increase in invasive medical procedures and associated harms. [12]

In the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), 202,638 postmenopausal women between the ages of 50 and 74 who were deemed to be at average risk for ovarian cancer were randomly assigned to undergo annual pelvic examination (control group), annual TVS (ultrasonography or USS group), or annual measurement of CA 125 plus TVS in cases in which the CA 125 level was elevated (multimodality or MMS group).[13] As compared with ultrasonography alone, multimodality screening had a significantly greater specificity (99.8% vs. 98.2%) and a higher positive predictive value (35.1% vs. 2.8%) ($P<0.001$); sensitivity did not differ significantly between the two groups. Both the USS and MMS arms demonstrated a higher proportion of stage I-II cancers. A report on the full results of the UKCTOCS trial, including impact on survival, is expected in 2014-15. Two major differences are apparent between the UKCTOCS and PLCO trials and may explain some of the disparity observed in the reported results, most notably the stage shift observed in the UCKTOCS trial and absent from the PLCO trial. The first difference involves the mode of diagnostic follow-up utilized in each trial wherein the suspicious cases identified in the PLCO trial were cared for by their physicians while in the UKCTOCS trial the majority were referred to a gynecologic oncologist. The second notable difference is the use of the Risk of Ovarian Cancer Algorithm (ROCA) for the interpretation of CA-125 measurements in the UKCTOCS trial in contrast to the set cutoff of 35 U/ml

utilized in the PLCO trial. The ROCA is based on annual measurements of CA-125 evaluated in a serial fashion so that each woman serves as her own baseline [14]. This method is currently under investigation in five separate clinical trials (reviewed in [15]) and a recent virtual cohort analysis demonstrated that the use of increasing serial CA-125 measurements to select for TVS screening among women at average risk of ovarian cancer reduces mortality by 13% and meets currently accepted cost-effectiveness guidelines [16]. However, another recent report determined that the use of ROCA in the PLCO trial would not have led to a significant mortality benefit from screening [17]. Thus, the results of the UKCTOCS trial utilizing ROCA are eagerly awaited.

2.2 Recent advances using multiplexed biomarker approaches

Although CA-125 remains the most useful individual biomarker of ovarian cancer, numerous efforts and strategies aimed at utilizing CA-125 for screening purposed have not proven fruitful. A popular strategy has emerged within ovarian cancer screening research wherein additional biomarkers are sought which are capable of complementing the performance of CA-125 in order to achieve levels of sensitivity and specificity worthy of clinical advancement. A number of notable reports are described below (Table 1).

A well publicized study by Mor et al. evaluated 169 proteins in serum samples obtained from ovarian cancer patients (n=158) by means of RCA immunoassay microarray and identified a subset of markers including CA 125, leptin, prolactin, OPN, IGF-II and MIF which, in combination, provided a classification power of 92% SN at 99% SP. This panel was equally sensitive to different histologic subtypes of primary epithelial ovarian cancers. [18,19] This panel was subsequently marketed under the trade name OvaSure, however deficiencies in study design were later identified which led to the eventual withdrawal of the kit and also illustrate the challenges facing biomarker development efforts in general. Most prominent among these deficiencies was the inaccurate calculation of PPV based on improper estimates of ovarian cancer prevalence. [20,21]

Our group utilized a subject cohort which included more than 2000 healthy women split among independent training and validation sets in an unbiased analysis of serum biomarker candidates to identify a four-biomarker panel comprised of CA 125, HE4, carcinoembryonic antigen (CEA), and vascular cell adhesion molecule-1 (VCAM-1) which could discriminate early-stage ovarian cancer (n=44) from the control group (n=929) with 86% SN at 98% SP [22] in the validation set. Our study utilized the Luminex multiplex liquid assay system which was also employed in a more recent analysis by Kim et al. [23]. In that study, the combination of CA-125, transthyretin (TTR), and ApoA1 provided a SN of 93.9% at a SP of 95% in a group of 118 stage I-II ovarian cancer patients and 61 healthy controls. In a multicenter case-control study, Zhang et al. utilized SELDI-TOF-MS to analyze sera obtained from 195 patients diagnosed with several types of ovarian cancer along with patients diagnosed with benign pelvic masses and healthy control women [24]. A four-biomarker panel consisting of CA 125, ApoA1, TTR and H418 was identified which provided a SN 74% at a SP of 97% for the discrimination of early stage ovarian cancer from healthy women. Su et al. also utilized SELDI-TOF-MS to identify a similar four-biomarker panel of CA 125, ApoA1, TTR and transferrin (TF) [25]. This panel provided a SN/SP of 89%/92% for the discrimination of early stage ovarian cancer patients (n=126) from healthy controls (n=82). In another multicenter study, Skates et al. utilized immunoassays and several statistical models to evaluate serum levels of four biomarkers in ovarian cancer patients [26]. The combination of CA 125, CA 15-3, CA 72-4 and M-CSF, identified using a mixture discriminant analysis model, performed best in the independent validation set, providing a SN of 70% at 98% SP for early stage ovarian cancer (n=60). Recently, Edgell et al. reported on a retrospective case-control study (phase II biomarker study) in which the authors utilized a multiplexed bead-based immunoassay platform to evaluate 5 ovarian

cancer biomarkers (CA 125, CRP, SAA, IL-6, IL-8) in 362 plasma samples obtained from ovarian cancer patients (n=150) and healthy controls (n=212) [27]. Through multivariate modeling the authors demonstrated a SN/SP of 92.3/91.3 for all early stage ovarian cancers. In another recent study, Amonkar et al. demonstrated the value of multiplexed analysis in their evaluation of 104 candidate biomarkers in a cohort of 176 ovarian cancer patients and 187 controls [28]. Their training analysis led to the identification of an 11-analyte profile consisting of CA 125, CA 19-9, EGFR, CRP, myoglobin, ApoA1, ApoCIII, MIP-1α, IL-6, IL-18, and tenascin C. In an independent validation set, this panel provided a SN of 91.3% at a SP of 88.5%.

Each of the reports presented above describe the performance of biomarkers evaluated in blood samples obtained near or after the time of ovarian cancer diagnosis. Biomarker panels of this type may therefore, be limited in their ability to detect a malignancy in its earliest stages. Nicole Urban and colleagues sought to overcome this limitation through the use of samples obtained prediagnostically through a prospective study design permitting the collection of samples prior to, at or after the time of ovarian cancer diagnosis. In a pair of reports, this group first describes elevated levels of CA 125, HE4, and mesothelin in the sera of 34 symptomatic ovarian cancer patients and then in the sera of patients 0-3 years prior to diagnosis, noting an optimal lead time of 1 year. [29,30] In a separate study, the investigators utilize a combinatorial approach including CA 125 and HE4 measurements in addition to the Symptom Index (SI) to prospectively classify 74 ovarian cancer patients from 137 healthy controls with a SN of 84% at a SP of 98.5%. [31]

3. Multiplexed biomarker assays in the triage of the pelvic mass

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 [32]. 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. 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 surgeons [33-35]. 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, providing SN/SP levels as low as 47%/77%.[36] Considerable effort has been focused on the identification of additional biomarkers capable of complementing the performance of CA 125. Several combinations have recently been approved for clinical use based on trial performance and these will be discussed below. Each of the FDA approved tests is intended to aid referring physicians in choosing the most appropriate specialist for surgical intervention for patients already planning to undergo surgery.

3.1 The CA 125/HE4 combination and ROMA

HE4, or human epididymal secretory protein 4, is a secreted glycoprotein product of the *WFDC2* gene. 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 125 [37]. HE4 has also demonstrated greater SN than CA 125 among early stage ovarian cancer patients and greater SP in comparison with benign ovarian lesions [37,38]. 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) [39]. 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 mass [37]. The combination of CA 125 and HE4 provided a greater overall classification accuracy that 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 patients with 93.8% of ovarian cancer patients correctly classified into the high risk group [38]. Several subsequent analysis by other groups further supported the superior performance of the CA 125/HE4 combination over either biomarker used alone [40-45].

Based on these findings, a scoring model was developed by Steven Skates and colleagues 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. 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 cancer [46]. 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 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 ROMA over CA 125 alone in various patient cohorts [47-52]. However, several groups have reported contrary results. Van Gorp et al.[53] 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 [38]. These differences include an increased proportion of overall cancers, mucinous tumors, borderline tumors, metastatic tumors, and postmenopausal women in the later study. Montagnana et al.[54] 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. That study design 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 them [55]. Thus, variability in the composition of the target population appears to impact the performance of the CA 125/HE4 combination. ROMA has also been compared to the well establish Risk of Malignancy Index (RMI) with inconsistent findings. In a comparison of ROMA and RMI in 467 patients, ROMA provided a higher SN (94.3% vs. 84.6%) at a SP of 75% [56]. This was particularly evident among stage I and II cancer, where ROMA detected 85% and RMI 65%. However, 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) [57]. In the latter study, both ROMA and RMI were outperformed by subjective assessment by ultrasound. In the most recent comparison of the two algorithms, RMI outperformed ROMA among all patients (ROC AUC .905 vs. .897) and premenopausal patients (ROC AUC .945 vs. .909) in a large

prospective study (n=1218) of women diagnosed with pelvic masses (4-7). However, the authors of that study concluded that the performance of ROMA is comparable to that of RMI and may offer advantages in that it is not reliant upon imaging, as is RMI.

3.2 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.) [58]. The test utilizes a five biomarker combination (CA 125, TTR, ApoA1, β-2 microglobulin, TF) identified through serum proteomics using SELDI-TOF-MS [59]. Following validation of these 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. 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 [60,61]. At the time of surgery there were 363 benign tumors and 161 malignancies of which 151 were ovarian cancers. 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 samples [62]. A similar finding by Cramer et al. in prediagnostic samples collected as part of the PLCO trial may indicate a potential limitation in the usefulness of the OVA1 test [63].

4. Conclusions

The search for biomarker based screening tools for the early detection of ovarian cancer has produced a number of biomarker panels offering levels of SN and SP exceeding 90% which have been identified through the use of a variety of analysis platforms and statistical models. Each of the panels identified demonstrates a clear performance advantage over the individual performance of CA 125. Clinical implementation of biomarker tools has been delayed, and sometimes reversed, in large part due to the stringent performance requirements associated with the detection of a rare disease and the lack of a demonstrated survival benefit. Decisions regarding implementation will require physicians, researchers and public health officials to weigh the potential survival benefits against the economic and social tolls associated with population-based screening. The continued improvement and refinement of screening tools should steadily tip the balance in favor of implementation. Among the reports presented here, some progress is evident in the use of several specific biomarkers, namely TTR and ApoA1, in combination with CA 125 for screening purposes. Several groups have independently achieved impressive results using this or closely derived

biomarker combinations, and such panels may offer the greatest promise for further clinical development.

The FDA approved ROMA and OVA1 tests are clear examples of advancement within the field of pelvic mass risk assessment and triage. 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. Success going forward will be measured by the ability of these kits to produce a meaningful reduction in morbidity and cost resulting from unnecessary surgical procedures and improved outcomes for ovarian cancer patients achieved by efficient referral to clinical centers of excellence. The ROMA and OVA1 test have not been compared directly in a single trial, and such an evaluation or comparative effectiveness study is eagerly awaited. 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. 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. 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. 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.

Acknowledgments

This work was supported by NIH grants: U01CA117452 (EDRN), RO1 570 CA098642, R01 CA108990, P50 CA083639, CA086381, CA105009, UPCI Hillman 571 Fellows Award, and The Frieda G. and Saul F. Shapira BRCA Cancer Research 572 Program Award (AEL).

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Table 1

Multiplex biomarker panels which discriminate ovarian cancer from healthy controls.

Panel	Cases/ Controls	SN/SP	Analytical platform	Year/Ref
CA 125, ApoA1, TTR	118/61	93.9/95	BBIA	2012/[23]
CA 125, HE4, CEA, VCAM-1	456/2000†	86-93/98	BBIA	2010/[22]
CA 125, CRP, SAA, IL6, IL8	150/212†	94.1/91.3	BBIA	2010/[27]
CA-125, CA 19-9, EGFR, CRP, myoglobin, Apo A1, Apo CIII, MIP1a, IL6, IL18, tenascin C	176/187†	91.3/88.5	BBIA	2009/[28]
CA 125, leptin, PRL, OPN, IGFII, MIF	156/362†	95.3/99.4	MS/ELISA	2008/[19]
CA 125, TTR, ApoA1, TF	126/82	89/92	MS	2007/[25]
CA 125, ApoA1, TTR	200/142†	74/97	MS	2004/[24]
CA 125, HE4, SI*	74/137‡	84/98.5	ELISA	2010/[31]
CA 125, CA 72-4, CA 15-3, MCSF	123/224†	70/98	ELISA	2004/[26]

*Symptom index;

†includes independent validation set;

‡prediagnostic samples; BBIA-bead-based immunoassay; MS – mass spectroscopy