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Current state of biomarker development for clinical application in epithelial ovarian cancer

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Abstract

Each year in the United States over 15,000 women die of epithelial ovarian cancer (EOC) and 22,000 are diagnosed with the disease. The incidence of ovarian cancer has remained stable over the past decade however, survival rates have improved steadily. Increases in survival rates can be attributed to the advances in surgical management, development of effective cytotoxic drugs and the route of administration of chemotherapy. Ovarian cancer survival rates could also be improved through screening and early detection. Disappointingly, effective screening methods have not been established and continue to be elusive. Historically the goal of a screening test was to achieve a positive predictive value (PPV) greater than 10% in order to be considered cost effective and have an acceptable risk for the population being screened. Despite the inability of currently available screening algorithms to achieve the desired PPV there may be an advantage in producing a stage migration to lower stages at the time of diagnoses, thereby resulting in improved survival. Equally important recent studies have demonstrated that women who have their initial surgery performed by gynecologic oncologists, and women who have their surgeries at centers experienced in the treatment of ovarian cancer have higher survival rates. For these reasons it is essential that all women at high risk for ovarian cancer receive their initial care by gynecologic oncologists and at centers with multidisciplinary teams experienced in the optimal care of ovarian cancer patients. With this in mind, methods that facilitate the accurate triage of women who will ultimately be diagnosed with ovarian cancer could play a significant role in improving survival rates for these patients. This review article will examine the current state of biomarker use in ovarian cancer screening, risk assessment and for monitoring ovarian cancer patients.

Each year in the United States over 15,000 women die of epithelial ovarian cancer (EOC) and 22,000 are diagnosed with the disease (1). The incidence of ovarian cancer has remained stable over the past decade. Survival has improved steadily with a five year survival rate of 45.9% in the most recent report of the *SEER* database(2). Increases in survival rates can be attributed to the advances in surgical management, development of effective cytotoxic drugs and the intraperitoneal administration of chemotherapy. Ovarian cancer survival rates could also be improved through screening and early detection. Effective screening methods have not been established and continue to be elusive. Historically the goal of a screening test was to achieve a positive predictive value (PPV) greater than 10% in order to be considered cost effective and have an acceptable risk for the population being screened. Despite the inability of currently available screening algorithms to achieve the desired PPV there may be an

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advantage in producing a stage migration to lower stages at the time of diagnoses, thereby resulting in improved survival.

Numerous studies have documented the impact that surgery has on the survival for patients with ovarian cancer. Aggressive cytoreductive surgery to achieve optimal tumor reduction has been shown to improve survival (3;4). Equally important recent studies have demonstrated that women who have their initial surgery performed by gynecologic oncologists, and women who have their surgeries at centers experienced in the treatment of ovarian cancer have higher survival rates (5-9). For these reasons it is essential that all women at high risk for ovarian cancer receive their initial care by gynecologic oncologists and at centers with multidisciplinary teams experienced in the optimal care of ovarian cancer patients. With this in mind, methods that facilitate the accurate triage of women who will ultimately be diagnosed with ovarian cancer could play a significant role in improving survival rates for these patients. Current triage guidelines by the American College of Obstetricians and Gynecologists and the Society of Gynecologic Oncologists stress the importance of accurate referral to gynecologic oncologists for women at high risk of having ovarian cancer (10;11). Algorithms such as the Risk of Malignancy Index (RMI) are tools that have also been created to help identify and triage such high risk patients(12). Improvement in the tools employed for triage of women at high risk for EOC will result in improved survival for these patients.

Advances in the route of delivery and agents employed in the treatment of women with ovarian cancer have made steady but slow progress. The advances in treatment regimens--to include platinum-based chemotherapy with the addition of taxanes, and more recently a change in the route of delivery to a combination of intraperitoneal and intravenous administration--has resulted in improved survival(13). The addition of biologics such as bevacizumab holds promise for further improvement in survival rates. However, drug development, testing and implementation may require a decade or more to impact survival rates. Although continued research and development for more effective chemotherapeutics are needed, much larger gains in survival will be achieved through improvements in screening, early detection and, appropriate triage of women diagnosed with ovarian cancer.

The challenge of early detection is, however, formidable, given the low prevalence of ovarian cancer even in the postmenopausal population (1:2500). A high sensitivity for early stage and, ideally, pre-clinical disease (>75%) is required. An extraordinarily high specificity (>99.6%) is also needed to achieve a positive predictive value (PPV) of 10%. i.e., 10 operations for each case of ovarian cancer detected. Progress has been made toward developing an appropriately sensitive and specific strategy for early detection, but even greater advances have occurred in the development of tools for more accurate triage.

The serum biomarker CA125 is the most widely used serum tumor marker for management of women with EOC. CA125 is a high molecular weight mucinous glycoprotein (MUC16) that was first identified in 1981 and has been extensively studied for its utility in screening and early detection, monitoring of disease status and its role as a prognostic indicator (14). Over the years, additional markers have been examined alone and in combination with CA125 for their potential role in the management of EOC (15). Complex statistical algorithms along with a further understanding of the velocity of biomarker changes over time have improved the sensitivity of the biomarker assays (16). The remainder of this manuscript will examine the serum biomarkers that are currently available clinically for risk assessment and the management of women with EOC.

Screening for Epithelial Ovarian Cancer

CA125 has been extensively evaluated in attempts to develop screening models for EOC. Up to 80% of epithelial ovarian cancers express CA125 that is shed into serum that can be quantitatively measured (14). Fixed serum thresholds for CA125 ($>35\text{U/mL}$) have, however, limited utility as a screening tool as only 50-60% of women with stage I EOC will have elevated serum levels, thereby limiting its sensitivity for detecting ovarian cancer when it is still confined to the ovary (17). CA125 specificity is also compromised by the fact that many benign gynecologic and medical conditions, as well as other malignancies can result in elevated serum CA125 levels. Many of the gynecologic conditions are encountered less frequently in postmenopausal women and here the specificity of a single value of CA125 can approach 99%, but not 99.6%.

The limitations of individual CA125 values and of transvaginal sonography for early detection of ovarian cancer have been highlighted by the Prostate, Lung, Colorectal and Ovarian (PLCO) screening trial. In this trial, 34,261 postmenopausal women were randomized to undergo CA125 and transvaginal sonography (TVS) screening annually for the first three years, followed by two more rounds of annual serum CA125 levels. Women were referred to a gynecologic surgeon if either the ultrasound or the CA125 was abnormal. The positive predictive value for CA125 alone was 3.7% and that for TVS 1%. If both were abnormal, the PPV was 23.5%, but 60% of invasive cancers would not have been detected if women had been referred for surgical consultation using this rule. Sensitivity for early stage disease was also a problem. Eighty-nine invasive ovarian or peritoneal cancers have been diagnosed, 60 of which were screen-detected. Of the 60 malignancies detected by the screening protocol, only 21% were stage I/II (18).

Greater specificity and PPV can be attained by observing the trend of CA125 values over time. In 1992, Einhorn et al reported the specificity of a rising CA125 during annual screening for ovarian cancer in a small prospective trial with healthy women in Sweden. Serum samples were obtained from 5550 women, 175 were found to have elevated levels of CA125 ($>35\text{U/mL}$) and ultrasound was performed. Among those with elevated CA125, six were diagnosed with ovarian cancer, although only 2 were in stage I. Three women were subsequently diagnosed with EOC who had normal CA125 levels. This resulted in a specificity of 98.5% in women over the age of 50, but only 94.5% in women under the age of 50 years (19). While this study resulted in a suboptimal predictive value, the important observation was made that women who had elevated CA125 levels in the setting of an ovarian malignancy demonstrated progressive elevations over time, whereas, women with erroneously elevated "false positive" serum CA125 levels, had CA125 values that were stable or declined over time (19).

More recently, Skates et al demonstrated in multiple serial samples from patients who eventually developed EOC had a change from their baseline serum CA125 levels up to two years prior to their clinical diagnosis of ovarian cancer. The phenomenon of a rising slope of serum CA125 levels as an early indicator for the development of an ovarian malignancy was used to develop the risk of ovarian cancer (ROC) algorithm, which takes into account a woman's age and rate of rise or velocity of change in serum CA125 levels over time (16;20;21). With this algorithm, women with an elevated ROC risk assessment are referred for ultrasound evaluation. This approach increased the sensitivity of a single CA125 value of 62% to 86% in a retrospective study of 9233 women with 33,621 serum samples (16). The ROC was subsequently validated in a prospective study that reported specificity for the detection of invasive EOC of 99.8% with a positive predictive value of 19%, i.e., 5 operations per case of ovarian cancer detected(21).

The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) is evaluating the ROC algorithm prospectively in a study powered to detect a difference in survival. The UKCTOCS has enrolled over 200,000 postmenopausal women and randomized them to screening (100,000) versus no screening (100,000). The women assigned to the screening arm were further randomized to undergo annual TVS alone (50,000) or multimodality screening (MMS) with annual CA125 values interpreted by the ROC with referral of approximately 2% to TVS if the CA125 were rising (50,000). Of the 50,078 women who underwent MMS during their initial 2 years on study, 97 underwent surgery (0.2%). In the ultrasound screening (USS) group, 845 of 48,230 women underwent surgery (1.8%). For primary invasive cancers of the ovary and fallopian tube, the MMS approach achieved a sensitivity, specificity and positive predictive value of 89.5%, 99.8% and 35.1% respectively. The USS group reported sensitivity, specificity and positive-predictive value of 75%, 98.2% and 2.8%. Of note, 48% of the 58 invasive malignancies diagnosed were stage I/II(22). Consequently, annual TVS required 36 operations per case of ovarian cancer detected, whereas the MMS with CA125 followed by TVS required only 2.8. It appears that the requirement for a rising CA125 precluded TVS detection of a great deal of benign disease which still requires surgery for a definitive diagnosis. Encouragingly, 48% of ovarian cancers were diagnosed in stage I or II in the prevalence phase of this trial, compared to the 25% of cases that one might expect. As the prevalence of the disease was twice its incidence, there may be as much as 2 years lead time, consistent with the practicality of annual screening. It still remains to be seen whether a stage shift will occur for incident cases during these next years and whether this will translate into a survival advantage. As treatment for early stage disease is not specified in the trial, it will be important to determine whether most participants had timely and appropriate chemotherapy in addition to surgery.

Over the last 6 years, the Ovarian Specialized Program of Research Excellence (SPORE) at U.T. M.D. Anderson has been conducting a multi-site trial of screening for ovarian cancer in the United States for postmenopausal women at conventional risk using the ROC algorithm to refer approximately 2% of participants for TVS. If TVS has been positive, women are referred to surgery. Preliminary data from more than 9,500 annual CA125 assays in more than 3,000 women, suggest that the ROC will have a specificity of 99.2% for each encounter and 97.8% over several years of screening. TVS further increases the specificity. Nine women have been referred for surgery based on the ROC and TVS, with six ovarian cancers detected (3 borderline and 3 invasive) all in early stage, assuring a PPV of at least 30%, consistent with observations in the UKCTOCS (Lu, et al, unpublished data).

As 20% of ovarian cancers express little or no CA125, many other serum tumor markers have been evaluated in combination with CA125 to improve the sensitivity, specificity and positive predictive value of the test (15;23). A panel of six tumor markers, including CA-125, leptin, prolactin, osteopontin, insulin-like growth factor II and macrophage migration inhibitory factor was compared to CA125 alone in a study evaluating the serum of 362 healthy controls and 156 women with ovarian cancer. The ELISA-based panel improved the sensitivity and specificity of CA125 alone (72% and 95%) to 95.3% and 98.7%. The authors reported that 221 of 224 women in a test set that included 43 women with ovarian cancer were classified appropriately (98.7%)(24). When the positive predictive value of this test was recalculated based on the prevalence of ovarian cancer in the general population, the result was 6.5%, as reported by Visintin et al which does not support using this test for screening of the general population.

Data on two other “panel” tests have reported sensitivities and specificities ranging from 91-96% and 88-96% for ovarian cancer (25;26). These panels have yet to be validated in prospective clinical trials. Another trial examining multiple markers looked at the various

combinations of 9 markers including CA125, HE4, SMRP, CA72-4, Osteopontin, ERBB2, Inhibin, Activin and EGFR and demonstrated dual marker combination of CA125 and HE4 had a greater sensitivity than either marker alone (15). Of some concern are the preliminary results from a collaborative trial from institutions that are funded by NCI SPORE grants in ovarian cancer. More than 50 biomarkers have been tested in the proximal serum samples taken from prior to diagnosis of ovarian cancer in the PLCO trial. In these preclinical samples, CA125 detected ovarian cancer in approximately 60% of patients within one year of diagnosis and little or no improvement was seen with multi-marker panels (27).

Novel approaches will be required to detect pre-clinical disease. Urinary markers have been evaluated for the early detection of EOC. At least in theory, small amounts of low molecular weight biomarkers (<50 kD) could pass through the glomerulus and be detected in urine before serum levels become elevated. Urine mesothelin (SMRP) exhibited greater sensitivity for early stage ovarian cancer than did hCG free beta subunit or beta subunit core fragment (28). The anti-apoptotic protein Bcl-2 has also been found elevated in urine from ovarian cancer patients (29) and complementarity has been detected between the two biomarkers (unpublished data). Novel urinary analytes deserve further attention as targets for screening.

Autoantibodies provide another approach to detecting preclinical disease with greater sensitivity. Small volumes of ovarian cancer could evoke a humoral immune response, breaking tolerance by the presentation of mutant, overexpressed or mal distributed tumor associated proteins (30). More than 60 different human protein target antigens have been reported in the literature over the last two decades. Recent studies have utilized protein arrays with 5,000-8,000 human proteins expressed in insect cells (31;32). Availability of arrays constructed from human proteins expressed in human cells may provide even more faithful representation of antigenic epitopes recognized by human autoantibodies. Substantial attention has been given to autoantibodies reactive with wild-type TP53 (33) that arise in patients with high grade, often late stage serous ovarian carcinomas with p53 mutations (34). Overall, approximately 20% of patients will have autoantibodies against the wild type TP53, although antibodies have generally not been measured against mutant protein or in pre-clinical samples. Autoantibodies might detect at least a fraction of patients with "type 2" high grade ovarian cancers, before metastasis occurs, complementing CA125 and other more traditional biomarkers, if autoantibodies prove sufficiently specific.

The use of biomarkers as part of screening protocols is clearly an important area of research in ovarian cancer. Final results of the UKCTOCS trial with CA125 followed by TVS will be important, but multiple markers are likely to be required to detect most early stage disease at the time of conventional diagnosis. In addition to panels of serum biomarkers, both urinary analytes and serum autoantibodies deserve more detailed attention.

The use of serum biomarkers in the evaluation of women with pelvic masses

Nearly 200,000 women will undergo surgery on an annual basis for the diagnosis of a pelvic mass, and anywhere from 13-21% of these women will be diagnosed with epithelial ovarian cancer (35;36). Women whose malignancy can be predicted preoperatively so that their surgery can be performed by a specialty-trained gynecologic oncologist in an appropriate referral center have been shown to have better outcomes (5-7;9;37). Accordingly, a reliable tool to estimate the risk of invasive epithelial ovarian cancer in women with pelvic masses is necessary to triage women to the appropriate care provider before their surgical intervention. Many tools used currently utilize serum tumor markers.

CA125 is the most commonly used serum biomarker for the evaluation of women with pelvic masses as 80% of women with EOC will have elevated serum levels. In fact, the sensitivity for elevated serum CA125 levels, when considered independently, for predicting EOC in women with a pelvic mass ranges from 43-81% (15;38-42). Unfortunately, the specificity of CA125 is limited by a number of factors. The CA125 glycoprotein is a coelomic epithelial antigen produced by the mesothelial cells that line the peritoneal, pleural and pericardial cavities. As such, serum concentrations of CA125 can be elevated by benign gynecologic and other medical conditions, such as normal menses, endometriosis, cirrhosis and congestive heart failure (43;44). In addition, mean levels of serum CA125 vary according to menopausal status, with higher values observed in premenopausal patients and a decreasing levels in postmenopausal women with increasing age (40;42-45). The limited sensitivity and specificity of CA125 alone has inspired the use of other modalities and novel tumor markers in combination with the test to improve the test's ability to predict malignancy in women with pelvic masses.

Human epididymis protein 4 (HE4) is made up of two whey acidic protein (WAP) domains and a 4 disulfide core and has been shown to be over-expressed by epithelial ovarian cancer tumors and circulate in the serum of patients with EOC (46). HE4 is less likely to be elevated falsely in the setting of benign neoplasms as compared to serum CA125, and can be used to differentiate endometriomas from malignant ovarian tumors (39;47).

As individual tests, CA125 and HE4 have equivalent sensitivities for detecting malignancy in women with pelvic masses (48). However, there is a subset of ovarian cancers that do not express CA125 that do express HE4, suggesting that the tests in combination could be complementary (15;49). In a prospective study evaluating the use of multiple serum tumor markers alone and in combination in the evaluation of women with pelvic masses, Moore et al reported a sensitivity of 72.9% for HE4 at a set specificity of 95%. Serum CA125 achieved a sensitivity of 43.3% at 95% specificity in this study. In combination, HE4 and CA125 achieved a sensitivity for detecting invasive epithelial ovarian cancer of 76.4% at a set specificity of 95%, higher than either test alone (15).

Even in combination serum biomarkers have limited sensitivities and specificities, so efforts to improve risk-stratification tools for women with pelvic masses have incorporated other factors from a woman's clinical evaluation.

One such tool is the Risk of Malignancy Index (RMI), which evaluates a woman's risk for invasive disease by considering the woman's menopausal status, ultrasound characteristics of her pelvic mass, and serum CA125 levels. The RMI was originally described in 1990 by Jacobs et al, and various versions of the calculations have been validated in several trials since. The reported sensitivity and specificity for the RMI in identifying epithelial ovarian cancer preoperatively ranges between 71-88.5% and 74.3-97% respectively (12;50-54).

Ultrasound evaluation of pelvic masses can be limited by subjective interpretation of imaging studies, and another recently published algorithm for risk stratification of women with pelvic masses excludes them. The Predictive Probability Index (PP) considers a patient's menopausal status along with serum CA125 and HE4 levels to determine the risk of malignancy in women with pelvic masses. In a prospective study evaluating 531 women with pelvic masses, the PP achieved a sensitivity of 88.7% for detecting invasive epithelial ovarian cancer and tumors of low malignant potential at a set specificity of 75%. When examining the ability to detect invasive epithelial ovarian cancer alone, the PP algorithm detect 94% of the invasive EOC in both pre and postmenopausal patients (89% premenopausal and 95% postmenopausal invasive EOC cases were detected) (55).

The serum biomarkers HE4 and CA125 are limited in detecting epithelial ovarian cancers of the rare mucinous cell type(15;49;55). Total inhibin is elevated in the serum of women with mucinous epithelial ovarian cancers(56;57). Another candidate serum tumor marker for mucinous tumors is CEA, which is used reliably in gastrointestinal tumors of mucinous histology. Immunohistochemical staining has demonstrated overexpression of CEA in a third of mucinous EOC tumors, and elevated serum levels have been documented in patients with mucinous epithelial ovarian cancer(58;59).

Currently two biomarkers that are available for clinical use are CA125 and HE4. These two markers when used in combination complement each other for the prediction of ovarian cancer in women with a pelvic mass. HE4 has a greater specificity in the premenopausal age group due to its lack of over expression in patients with benign gynecologic diseases. CA125 and HE4 as a dual marker assay is a valuable tool for the risk assessment of a patient with an ovarian cyst or pelvic mass. The dual marker combination of HE4 and CA125 continues to be studied in a second large multicenter trial examining the predictive probability algorithms utility in a low risk population of patients presenting to general obstetrician and gynecologist.

Biomarkers for Monitoring EOC Disease status

The only two serum biomarkers currently clinically available and approved by the FDA for monitoring patients with EOC are CA125 and HE4. Approximately 90% of patients with EOC have tumors that overexpress either CA125 or HE4, and the changes in a patients serum levels accurately reflect regression or progression of disease(60-63). Reliable tumor markers can be used to monitor response to treatment provided the biomarker serum levels are elevated at the time of diagnosis. The most commonly used biomarker for monitoring treatment of EOC is CA125, as changes in serum levels have been demonstrated to correspond to progression or regression of disease(60;61;64). CA125 can be expressed not only by ovarian cancers, but also by inflamed peritoneum. Consequently approximately 90% of advanced ovarian cancer can be monitored with CA125, but some 10% of ovarian cancer patients will not have elevated serum CA125 levels and are therefore left without a reliable marker for monitoring of disease. There is a substantial correlation between HE4 and CA125 for monitoring the biologic activity of epithelial ovarian cancer(65). Recently, the novel serum biomarker HE4 has been shown to be elevated in over half of the EOC where CA125 was not a marker resulting in over 90% of all EOC having at least CA125 or HE4 as a biomarker for monitoring disease status(62;63).

CA125 is the most well studied serum biomarker for monitoring EOC and a 50% decrease in serum CA125 levels has been shown to correlate with disease response as measured by conventional criteria in phase II clinical trials (64). Equally, a doubling of CA125 from baseline has been shown to be consistent with failure of a drug and progressive disease. Because a rise in serum biomarkers can indicate recurrence or progression of disease, evaluating serum markers as part of cancer surveillance has become a clinical standard. In patients whose CA125 is initially elevated and normalizes after treatment, the Gynecologic Cancer Intergroup (GCIg) defines progression based on two CA125 levels, one week apart, that are each at least twice the upper limit of normal for the specific assay being used. In patients with serum CA125 levels that never normalized, progression is defined as two values that are at least twice the patients' CA125 nadir. In a study evaluating 255 patients with elevated CA125 levels undergoing treatment for EOC, Rustin et al reported that a doubling of a patient's post-treatment nadir or values twice that of the upper limit of normal predicted recurrent EOC with a sensitivity of 86%, specificity of 91%, PPV 95% and NPV 78%(66).

The only other tumor marker approved by the United States FDA and clinically available for monitoring patients with EOC for recurrence or progression is HE4. In a study evaluating serum HE4 levels of women with EOC, serial HE4 levels from 80 patients either undergoing treatment for EOC or being monitored for recurrence showed that changes in HE4 values were not inferior to changes in CA125 values in accordance with clinical status. CA125 correlated with clinical status in 78.8% (63/80) and HE4 correlated in 76.2% (61/80). In consideration of all cases either CA125 or HE4 correlated with clinical status for 83.8% (67/80) of patients (65).

Serial measurements of serum biomarkers are helpful for detecting early recurrence of disease, and serum elevations may precede clinical or radiological detectable disease by a median time of 2 to 6 months (67;68). However, benefit of the early detection of recurrent disease based on serologic recurrence has recently come under question. At the 2009 American Society of Clinical Oncologists, Rustin, et al, presented a trial conducted over the last decade in the United Kingdom and several centers in Europe comparing the impact on ovarian cancer survival of a physician's knowing or not knowing that CA125 was rising in the setting of disease recurrence. No survival advantage was found for patients treated for recurrent disease based on a rising CA125 when compared to patients treated on the basis of subsequent symptoms and signs (69). CA125 accurately predicted disease recurrence, but three months of lead time did not appear to impact on survival based upon the treatment given. Each participating physician was, however free to choose how patients were treated. Only one third of the patients received a combination of a taxane and platinum compounds in either arm. As carboplatin and paclitaxel have been shown to provide superior survival over carboplatin alone in recurrent disease (70), it appears that two thirds of patients received suboptimal therapy by today's standards. With only three months lead time, suboptimal chemotherapy would have minimized any advantage to early treatment. In addition, patients were not stratified based on the extent of primary cytoreduction nor were they staged with CT scans at the completion of chemotherapy, so that balance of the two treatment arms was not certain. Thus, the value of detecting recurrent EOC before it is clinically evident needs further evaluation. This study certainly does point to the importance of improving therapy for recurrent disease. As many patients have only a year of life after recurrence, earlier detection of disease would provide a greater opportunity to participate in clinical trials or to utilize the several conventional agents currently available for treatment. As newer classes of drugs and biological agents become available there may be an advantage to treating patients with low volume disease.

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Reference List

1. American Cancer Society Cancer Facts & Figures 2009. Atlanta: American Cancer Society; 2009. p. 1-68.
2. Horner, MJ.; Ries, LAG.; Krapcho, M.; Neyman, N.; Aminou, R.; Howlader, N., et al. SEER Cancer Statistics Review, 1975-2006, National Cancer Institute. Bethesda, MD: SEER Cancer Statistics Review; 2009.
3. Hoskins WJ, McGuire WP, Brady MF, Homesley HD, Creasman WT, Berman M, et al. The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. *Am J Obstet Gynecol.* 1994; 170(4):974-979. [PubMed: 8166218]
4. Young RC, Decker DG, Wharton JT, Piver MS, Sindelar WF, Edwards BK, et al. Staging laparotomy in early ovarian cancer. *JAMA.* 1983; 250(22):3072-3076. [PubMed: 6358558]

5. Carney ME, Lancaster JM, Ford C, Tsodikov A, Wiggins CL. A population-based study of patterns of care for ovarian cancer: who is seen by a gynecologic oncologist and who is not? *Gynecol Oncol.* 2002; 84(1):36–42. [PubMed: 11748973]
6. Earle CC, Schrag D, Neville BA, Yabroff KR, Topor M, Fahey A, et al. Effect of surgeon specialty on processes of care and outcomes for ovarian cancer patients. *J Natl Cancer Inst.* 2006; 98(3):172–180. [PubMed: 16449677]
7. Goff BA, Matthews BJ, Wynn M, Muntz HG, Lishner DM, Baldwin LM. Ovarian cancer: patterns of surgical care across the United States. *Gynecol Oncol.* 2006; 103(2):383–390. [PubMed: 17005244]
8. Goff BA, Matthews BJ, Larson EH, Andrilla CH, Wynn M, Lishner DM, et al. Predictors of comprehensive surgical treatment in patients with ovarian cancer. *Cancer.* 2007; 109(10):2031–2042. [PubMed: 17420977]
9. Paulsen T, Kjaerheim K, Kaern J, Tretli S, Trope C. Improved short-term survival for advanced ovarian, tubal, and peritoneal cancer patients operated at teaching hospitals. *Int J Gynecol Cancer.* 2006; 16(Suppl 1):11–17. [PubMed: 16515561]
10. Guidelines for referral to a gynecologic oncologist: rationale and benefits. The Society of Gynecologic Oncologists. *Gynecol Oncol.* 2000; 78(3 Pt 2):S1–13. [PubMed: 10985904]
11. ACOG Committee Opinion: number 280, December 2002. The role of the generalist obstetrician-gynecologist in the early detection of ovarian cancer. *Obstet Gynecol.* 2002; 100(6):1413–1416. [PubMed: 12468197]
12. Jacobs I, Oram D, Fairbanks J, Turner J, Frost C, Grudzinskas JG. A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. *Br J Obstet Gynaecol.* 1990; 97(10):922–929. [PubMed: 2223684]
13. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med.* 2006; 354(1):34–43. [PubMed: 16394300]
14. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest.* 1981; 68(5):1331–1337. [PubMed: 7028788]
15. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol.* 2007; 108:402–408. [PubMed: 18061248]
16. Skates SJ, Menon U, MacDonald N, Rosenthal AN, Oram DH, Knapp RC, et al. Calculation of the Risk of Ovarian Cancer From Serial CA-125 Values for Preclinical Detection in Postmenopausal Women. *J Clin Oncol.* 2003; 21(10 Suppl):206–210.
17. Woolas RP, Xu FJ, Jacobs IJ, Yu YH, Daly L, Berchuck A, et al. Elevation of multiple serum markers in patients with stage I ovarian cancer. *J Natl Cancer Inst.* 1993; 85(21):1748–1751. [PubMed: 8411259]
18. Partridge E, Kreimer AR, Greenlee RT, Williams C, Xu JL, Church TR, et al. Results from four rounds of ovarian cancer screening in a randomized trial. *Obstet Gynecol.* 2009; 113(4):775–782. [PubMed: 19305319]
19. Einhorn N, Sjøvall K, Knapp RC, Hall P, Scully RE, Bast RC Jr, et al. Prospective evaluation of serum CA 125 levels for early detection of ovarian cancer. *Obstet Gynecol.* 1992; 80(1):14–18. [PubMed: 1603484]
20. Skates SJ, Xu FJ, Yu YH, Sjøvall K, Einhorn N, Chang Y, et al. Toward an optimal algorithm for ovarian cancer screening with longitudinal tumor markers. *Cancer.* 1995; 2004–2010; 76(10 Suppl)
21. Menon U, Skates SJ, Lewis S, Rosenthal AN, Rufford B, Sibley K, et al. Prospective study using the risk of ovarian cancer algorithm to screen for ovarian cancer. *J Clin Oncol.* 2005; 23(31):7919–7926. [PubMed: 16258091]
22. Menon U, Gentry-Maharaj A, Hallett R, Ryan A, Burnell M, Sharma A, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). *Lancet Oncol.* 2009; 10(4):327–340. [PubMed: 19282241]
23. Bast RC Jr, Badgwell D, Lu Z, Marquez R, Rosen D, Liu J, et al. New tumor markers: CA125 and beyond. *Int J Gynecol Cancer.* 2005; 15(Suppl 3):274–281. [PubMed: 16343244]

24. Visintin I, Feng Z, Longton G, Ward DC, Alvero AB, Lai Y, et al. Diagnostic markers for early detection of ovarian cancer. *Clin Cancer Res.* 2008; 14(4):1065–1072. [PubMed: 18258665]
25. Amonkar S, Bertenshaw G, Chen T. Development and preliminary evaluation of a multivariant index assay for ovarian cancer. *PLoS ONE.* 2009; 4:e4599. [PubMed: 19240799]
26. Nosov V, Su F, Amneus M, Birrer M, Robins T, Kotlerman J, et al. Validation of serum biomarkers for detection of early-stage ovarian cancer. *Am J Obstet Gynecol.* 2009; 200(6):639–5. [PubMed: 19285648]
27. Cramer DW, Bast RC, Clark C, Coombes K, Diamandis E, Fung E, et al. Phase III validation of ovarian cancer biomarkers in pre-diagnostic specimens from the PLCO screening trial. *Proc Am Assoc Cancer Res.* 2009:LB–96.
28. Badgwell D, Lu Z, Cole L, Fritsche H, Atkinson EN, Somers E, et al. Urinary mesothelin provides greater sensitivity for early stage ovarian cancer than serum mesothelin, urinary hCG free beta subunit and urinary hCG beta core fragment. *Gynecol Oncol.* 2007; 106(3):490–497. [PubMed: 17532030]
29. Anderson NS, Bermudez Y, Badgwell D, Chen R, Nicosia SV, Bast RC Jr, et al. Urinary levels of Bcl-2 are elevated in ovarian cancer patients. *Gynecol Oncol.* 2009; 112(1):60–67. [PubMed: 19007973]
30. Goodell V, Salazar LG, Urban N, Drescher CW, Gray H, Swensen RE, et al. Antibody immunity to the p53 oncogenic protein is a prognostic indicator in ovarian cancer. *J Clin Oncol.* 2006; 24(5):762–768. [PubMed: 16391298]
31. Hudson ME, Pozdnyakova I, Haines K, Mor G, Snyder M. Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. *Proc Natl Acad Sci U S A.* 2007; 104(44):17494–17499. [PubMed: 17954908]
32. Gunawardana CG, Memari N, Diamandis EP. Identifying novel autoantibody signatures in ovarian cancer using high-density protein microarrays. *Clin Biochem.* 2009; 42(4-5):426–429. [PubMed: 19094976]
33. Soussi T. p53 Antibodies in the sera of patients with various types of cancer: a review. *Cancer Res.* 2000; 60(7):1777–1788. [PubMed: 10766157]
34. Tsai-Turton M, Santillan A, Lu D, Bristow RE, Chan KC, Shih I, et al. p53 autoantibodies, cytokine levels and ovarian carcinogenesis. *Gynecol Oncol.* 2009; 114(1):12–17. [PubMed: 19398128]
35. Curtin JP. Management of the adnexal mass. *Gynecol Oncol.* 1994; 55(3 Pt 2):S42–S46. [PubMed: 7835810]
36. National Institutes of Health Consensus Development Conference Statement. Ovarian cancer: screening, treatment, and follow-up. *Gynecol Oncol.* 1994; 55(3 Pt 2):S4–14. [PubMed: 7835809]
37. McGowan L, Leshner LP, Norris HJ, Barnett M. Misstaging of ovarian cancer. *Obstet Gynecol.* 1985; 65(4):568–572. [PubMed: 3982731]
38. Einhorn N, Knapp RC, Bast RC, Zurawski VR Jr. CA 125 assay used in conjunction with CA 15-3 and TAG-72 assays for discrimination between malignant and non-malignant diseases of the ovary. *Acta Oncol.* 1989; 28(5):655–657. [PubMed: 2590540]
39. Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res.* 2003; 63(13):3695–3700. [PubMed: 12839961]
40. Maggino T, Gadducci A, D'Addario V, Pecorelli S, Lissoni A, Stella M, et al. Prospective multicenter study on CA 125 in postmenopausal pelvic masses. *Gynecol Oncol.* 1994; 54(2):117–123. [PubMed: 8063233]
41. Skates SJ, Horick N, Yu Y, Xu FJ, Berchuck A, Havrilesky LJ, et al. Preoperative sensitivity and specificity for early-stage ovarian cancer when combining cancer antigen CA-125II, CA 15-3, CA 72-4, and macrophage colony-stimulating factor using mixtures of multivariate normal distributions. *J Clin Oncol.* 2004; 22(20):4059–4066. [PubMed: 15381683]
42. Malkasian GD Jr, Knapp RC, Lavin PT, Zurawski VR Jr, Podratz KC, Stanhope CR, et al. Preoperative evaluation of serum CA 125 levels in premenopausal and postmenopausal patients with pelvic masses: discrimination of benign from malignant disease. *Am J Obstet Gynecol.* 1988; 159(2):341–346. [PubMed: 2457318]

43. Alagoz T, Buller RE, Berman M, Anderson B, Manetta A, DiSaia P. What is a normal CA125 level? *Gynecol Oncol.* 1994; 53(1):93–97. [PubMed: 8175027]
44. Bon GG, Kenemans P, Verstraeten R, van Kamp GJ, Hilgers J. Serum tumor marker immunoassays in gynecologic oncology: establishment of reference values. *Am J Obstet Gynecol.* 1996; 174(1 Pt 1):107–114. [PubMed: 8571992]
45. Einhorn N, Bast RC Jr, Knapp RC, Tjernberg B, Zurawski VR Jr. Preoperative evaluation of serum CA 125 levels in patients with primary epithelial ovarian cancer. *Obstet Gynecol.* 1986; 67(3): 414–416. [PubMed: 2418395]
46. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res.* 2005; 65(6):2162–2169. [PubMed: 15781627]
47. Huhtinen K, Suvitie P, Hiissa J, Junnila J, Huvila J, Kujari H, et al. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *Br J Cancer.* 2009; 100(8):1315–1319. [PubMed: 19337252]
48. Shah CA, Lowe KA, Paley P, Wallace E, Anderson GL, McIntosh MW, et al. Influence of ovarian cancer risk status on the diagnostic performance of the serum biomarkers mesothelin, HE4, and CA125. *Cancer Epidemiol Biomarkers Prev.* 2009; 18(5):1365–1372. [PubMed: 19423517]
49. Rosen DG, Wang L, Atkinson JN, Yu Y, Lu KH, Diamandis EP, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. *Gynecol Oncol.* 2005; 99(2):267–277. [PubMed: 16061277]
50. Bailey J, Tailor A, Naik R, Lopes A, Godfrey K, Hatem HM, et al. Risk of malignancy index for referral of ovarian cancer cases to a tertiary center: does it identify the correct cases? *Int J Gynecol Cancer.* 2006; 16(Suppl 1):30–34. [PubMed: 16515564]
51. Enakpene CA, Omigbodun AO, Goecke TW, Odukogbe AT, Beckmann MW. Preoperative evaluation and triage of women with suspicious adnexal masses using risk of malignancy index. *J Obstet Gynaecol Res.* 2009; 35(1):131–138. [PubMed: 19215560]
52. Geomini P, Kruitwagen R, Bremer GL, Cnossen J, Mol BW. The accuracy of risk scores in predicting ovarian malignancy: a systematic review. *Obstet Gynecol.* 2009; 113(2 Pt 1):384–394. [PubMed: 19155910]
53. Tingulstad S, Hagen B, Skjeldestad FE, Onsrud M, Kiserud T, Halvorsen T, et al. Evaluation of a risk of malignancy index based on serum CA125, ultrasound findings and menopausal status in the pre-operative diagnosis of pelvic masses. *Br J Obstet Gynaecol.* 1996; 103(8):826–831. [PubMed: 8760716]
54. Tingulstad S, Hagen B, Skjeldestad FE, Halvorsen T, Nustad K, Onsrud M. The risk-of-malignancy index to evaluate potential ovarian cancers in local hospitals. *Obstet Gynecol.* 1999; 93(3):448–452. [PubMed: 10074998]
55. Moore RG, McMeekin DS, Brown AK, Disilvestro P, Miller MC, Allard WJ, 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.* 2009; 112(1):40–46. [PubMed: 18851871]
56. Healy DL, Burger HG, Marners P, Jobling T, Bangah M, Quinn M, et al. Elevated serum inhibin concentrations in postmenopausal women with ovarian tumors. *N Engl J Med.* 1993; 329(21): 1539–1542. [PubMed: 8413476]
57. Tsigkou A, Marrelli D, Reis FM, Luisi S, Silva-Filho AL, Roviello F, et al. Total inhibin is a potential serum marker for epithelial ovarian cancer. *J Clin Endocrinol Metab.* 2007; 92(7):2526–2531. [PubMed: 17473066]
58. Hogdall EV, Christensen L, Kjaer SK, Blaakaer J, Jarle C I, Gayther S, et al. Protein expression levels of carcinoembryonic antigen (CEA) in Danish ovarian cancer patients: from the Danish ‘MALOVA’ ovarian cancer study. *Pathology.* 2008; 40(5):487–492. [PubMed: 18604735]
59. Tholander B, Taube A, Lindgren A, Sjoberg O, Stendahl U, Tamsen L. Pretreatment serum levels of CA-125, carcinoembryonic antigen, tissue polypeptide antigen, and placental alkaline phosphatase in patients with ovarian carcinoma: influence of histological type, grade of differentiation, and clinical stage of disease. *Gynecol Oncol.* 1990; 39(1):26–33. [PubMed: 2227570]

60. Bast RC Jr, Klug TL, St John E, Jenison E, Niloff JM, Lazarus H, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med*. 1983; 309(15):883–887. [PubMed: 6310399]
61. Hawkins RE, Roberts K, Wiltshaw E, Mundy J, McCready VR. The clinical correlates of serum CA125 in 169 patients with epithelial ovarian carcinoma. *Br J Cancer*. 1989; 60(4):634–637. [PubMed: 2803938]
62. Moore RG, Brown AK, Miller CM, Badgwell D, Lu Z, Verch T, Lu KH, et al. A novel multiple biomarker assay for the detection of ovarian carcinoma. *J Clin Oncol*. 2006; 24:5023.
63. Brown AK, Miller MC, Robison K, Somers E, Altaner S, Granai CO, et al. Differential expression of CA125 and a novel serum tumor marker HE4 in epithelial ovarian cancer. *J Clin Oncol*. 2009; 26(suppl) abstr 5533.
64. Rustin GJ, Bast RC Jr, Kelloff GJ, Barrett JC, Carter SK, Nisen PD, et al. Use of CA-125 in clinical trial evaluation of new therapeutic drugs for ovarian cancer. *Clin Cancer Res*. 2004; 10(11):3919–3926. [PubMed: 15173101]
65. Allard WJ, Somers E, Theil R, Moore RG. Use of a novel biomarker HE4 for monitoring patients with epithelial ovarian cancer. *J Clin Oncol*. 2009 May 20.26(suppl) abstr 5535.
66. Rustin GJ, Nelstrop AE, Tuxen MK, Lambert HE. Defining progression of ovarian carcinoma during follow-up according to CA 125: a North Thames Ovary Group Study. *Ann Oncol*. 1996; 7(4):361–364. [PubMed: 8805927]
67. Rustin GJ, Nelstrop AE, McClean P, Brady MF, McGuire WP, Hoskins WJ, et al. Defining response of ovarian carcinoma to initial chemotherapy according to serum CA 125. *J Clin Oncol*. 1996; 14(5):1545–1551. [PubMed: 8622070]
68. Partridge EE, Barnes MN. Epithelial ovarian cancer: prevention, diagnosis, and treatment. *CA Cancer J Clin*. 1999; 49(5):297–320. [PubMed: 11198956]
69. Rustin GJ, van der Burg ME. A randomized trial in ovarian cancer (OC) of early treatment of relapse based on CA125 level alone versus delayed treatment based on conventional clinical indicators (MRC OV05/EORTC 55955 trials). *J Clin Oncol*. 2009; 27(18s)
70. Parmar MK, Ledermann JA, Colombo N, du BA, Delaloye JF, Kristensen GB, et al. Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON4/AGO-OVAR-2.2 trial. *Lancet*. 2003; 361(9375):2099–2106. [PubMed: 12826431]