Ca-125 and ovarian cancer markers

by Balasubramaniam Iyer

Transcript

Foreword

Of all gynecologic malignancies, the most lethal is ovarian cancer. The lack of symptoms in the early stages of the disease and the intraabdominal location make early detection and monitoring of the disease, by conventional methods, difficult. More than 70% of ovarian cancers are diagnosed in the third or fourth stage of the disease when the 5-year survival rate is less then 20%, even with extensive surgery and chemotherapy. It is widely accepted that early diagnosis is the cornerstone of successful treatment of ovarian cancer. Early diagnosis requires new approaches. An attractive direction is the use of serum tumor markers.

Introduction

One of the most promising approaches to management of ovarian cancer is early detection. Stage I ovarian cancer can be cured with currently available therapy in more than 90% of patients. However, fewer than 25% of ovarian cancers are currently detected in stage I. Detection of a greater fraction of cancers at an early stage might improve clinical outcome. Given a prevalence of one patient with ovarian cancer among 2,500 asymptomatic postmenopausal women in the general population, a successful screening strategy must have a sensitivity of more than 75% and a specificity of more than 99.6% to achieve a positive predictive value of 10%. Approaches to screening include transvaginal sonography, serum markers, and two-stage strategies that use alterations in serum markers to prompt sonographic examination. The ideal marker is a substance secreted only by cancerous cells (and not by normal cells), and should be detectable in a bodily fluid, in constant levels. Additionally, it should be determined by sufficiently noninvasive and inexpensive methods, that can be used in a widespread screening process for the detection of the disease in asymptomatic women. Considering the known prevalence data for ovarian cancer, tests used for his detection, in the early stage, must have a high sensitivity (proportion of cancers detected by a positive test), as well as an extremely high specificity (proportion of those without cancer identified by a negative test), to attain a positive predictive value (PPV) of at least 10%. Currently, for early-stage detection, there are no markers who are fully satisfying. Only a few markers for ovarian cancer have a sufficiently high sensitivity and even among them, most have a very poor specificity. The most limiting factor is the lack of specificity. Many markers are tumor-associated rather than tumor-specific and are elevated in multiple cancers, benign and physiological conditions also. Among the serum markers, CA-125 has been studied most extensively.
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Introduction

One of the most promising approaches to management of ovarian cancer is early detection. Stage I ovarian cancer can be cured with currently available therapy in more than 90% of patients. However, fewer than 25% of ovarian cancers are currently detected in stage I. Detection of a greater fraction of cancers at an early stage might improve clinical outcome. Given a prevalence of one patient with ovarian cancer among 2,500 asymptomatic postmenopausal women in the general population, a successful screening strategy must have a sensitivity of more than 75% and a specificity of more than 99.6% to achieve a positive predictive value of 10%. Approaches to screening include transvaginal sonography, serum markers, and two-stage strategies that use alterations in serum markers to prompt sonographic examination. The ideal marker is a substance secreted only by cancerous cells (and not by normal cells), and should be detectable in a bodily fluid, in constant levels. Additionally, it should be determined by sufficiently noninvasive and inexpensive methods, that can be used in a widespread screening process for the detection of the disease in asymptomatic women. Considering the known prevalence data for ovarian cancer, tests used for his detection, in the early stage, must have a high sensitivity (proportion of cancers detected by a positive test), as well as an extremely high specificity (proportion of those without cancer identified by a negative test), to attain a positive predictive value (PPV) of at least 10%. Currently, for early-stage detection, there are no markers who are fully satisfying. Only a few markers for ovarian cancer have a sufficiently high sensitivity and even among them, most have a very poor specificity. The most limiting factor is the lack of specificity. Many markers are tumor-associated rather than tumor-specific and are elevated in multiple cancers, benign and physiological conditions also. Among the serum markers, CA-125 has been studied most extensively.
pelvic inflammatory disease all produce higher levels of CA-125. 70% of people with cirrhosis, 60% of people with pancreatic cancer, and 20%-25% of people with other malignancies have elevated levels of CA-125. Combining detection methods with the CA-125 test lowers the number of false positive results and ideally should be done serially for best accuracy. One alternative approach is to not do the CA-125 test alone to detect ovarian cancer, but rather in conjunction with transvaginal sonography and rectovaginal pelvic examination for greater accuracy.

Interpretation

• A CA-125 test result of greater than 35 U/ml is generally accepted as being elevated.

True

• A true positive result is when the CA-125 test identifies a patient as having ovarian cancer, and they do have ovarian cancer.

• A true negative result is when the CA-125 test identifies a patient as not having ovarian cancer, and they do not have ovarian cancer.

False

• A false positive result is when the CA-125 test identifies a patient as having ovarian cancer, and they do not have ovarian cancer.

• A false negative result is when the CA-125 test identifies a patient as not having ovarian cancer, and they do have ovarian cancer.

• Elevated CA-125 levels can be a false positive, benign tumor, ovarian cancer, or another type of cancer.

• A false positive patient will most likely be identified by a physician as being cancer free. The possibility that normal ovaries are surgically removed due to a false positive result does exist.

HE4 (Human Epididymal Protein 4) HE4 is a protein that was first found in the epididymal epithelial cells but is also expressed in other epithelial cells. As a marker, HE4 is the product of the WFDC2 (HE4) gene that is over-expressed in patients with ovarian carcinoma and so was proposed as marker for ovarian cancer. Studies have shown that levels of the protein HE4, while not elevated in benign gynecologic conditions, is elevated in epithelial ovarian cancers (EOC), the most common type of ovarian cancer. In 2009, in the USA, HE4 was approved for monitoring women who had been diagnosed with epithelial ovarian cancer, for indications similar to CA-125. In 2011, use of the HE4 Test along with the CA-125 test was approved in the USA. These tests were combined in the Risk of Ovarian Malignancy Algorithm, called ROMA, to determine the likelihood of finding malignancy at surgery in women who have an adnexal mass. The diagnostic performance of ROMA was advocated for the
first time by Moore et al. [4] who sustained that CA-125 combined with HE4 reveals the highest sensitivity and specificity among 9 markers studied. HE4 serum concentrations vary significantly on the basis of age and these variations must be considered when the upper limit of normal for HE4 is determined. The Risk of Ovarian Malignancy Algorithm (ROMA) combines the result of the determinations of CA-125 and HE4, by taking in consideration of the premenopausal or the menopausal status, and converting them into a numerical score. ROMA is interpreted in conjunction with an independent clinical and radiological assessment. The aim is to aid the assessing for premenopausal or postmenopausal woman, who presents an ovarian adnexal mass and to establish if there is a high or low likelihood of finding malignancy at surgery. The ROMA test is indicated for women with age over 18, who have an ovarian pelvic mass for which surgery is planned and are not yet referred to an oncologist. HE4 and ROMA helps differentiating Ovarian Cancer from other pelvic masses, even in early stage OC. ROMA performs equally well as the ultrasound depending risk of malignancy index (RMI) and might be valuable as a first line biomarker for selecting high risk patients for referral to a tertiary center and further diagnostics[5].

Reference:


5. Gynecol Oncol. 2012 Nov;127(2):379-83. Evaluation of HE4, CA125, risk of ovarian malignancy algorithm (ROMA) and risk of malignancy index (RMI) as diagnostic tools of epithelial ovarian cancer in patients with a pelvic mass, Karlsen MA et al.
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Discussion: ‘Biomarkers for detection of early ovarian cancer’ by Nosov et al

In the roundtable that follows, clinicians discuss a study published in this issue of the Journal in light of its methodology, relevance to practice, and implications for future research. Article discussed:


DISCUSSION QUESTIONS

- What was the study design?
- How were candidate markers selected?
- How were patient samples selected?
- Which outcome was assessed?
- Which statistical approaches were applied?
- What are the clinical implications of this article?

INTRODUCTION

Ovarian cancer is a devastating disease with a high case-fatality rate. The expected incidence for 2008 was 21,650; 15,520 deaths were predicted. This high ratio of deaths to total cases drives the search for a screening test. But because the disease prevalence is relatively low—about 40/100,000 in postmenopausal women—development of a valid tool is problematic. Blood levels of the 1 currently available biomarker, cancer antigen-125 (CA-125) do not reliably pinpoint early malignancies. Nosov and colleagues studied how effectively 2 panels of candidate biomarkers—1 with CA-125; 1 without—detected ovarian cancer, a subject of great interest to this month’s Journal Club participants.

L. Stewart Massad, MD and George A. Macones, MD, MSCE

BACKGROUND

Massad: Judging the utility of a proposed screening test means assessing that test against several criteria. Screening tests must address a disease that causes significant morbidity and mortality, and ovarian cancer certainly meets that criterion. A presymptomatic stage must exist, and that is more controversial. The serendipitous discovery of early cancers in asymptomatic women suggests that at least some ovarian cancers have a presymptomatic stage, though the duration of this stage may be fairly short relative to screening intervals.

Outcomes after presymptomatic diagnosis and treatment must be better than outcomes for symptomatic disease. In fact, women with early stage ovarian cancers do better than those with advanced disease. The screening tests must be acceptable. Most screening tests proposed for ovarian cancer have involved blood or imaging tests, which are generally acceptable to patients, though cost may be unacceptable to payers. The reduced morbidity and mortality achieved through screening must outweigh the potential harm from false-positive tests. Proposed ovarian cancer screens, such as CA-125 levels or transvaginal ultrasound scans, have usually done poorly in this regard. The low prevalence of the disease means that most positive tests are false-positives, and since the validation test for most screens is oophorectomy, a relatively high-cost and high-morbidity procedure, a low positive predictive value means that risks for surgical morbidity and even mortality outweigh benefits from screening.

This problem has led some to suggest that more sensitive and specific markers of ovarian cancer will overcome this problem. One common strategy for marker discovery has been proteomics, the evaluation of the full range of serum or cell proteins. By comparing protein expression in normal women with that of ovarian cancer patients, candidate markers have emerged. Nosov and colleagues have identified a panel of serum proteins as potentially good markers for ovarian cancer screening.

STUDY DESIGN

Massad: What was the authors’ objective?

Ogutha: Previous studies have examined the use of serum biomarkers to identify early stage ovarian cancer. This study had 2 objectives:

- To test whether panels of 3 or 4 serum biomarkers—Apolipoprotein A1 (Apo A1), transthyretin (TTR), and transferrin (TF) or Apo A1, TTR, TF and...
CA-125 can detect early-stage endometrioid and serous ovarian cancers.

To evaluate whether diluted serum samples, when serum sample size is limited, retain adequate sensitivity and specificity.

Massad: What study design was employed to meet this objective?

Parks: First, in order to evaluate the ability of the panels to screen for early ovarian cancer, the authors looked at Apo A1, TTR, and TF, with and without CA-125 levels. They obtained serum samples from the Gynecologic Oncology Group (GOG) Tissue Bank. These came from healthy controls and patients with benign or malignant pelvic masses. Levels of the 4 protein markers were measured. Then, a multiple logistic regression model was built to predict the occurrence of normal results vs benign growths, early-stage ovarian cancer, and late-stage ovarian cancer. Next, they calculated the sensitivity and specificity of these tests for Apo A1, TTR, and TF vs those 3 plus CA-125. Finally, they calculated the sensitivity and specificity data separately for serous and endometrioid ovarian tumors as compared to all tumors.

The second objective was to determine if representative serum marker values could be obtained from diluted serum samples. To evaluate the efficacy of these dilutions, they looked at a subset of 20 serum samples at 1:10 and 1:5 dilutions. The 1:5 dilutions had high correlations to the nondiluted samples and did not show any significant loss of percent recovered. Therefore, in their main study of 358 samples, they used a 1:5 dilution.

Massad: How were candidate markers selected?

Kizer: The authors of the paper had previously analyzed protein profiles using surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy (SELDI-TOF-MS) and successfully identified 3 differentially expressed serum proteins for the detection of ovarian cancer: Apo A1, TTR, and TF. They demonstrated in a previous publication that these biomarkers allowed researchers to distinguish normal samples from tumors of low malignant potential (LMP) with 91% sensitivity and normal samples from early-stage ovarian cancer with a sensitivity of 89%. Collectively, Apo A1, TTR and TF (excluding CA-125) could be used to distinguish normal samples from early-stage ovarian cancer with a sensitivity of 84% and normal samples from late-stage ovarian cancer with a sensitivity of 97%. However, these biomarkers had not been previously evaluated in patients with other types of early ovarian cancer histologies, and thus, they were chosen for this study.

Massad: How were patient samples selected?

Kizer: As noted, serum samples were obtained from the GOG Tissue Bank. These were collected preoperatively from patients with benign or malignant pelvic masses. Serum samples from healthy controls were also included in the study. All specimens were obtained and processed according to GOG Tissue Bank serum standard operating procedures. Ultimately, 358 samples were randomly selected from 1680 possible specimens with adequate volume. Four categories of patients were included in the analysis: healthy controls, patients with benign adnexal masses (with exclusion of LMP tumors), patients with early-stage ovarian cancer (stages I and II), and patients with late stages (III and IV) of ovarian cancer. Four hundred samples were available for initial analysis; however, 22 of them lacked complete information regarding surgical stage or pathology. The data on these 22 specimens were considered incomplete, and therefore, these samples were excluded from the analysis. Additionally, 20 tumors of LMP were excluded from the analysis. The distribution of the remaining 358 specimens was as follows: 93 represented normal controls, 79 samples came from patients with benign ovarian conditions, 90 samples were from patients with early-stage ovarian cancer, and 96 samples were from patients with late-stage ovarian cancer.

Massad: What outcome was assessed?

Rimel: The outcome assessed was the presence or absence of ovarian cancer in patients with a positive screen.

Massad: How is this outcome determined in clinical practice?

Rimel: In clinical practice, pathologic review of tissue from either biopsy or oophorectomy is the only way to definitively determine the presence or absence of malignant tissue.

Massad: What are the clinical implications of selecting these markers?

Rimel: Since surgery has risks but is required for definitive diagnosis of ovarian cancer, the screening test must meet the high bar of being both very sensitive and very specific to reduce the number of unnecessary procedures.

Massad: What statistical approaches were applied?

Rimel: Descriptive statistics were reported for each of the markers. Multiple logistic regression models were used to evaluate the ability of the markers to discriminate between normal and disease states. Sensitivity, specificity, and area under the receiver operator curve were also calculated.

Massad: Were these statistics appropriate?

Rimel: These statistical tests were appropriate for individual and combination evaluation of the biomarkers to arrive at the most precise cut-off points for each and to define the most useful algorithm for differentiation between normal and early-stage malignancy.

Massad: Were additional analyses needed to determine clinical relevance?

Rimel: Yes. I believe that calculating the number needed to treat to a more rational and cost-effective range.

Conclusions

Massad: What are the clinical implications of this paper?

Parks: Currently, there is not a good screening test for ovarian cancer, which is the eighth most common cancer in women. Many tests have been looked at but found to be lacking in sensitivity,
specificity, or both. This becomes even more difficult as ovarian cancer is so rare in the general population, with an estimated prevalence of 0.04%. In this paper, the authors identified and tested the sensitivity and specificity of 3 serum tumor markers—in a 3-marker panel and in a 4-marker panel that included CA-125—to aid recognition of early ovarian cancer. The 4-marker panel was superior, identifying early-stage ovarian cancer with a sensitivity and specificity of 96%; late-stage malignancies were detected with a sensitivity and specificity of 98%. The 4-marker panel recognized serous tumors with a sensitivity and specificity of 94%; identification of endometrioid tumors was associated with a sensitivity and specificity of 98%.

To date, 96% is the highest sensitivity recorded for the early detection of ovarian cancer. However, because ovarian cancer is so rare, a sensitivity and specificity of 96% would only detect 1 case of cancer per 100 oophorectomies done for positive screens due to the high false-positive rate. While these results are promising, the risk vs benefit of unnecessary oophorectomies is very concerning. Future research needs to be conducted to find a tumor marker panel that has an even higher sensitivity and specificity for the early detection of ovarian cancer.

REFERENCES

Progress and Challenges in Screening for Early Detection of Ovarian Cancer*

Ian J. Jacobs‡ and Usha Menon

Ovarian cancer is characterized by few early symptoms, presentation at an advanced stage, and poor survival. As a result, it is the most frequent cause of death from gynecological cancer. During the last decade, a research effort has been directed toward improving outcomes for ovarian cancer by screening for preclinical, early stage disease using both imaging techniques and serum markers. Numerous biomarkers have shown potential in samples from clinically diagnosed ovarian cancer patients, but few have been thoroughly assessed in preclinical disease and screening. The most thoroughly investigated biomarker in ovarian cancer screening is CA125. Prospective studies have demonstrated that both CA125 and transvaginal ultrasound can detect a significant proportion of preclinical ovarian cancers, and refinements in interpretation of results have improved sensitivity and reduced the false-positive rate of screening. There is preliminary evidence that screening can improve survival, but the impact of screening on mortality from ovarian cancer is still unclear. Prospective studies of screening are in progress in both the general population and high-risk population, including the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), a randomized trial involving 200,000 postmenopausal women designed to document the impact of screening on mortality. Recent advances in technology for the study of the serum proteome offer exciting opportunities for the identification of novel biomarkers or patterns of markers that will have greater sensitivity and lead time for preclinical disease than CA125. Considerable interest and controversy has been generated by initial results utilizing surface-enhanced laser desorption/ionization (SELDI) in ovarian cancer. There are challenging issues related to the design of studies to evaluate SELDI and other proteomic technology, as well as the reproducibility, sensitivity, and specificity of this new technology. Large serum banks such as that assembled in UKCTOCS, which contain preclinical samples from patients who later developed ovarian cancer and other disorders, provide a unique resource for carefully designed studies of proteomic technology. There is a sound basis for optimism that further developments in serum proteomic analysis will provide powerful methods for screening in ovarian cancer and many other diseases. Molecular & Cellular Proteomics 3:355–366, 2004.

THE RATIONALE OF OVARIAN CANCER SCREENING

Ovarian cancer is the most frequent cause of death from gynecological cancer and the fourth most frequent cause of death from cancer in women in Europe and the United States. Most ovarian cancers occur after menopause when the ovaries have no physiological role and consequently abnormal ovarian function causes no symptoms. As a result of this factor, combined with the anatomical location of the ovaries deep in the pelvis, ovarian cancers typically cause few symptoms until they reach a large size or have disseminated. As a result, ovarian cancer is usually diagnosed at an advanced stage when despite advances in surgical and chemotherapeutic management during the last decade survival rates are poor. Almost 90% of patients are diagnosed with metastatic disease in the pelvis or abdomen and for these patients 5-year survival rates are less than 30%. In contrast, the small proportion of patients diagnosed with stage I ovarian cancer confined to the ovaries have a 5-year survival rate in excess of 90%.

A premalignant precursor lesion for ovarian cancer has not been identified, limiting the focus of screening at present to detection of asymptomatic, early stage disease (1). The relationship between stage at presentation and survival in ovarian cancer has long provided a rationale for efforts to improve outcome by detection of early stage disease. Ovarian cancer satisfies many of the World Health Organization criteria (2) for population screening. However, it remains uncertain whether the currently available screening tests can detect ovarian cancer sufficiently early to allow intervention to alter the natural history of the disease. A major effort has been made during the last 20 years to evaluate the tumor marker CA125 and ultrasound scanning in screening for ovarian cancer. Considerable success has been achieved in refining these tests, and large prospective trials are currently in progress to assess the impact of general population screening. Recent progress in serum proteomic analysis has generated much interest in the prospect of novel and sensitive combinations of serum protein markers.

THE CHALLENGE OF OVARIAN CANCER SCREENING

The consequence of a positive screening test for ovarian cancer is surgical intervention of some kind (either laparoscopy or laparotomy). Although ovarian cancer is an important cause of mortality, it is still a relatively uncommon disease, with an incidence no greater than 40 per 100,000 per year even in the postmenopausal population. There is therefore a
screening at Bart forms part of the multimodal screening strategy in the recently preclinical detection of ovarian cancer (68). This approach levels are not classified as low risk. For a target specificity of specificity is improved as women with static but elevated are identified as being at increased risk. At the same time, cut-off value because women with normal but rising values ovarian cancer (ROC) (67, 68) (Table II). The ROC algorithm ovarian cancer and serial CA125 profile to estimate her risk of ovarian cancer and her CA125 profile with time (24, 25).

The ROC for an individual is calculated using a computerized algorithm based on the Bayes theorem, which compares each individuals serial CA125 levels to the pattern in cases compared to controls.

The closer the CA125 profile to the CA125 behavior of known cases of ovarian cancer, the greater the risk of ovarian cancer. The final result is presented as the individual’s estimated risk of having ovarian cancer so that a ROC of 2% implies a risk of 1 in 50.

| TABLE II |
| Risk of ovarian cancer algorithm |

- Detailed analysis of over 5,000 serum CA125 values involving 22,000 volunteers followed up for a median of 8.6 years in the study by Jacobs et al. (6, 20) revealed that CA125 levels in women without ovarian cancer were static or decreased with time while preclinical levels associated with malignancy tended to rise.
- This allowed the formulation of separate complex change-point statistical models of the behavior of serial preclinical CA125 levels for cases and controls. These models take into account a woman’s age-related risk of ovarian cancer and her CA125 profile with time (24, 25).
- The ROC for an individual is calculated using a computerized algorithm based on the Bayes theorem, which compares each individuals serial CA125 levels to the pattern in cases compared to controls.
- The closer the CA125 profile to the CA125 behavior of known cases of ovarian cancer, the greater the risk of ovarian cancer. The final result is presented as the individual’s estimated risk of having ovarian cancer so that a ROC of 2% implies a risk of 1 in 50.

phisticated approach to interpretation of CA125 results than a fixed absolute cut-off level. It was observed that elevated CA125 levels in women without ovarian cancer were static or decreased with time while levels associated with malignancy tended to rise. This finding has been incorporated into an algorithm that uses an individual’s age-specific incidence of ovarian cancer and serial CA125 profile to estimate her risk of ovarian cancer (ROC) (67, 68) (Table II). The ROC algorithm increases the sensitivity of CA125 compared with a single cut-off value because women with normal but rising values are identified as being at increased risk. At the same time, specificity is improved as women with static but elevated levels are not classified as low risk. For a target specificity of 98%, the ROC calculation achieved a sensitivity of 86% for preclinical detection of ovarian cancer (68). This approach forms part of the multimodal screening strategy in the recently completed pilot randomized control trial of ovarian cancer screening at Bart’s London and is part of the ongoing United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS; www.ukctocs.org.uk). The ROC algorithm is also being evaluated prospectively in a pilot ovarian cancer screening trial in “high-risk” women under the auspices of the Cancer Genetics Network in the United States.

OVARian CANcer SCREENing TRIALS IN PROGRESS

Two distinct screening strategies have emerged, one ultrasound based and the other based on measurement of the serum tumor marker CA125 with ultrasound as the secondary test (multimodal screening). Overall, the data from large prospective studies of screening for ovarian cancer in the general population (Table III) suggests that sequential multimodal screening has superior specificity and PPV compared with strategies based on transvaginal ultrasound alone. However, ultrasound as a first-line test may offer greater sensitivity for early stage disease.

Trials in the General Population—Randomized controlled trials are now underway in the general population to assess the impact of screening on ovarian cancer mortality. The UKCTOCS has recruited over 120,000 postmenopausal women from 13 centers in the United Kingdom. A total of 200,000 women in all will be randomized to either control, screening with ultrasound, or multimodal screening. The primary endpoint is impact of screening on ovarian cancer mortality. The study also addresses the issues of target population, compliance, health economics, and physical and psychological morbidity of screening. Results are expected in 10 years (www.ukctocs.org.uk). The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial has completed enrolling 74,000 women aged 55–74 at 10 screening centers in the United States with balanced randomization to intervention and control arms. For ovarian cancer, women are screened using both CA125 and transvaginal ultrasound for 3 years and CA125 alone for a further 2 years. Follow-up will continue for at least 13 years from randomization to assess health status and cause of death (78).

Trials in the High-Risk Population—Screening this population can be problematic as they are mainly premenopausal women who have a variety of both physiological (e.g. menstrual cycle variations) and benign conditions (e.g. endometriosis, ovarian cysts) that can give rise to false-positive abnormalities on ultrasound and CA125. Hence criteria for interpretation of the screening tests need to be different from that developed for postmenopausal women in the general population. To date, nine prospective studies have reported on screening for familial ovarian cancer (Table IV). Over 5,000 women have been screened and 33 primary invasive epithelial ovarian and peritoneal cancers detected using mainly ultrasound and CA125 as first-line tests. Criteria for interpreting the test results vary, and screening protocols are not always clearly reported. Only three of the studies have reported interval cancers, which presented between 2 and 24 months following the last screen (79, 86, 88). Multifocal peritoneal serous papillary carcinoma may be a phenotypic variant of familial ovarian cancer and screening strategies using ultrasonography and CA125 testing are not reliable in detecting this disease (86, 90). The other option for these women at high risk is risk-reducing salpingo-oophorectomy after completion of their families (91, 92). In order to develop an optimal screening strategy in the high-risk population, a multicenter National Familial Ovarian Cancer Screening Study (UKFOCSS) has started recruiting “high-risk” women in the United Kingdom. This is a prospective study based on annual screening with CA125 measurement and transvaginal ultrasound. The trial design includes collecting and storing serial serum samples every 4 months for retrospective analysis of CA125 and other markers (93). A similar trial is underway in the United States under the auspices of the Cancer Genetics Network of the National Cancer Institute with the scope for meta-analysis in the future. In the U.S. trial, screening is
Finding the right biomarkers for cancer is a little like looking for love. You could waste a lot of time on worthless contenders. The quest can seem endless, and you may have to reconsider candidates you rejected the first time around. People you respect may strongly disagree with your choice once you find one you like. And even after making a commitment, there’s still no guarantee that what you’ve found is the real thing. Worst-case-scenario: You’re looking for Mr. Goodbar.

Few are willing to call off the search in either love or medicine, though—the potential reward is just too magnificent. That’s one of the reasons for the excitement over the discovery of three potential biomarkers for detecting early-stage ovarian cancer (Cancer Res. 2004;64: 5882-5890).

The biomarkers, identified from serum proteomic analysis, are apolipoprotein A1 (down-regulated in cancer), a truncated form of transthyretin (also down-regulated), and a cleavage fragment of inter-XXX-trypsin inhibitor heavy chain H4 (up-regulated). Their discovery “is an excellent step toward identifying biomarkers for early detection of ovarian cancer,” says Steven Skates, PhD, assistant professor of medicine, Harvard Medical School, and a biostatistician at Massachusetts General Hospital, whose own ovarian cancer research has led to the development of an algorithm for using longitudinal measurements of the CA 125 biomarker.

But just as exciting—perhaps even more so—is the means by which the biomarkers were discovered. The study was designed to take into account confounding variables that many previous studies may have overlooked, say the study’s authors, making it a more sophisticated approach to conducting proteomics-based biomarker research.

The markers emerged from a five-center case-control study, which was designed to contain internal as well as external validation based on the origin of samples. This is important for several reasons, say the researchers.

Previous proteomic studies have pointed to "an unfortunate fact of life," says Eric T. Fung, MD, PhD, vice president of clinical affairs for the diagnostics division of Ciphergen Biosystems Inc., Fremont, Calif., and one of the study’s coauthors. Studies in which samples are obtained from a single site make it possible for researchers to obtain results that suggest very high levels of accuracy. But like a great first date, they can be hard to replicate later on. "When these results are attempted to be validated on a sample obtained from a different hospital, they are generally unsuccessful," says Dr. Fung.

First, there may be demographic and epidemiological differences between hospitals. Each institution may also have its own protocols for sample processing, and those differences, no matter how subtle, can be reflected in proteomic profiles. Finally, says Dr. Fung, the power of bioinformatics and biostatistics as applied to the complex data generated by proteomics platforms makes it possible "to create what we call 'overfit solutions'”—that is, mathematical solutions that are tailored to the data that are given."
Finding the right biomarkers for cancer is a little like looking for love. You could waste a lot of time on worthless contenders. The quest can seem endless, and you may have to reconsider candidates you rejected the first time around. People you respect may strongly disagree with your choice once you find one you like. And even after making a commitment, there’s still no guarantee that what you’ve found is the real thing. Worst-case-scenario: You’re looking for Mr. Goodbar.

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Editorial

Ovarian cancer: role of ultrasound in preoperative diagnosis and population screening

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Four papers in this issue of the Journal are concerned with the role of ultrasound in the diagnosis and early detection of ovarian cancer. Three of them1–3 address the problem of the accurate diagnosis of ovarian cancer in women who have a presumed ovarian mass identified by ultrasound. Since the seminal paper by Granberg et al.4 on morphological characterization of ovarian cysts by transvaginal scanning there has been an explosion of interest in this subject, with ultrasound algorithms based on morphological indices and Doppler being produced on an industrial scale. Geomini et al.5 reviewed 109 studies on 83 different prediction models but did not include in their analysis any of the well-known International Ovarian Tumor Analysis (IOTA) studies which have evaluated a further 11 logistic regression models in 28 papers. There have also been models developed in non-English language journals6 that have somehow crept under the radar. Not to mention the vast hinterland of literature concerned with modifying, evaluating and comparing these algorithms in different populations and subsets of these populations. This Editorial is an attempt to put our three new papers into some kind of context and also to address the question as to where we go from here.

Running parallel with diagnostic studies, there have been numerous papers addressing the role of ultrasound in detecting ovarian cancer in an unselected population of women, and the fourth paper in this issue7 analyzes the risk of malignancy in masses detected during an ovarian cancer screening program in a general population of women over the age of 50 years. The aim of these screening studies is to detect ovarian cancer at an early treatable stage and reduce mortality, but, as ovarian cysts are common in postmenopausal women, ultrasound has a dual role in detecting ovarian cysts and then making an accurate diagnosis of malignancy in these cysts. Population screening studies began in the early 1980s with programs based on abdominal scanning8 and, as there is now a large amount of data on screening by transvaginal ultrasound in healthy volunteers over the age of 50, it is appropriate at this juncture to try to evaluate the role of ultrasound in early cancer detection.

The Problem

Ovarian cancer is one of the greatest health problems in gynecology. In developed countries it is the most common genital tract malignancy, with women having a 1–2% lifetime risk of developing the disease. It is also the most lethal gynecological malignancy, with an overall 5-year survival of 45%10. For example, in the United States approximately 21,550 women develop ovarian cancer each year and 14,600 women die from the disease. In Europe, the corresponding figures are 66,700 and 41,900, respectively11. Over 90% of ovarian cancers are sporadic and occur in the general population, mainly in women over 50 years of age. Familial predisposition has been described in 5–10% of a younger subset of women who develop ovarian cancer and most of these cases are associated with mutations in the BRCA1, BRCA2 and MMR genes12,13. Between 80 and 85% of cancers are epithelial in origin (EOC), the most common histological subtype being serous ovarian cancer, which usually presents at advanced stages and has the poorest outcome14. Ovarian cancer presents late as early symptoms are often vague and the condition is usually first identified as abdominal distension, a feeling of bloatedness15 or as an abdominal mass. Sixty per cent of women are diagnosed at an advanced stage, which has a 5-year survival as low as 10%. When the disease is diagnosed at Stage 1 (i.e. confined to the ovaries), the 5-year survival is in excess of 90%16. This forms the rationale for ovarian cancer screening programs, the premise being that early detection may affect long-term survival.

Recent studies on the origin and pathogenesis of ovarian cancer may have implications for the screening and diagnosis of this condition. EOC presents as a heterogeneous group of tumors that can be classified on a morphologic and molecular genetic basis into two types. Type I are slow-growing cancers with good prognosis, such as low-grade serous, low-grade endometrioid, clear cell, mucinous and Brenner carcinomas and borderline tumors. They are easily detected by pelvic examination and/or transvaginal ultrasound; however, they constitute only 25% of ovarian cancers and account for approximately 10% of ovarian cancer deaths. Type-II tumors are more aggressive and include high-grade serous, high-grade endometrioid and undifferentiated tumors and carcinosarcomas. Type-II tumors represent approximately 75% of all ovarian carcinomas and are responsible for 90% of ovarian cancer deaths. They are more difficult to detect due to their rapid growth and dissemination. They display p53 mutations in over 80% of cases and rarely harbor the mutations that are found in the Type I-tumors. Recent advances in our understanding of the cell of origin of ovarian cancer may help us to explain the biological differences between Type-I and Type-II cancers.
had a low sensitivity for Stage 1 disease\textsuperscript{39}. In order to improve sensitivity, Skates \textit{et al.}\textsuperscript{51} introduced a more sophisticated approach by rejecting a fixed cut-off CA 125 level and taking into account the serial values that are available in the screening context. They demonstrated that elevated CA 125 levels in women without ovarian cancer had a flat or static profile or decreased with time, whereas levels associated with malignancy tended to rise. This led to the development of the ROC algorithm which estimates a woman’s risk of ovarian cancer based on the rise in CA 125 and allows women to be triaged into low-, intermediate- or high-risk categories. It is important to realize that, for example, a rise in value from 8 to 16 U/mL (i.e. a value which would usually be regarded as normal) over a period of 3 months could put a woman in the high-risk category. Jacobs \textit{et al.}\textsuperscript{60} then introduced the ROC algorithm into a randomized controlled screening study using transvaginal ultrasound to visualize the ovaries of women in the high-risk group in order to improve specificity. This was called multimodal screening. The trial of 22,000 postmenopausal women showed a significantly increased median survival in women who developed ovarian cancer in the screened group compared with the control group. These results prompted the UKCTOCS multicenter trial which is discussed below.

Recent studies

There are now four large ongoing or recently completed trials on ovarian cancer screening by means of transvaginal scanning and CA 125 that have published data in the last decade:

1. The University of Kentucky ovarian cancer screening trial\textsuperscript{56} is a single-arm (i.e. uncontrolled) annual ultrasound screening study of 25,327 volunteers over a period of 9 years, in which 120,569 scans (mean, 4.8 per participant) were performed. An ovarian volume $> 20$ mL (premenopause) or $> 10$ mL (postmenopause) or any cystic ovarian tumor with a solid or papillary projection into its lumen was considered abnormal. The mean age of the cohort was 55 years. The reported sensitivity for primary EOC was 81%, with 9.3 operations carried out per case detected. When restricted to primary invasive ovarian cancer, the sensitivity decreased to 76.3%. Most (82%) of the primary ovarian cancers were early stage (Stage I/II). Serum CA 125 levels were increased ($> 35$ U/mL) at the time of detection in 13 of 15 (87%) patients who had Stage 3 EOC but in only three of 15 (20%) patients who had Stage 1 or 2 disease. At a mean follow up of 5.8 years, the women in the trial had a significantly longer 5-year survival (74.8 $\pm$ 6.6%) compared to the women from the same institution, treated by the same surgical and chemotherapeutic protocols, who were not screened ($53.7 \pm 2.3\%$)\textsuperscript{61}.

2. The Japanese Shizuoka Cohort Study of Ovarian Cancer Screening\textsuperscript{54} is a randomized controlled trial of 82,487 low-risk postmenopausal women from 212 hospitals in 35 townships carried out over a 15-year period. Women with a median age of 58 years were screened by annual transvaginal ultrasound exam and CA 125 using a cut-off of 35 U/mL. The mean number of screens per woman was 5.4; the uptake of screening fell from 82% to 56% from the second to the fifth screen. Abnormal ovarian morphology was classified as simple cyst (single, thin walled, anechoic cyst with no septa or papillary projections) or complex cyst (abnormal ovarian morphology other than simple cyst). The screening strategy achieved a sensitivity for malignancy of 77.1% and a specificity of 99.9%. The proportion of Stage-1 ovarian cancer was higher in the screened group (63%) than in the control group (38%) but the difference was not statistically significant. The effect on mortality has not yet been reported.

3. The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO)\textsuperscript{57}. This is a randomized controlled trial of 78,216 women aged 55 to 74 years assigned to undergo either annual screening or usual care at 10 screening centers across the United States between November 1993 and July 2001. Women were screened by serum CA 125, using a cut-off of 35 U/mL, and transvaginal ultrasonography for 3 years, followed by CA 125 alone for a further 2 years. The following transvaginal ultrasound results were classified as abnormal: (1) ovarian volume greater than 10 mL; (2) cyst volume greater than 10 mL; (3) any solid area or papillary projection extending into the cavity of a cystic ovarian tumor of any size; and (4) any mixed (solid and cystic) component within a cystic ovarian tumor. Evaluation and management of positive screening tests was at the discretion of the participant’s clinician. Women were followed up for a median of 12.4 years. During four rounds of incidence screening\textsuperscript{62}, 89 invasive ovarian or peritoneal cancers were diagnosed, of which 60 were detected by screening (sensitivity of 68.2%), with 13 surgeries carried out per case of ovarian cancer. A total of 72% of the screen-detected cancers were late stage (Stage 3/4). Recently, mortality data have been reported\textsuperscript{57}. A total of 212 women had a screen-detected cancer in the intervention arm and 176 were identified in the control arm. The screening and control arms included 118 and 100 deaths, respectively, with a mortality rate ratio of 1.18. These data showed that simultaneous screening with CA 125 using an absolute cut-off and transvaginal scanning did not reduce mortality from the disease. Moreover, the excess morbidity of carrying out surgery in women with false-positive results was 5.1%.

4. The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS)\textsuperscript{63}. In this trial, 202,638 postmenopausal women aged 50–74 years were randomized to either control or annual screening with ultrasound or a multimodal strategy in a 2:1:1 fashion. In the multimodal group, CA 125 was interpreted using the ROC algorithm to triage the
Validation of Candidate Serum Ovarian Cancer Biomarkers for Early Detection

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Abstract

Objective: We have previously analyzed protein profiles using Surface Enhanced Laser Desorption and Ionization Time-Of-Flight Mass Spectroscopy (SELDI-TOF-MS) [Kozak et al. 2003, Proc. Natl. Acad. Sci. U.S.A. 100:12343–8] and identified 3 differentially expressed serum proteins for the diagnosis of ovarian cancer (OC) [Kozak et al. 2005, Proteomics, 5:4589–96], namely, apolipoprotein A-I (apoA-I), transthyretin (TTR) and transferin (TF). The objective of the present study is to determine the efficacy of the three OC biomarkers for the detection of early stage (ES) OC, in direct comparison to CA125.

Methods: The levels of CA125, apoA-I, TTR and TF were measured in 392 serum samples [82 women with normal ovaries (N), 24 women with benign ovarian tumors (B), 85 women with ovarian tumors of low malignant potential (LMP), 126 women with early stage ovarian cancer (ESOC), and 75 women with late stage ovarian cancer (LSOC)], obtained through the GOG and Cooperative Human Tissue Network. Following statistical analysis, multivariate regression models were built to evaluate the utility of the three OC markers in early detection.

Results: Multiple logistic regression models (MLRM) utilizing all biomarker values (CA125, TTR, TF and apoA-I) from all histological subtypes (serous, mucinous, and endometrioid adenocarcinoma) distinguished normal samples from LMP with 91% sensitivity (specificity 92%), and normal samples from ESOC with a sensitivity of 89% (specificity 92%). MLRM, utilizing values of all four markers from only the mucinous histological subtype showed that collectively, CA125, TTR, TF and apoA-I, were able to distinguish normal samples from mucinous LMP with 90% sensitivity, and further distinguished normal samples from early stage mucinous ovarian cancer with a sensitivity of 95%. In contrast, in serum samples from patients with mucinous tumors, CA125 alone was able to distinguish normal samples from LMP and early stage ovarian cancer with a sensitivity of only 46% and 47%, respectively. Furthermore, collectively, apoA-I, TTR and TF (excluding CA-125) distinguished i) normal samples from samples representing all histopathologic subtypes of LMP, with a sensitivity of 73%, ii) normal samples from ESOC with a sensitivity of 84% and iii) normal samples from LSOC with a sensitivity of 97%. More strikingly, the sensitivity in distinguishing normal versus mucinous ESOC, utilizing apoA-I, TF and TTR (CA-125 excluded), was 95% (specificity 86%; AUC 95%).

Conclusions: These results suggest that the biomarker panel consisting of apoA-I, TTR and TF may significantly improve early detection of OC.

Keywords: Ovarian cancer, Serum biomarker, Serous, Mucinous

Introduction

Ovarian cancer has the highest mortality rate of all the gynecologic malignancies worldwide. With no adequate screening tests, early diagnosis—the most significant prognostic factor—continues to elude the clinician. Presently, over 85% of patients with ovarian cancer are diagnosed with Stage III or IV disease [1].

Serum cancer antigen 125 (CA125), a high molecular weight glycoprotein, is currently the best clinical marker for papillary serous adenocarcinoma of the ovary in the postmenopausal age group.
However it is a consistently poor diagnostic tumor biomarker in premenopausal women, non-serous histologies, and early stage diseases. Only 50%-60% of women with early stage ovarian cancer will demonstrate elevated serum levels of CA125 [2]. Falsely elevated levels are common in a number of benign conditions such as pregnancy, uterine fibroids, or intra-abdominal infections and other intraperitoneal pathology [3]. The identification of more sensitive and specific biomarkers for the early detection of ovarian cancer would clearly be immediately beneficial.

Proteomic-based approaches have been utilized in an attempt to detect early-stage ovarian cancer patients, and monitor biologic responses to therapy [4], [5]. Serum protein profiling at different stages in disease progression, or along the course of therapy, offers a new paradigm for detecting and treating ovarian cancer [6–10]. We have previously analyzed protein profiles using Surface Enhanced Laser Desorption and Ionization Time-Of-Flight Mass Spectroscopy (SELDI-TOF-MS) and identified 3 differentially expressed serum proteins for the detection of ovarian cancer [6], [7]. These were apoA-I, TTR, and TF. In the present study, we analyzed an additional 392 serum samples from patients obtained through the GOG and Cooperative Human Tissue Network for the levels of markers that included CA125, in addition to the previously described markers.

### Materials and Methods

Serum samples were obtained through the Gynecological Oncology Group (GOG) and Cooperative Human Tissue Network. Samples were collected preoperatively following the standard GOG protocol (GOG 199 protocol) from patients with benign, borderline and malignant ovarian tumors. The 392 serum samples utilized in the present study included 82 women with normal ovaries (N), 24 women with benign ovarian tumors (B), 85 women with ovarian tumors of low malignant potential (LMP), 126 women with early stage ovarian cancer (ESOC), and 75 women with late stage ovarian cancer (LSOC). The age and pathology distribution of the samples are provided in Table 1.

The levels of each individual protein marker (CA125, apoA-I, TTR, TF) were measured on all serum samples. The Immulite 1000 was used to measure CA125 level by using chemiluminescence technology and the Hitachi 912 was used to measure apoA-I, TTR and TF levels based on immunoturbimetry technology. The reagents were purchased from Diagnostics Product Corporation and Roche. A separate dataset was compiled for external-validation purposes from serum collected from patients with breast cancer, colon cancer and atherosclerosis.

Statistical analysis of the levels of each of the individual markers (apoA-I, TTR, TF, and CA125) was performed using the Kruskal-Wallis non-parametric rank sum test and Mann-Whitney U tests to compare marker levels across ovarian cancer stage. Multivariate logistic regression models (MLRM) were built to predict N vs. low malignant potential (LMP) and N vs. ESOC and LSOC. Model prediction ‘cut-points’ were also determined by maximizing specificity and sensitivity with equal weight. We then compared MLRM sensitivity, specificity and area under the receiver operator curve (AUC). AUC is a cut-point independent measure of predictive value.

Age-matched (51.5 ± 7.5) sera from a separate dataset that included normals, patients with early stage ovarian cancer, breast and colon cancers, and atherosclerosis were then standardized based on the normals in each dataset, assuming a scalar multiplier for each type of measurement (CA125, apoA-I, TF, and TTR). To compute the standardization, multipliers and perform multivariate statistical tests,

<table>
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<tr>
<th>Diagnostic group</th>
<th>Number</th>
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<th>SD</th>
<th>Median</th>
<th>Clear cell</th>
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<td>Endometrioid</td>
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<tr>
<td>N</td>
<td>82</td>
<td>42.5</td>
<td>10.7</td>
<td>43</td>
<td></td>
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<td>53.0</td>
<td>18.6</td>
<td>50</td>
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<td>59.1</td>
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To further validate the disease-specificity of the three biomarkers, we examined serum levels for apoA-I, TTR and TF in 71 additional subjects that included normal (18), breast cancer (18), colon cancer (8), atherosclerosis (9), and early stage OC (18) (Fig. 2). Multivariate comparison of apoA-I, TF and TTR demonstrate notable differences between diseases (Fig. 2). Using the MLRM constructed to make predictions on these independent data resulted in the ROC curve (Fig. 3), and demonstrated a specificity of 92%, sensitivity of 94% and AUC of 0.98.

**Discussion**

The majority of patients with ovarian cancer are diagnosed with Stage III or IV disease. Unfortunately, there are no adequate screening tests for the early detection of ovarian cancer and as a result, the diagnosis of ovarian cancer eludes the clinician. Not surprisingly, ovarian cancer is associated with the highest mortality rate among gynecologic malignancies. [1].

Serum cancer antigen 125 (CA125), a high molecular weight glycoprotein, is currently the best clinical marker for papillary serous adenocarcinoma of the ovary in the postmenopausal age group. However it is a consistently poor diagnostic tumor biomarker in premenopausal women, non-serous histologies, and early stage diseases. Only 50%–60% of women with early stage ovarian cancer will demonstrate elevated serum levels of CA125 [2]. Falsely elevated levels are common in a number of benign conditions such as pregnancy,
Validation of candidate serum ovarian cancer biomarkers for early detections

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uterine fibroids, or intra-abdominal infections and other intraperitoneal pathology [3]. The identification of more sensitive and specific biomarkers for the early detection of ovarian cancer would clearly be immediately beneficial.

Since CA125 is the gold standard biomarker for ovarian cancer, we measured CA125 levels in all the study samples. CA125 levels alone distinguished N from LMP with a sensitivity of 62% and N from ESOC with a sensitivity of 76% (Table 2). Furthermore, when the mucinous subsets were analyzed, CA125 levels distinguished N from LMP and ESOC with a sensitivity of 46% and 47% respectively (Table 2). These numbers are in agreement with previously reported data for CA125 [12]. As one of the goals of this study was to test the efficacy of the three biomarkers we recently identified for the detection of OC, we examined whether the three markers, apoA-I, TTR and TF could improve upon the CA125 based measurements. Using all the four markers (apoA-I, TTR, TF and CA125) and all of the 392 samples we analyzed for this study, we observed a 29% improvement in sensitivity for the detection of LMP, and a 13% improvement in sensitivity for the detection of ESOC (Table 3). More importantly, the four markers collectively improved the detection of LMP and ESOC of the mucinous subtype by 44% and 48%, respectively, compared to normal subjects (Table 3). These results warrant further studies to evaluate the new biomarkers in the early detection of OC.

Interestingly, there exists a link between OC and each of the three biomarkers, apoA-I, TTR and TF [13], [14], [15]. ApoA-I (28 kDa) is the major protein constituent of high density lipoprotein. Decreased apoA-I levels were previously reported in the serum of patients with both ovarian cancer [13], [14], [15] as well as atherosclerosis [16]. Serum lipid and lipoprotein association with cancer has been reported in numerous studies [17], [18], [19]. The mechanism of this association remains unclear at this time, however it has been proposed to be associated with free radical-mediated damage to cellular biomembranes resulting in lipid peroxidation. Malondiadehyde (MDA) is a byproduct of lipid degradation. MDA-DNA adducts appear to be promutagenic, inducing mutations in oncogenes and tumor suppressor genes seen in human tumors [20]. TTR (13.9 kDa) is a secreted protein that functions as a binding protein to transport serum thyroxine, tri-iodothyronine and retinol (vitamin A). TTR levels have

Table 4. Multivariate logistic regression models using apoA-I, TF and TTR (CA-125 excluded), for either all histopathologic subtypes or for mucinous subtype alone from 82 normal samples.

<table>
<thead>
<tr>
<th>Groups</th>
<th>All histological subtypes</th>
<th>Mucinous subtype</th>
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<tr>
<td></td>
<td>n</td>
<td>Specificity</td>
</tr>
<tr>
<td>N vs. LMP</td>
<td>85</td>
<td>0.83</td>
</tr>
<tr>
<td>N vs. ESOC</td>
<td>126</td>
<td>0.85</td>
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<tr>
<td>N vs. LSOC</td>
<td>75</td>
<td>0.86</td>
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Figure 2. The standardized comparisons of differential protein expression in serum across different diseases. Plotted values are 1 standard error. Using a robust MANOVA analysis, CA125, apoA-I, TTR and TF are significantly different only in early stage ovarian cancer from normal samples, all p-values < 0.0001.
Early Detection of Cancer: Immunoassays for Plasma Tumor Markers

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Abstract

Background—Plasma tumor biomarkers are widely used clinically for monitoring response to therapy and detecting cancer recurrence. However, only a limited number of them have been effectively used for the early detection of cancer.

Objective—To review plasma tumor markers used clinically for the early detection of cancer and to provide expert opinion about future directions.

Methods—Literature review, as well as our expert opinion, of plasma tumor markers that have been widely accepted for the early detection of cancer.

Results—In the United States, only prostate specific antigen (PSA), cancer antigen 125 (CA125), and alpha-fetoprotein (AFP) have been clinically used for the early detection of prostate, ovarian, and liver cancers, respectively. Both analytical and clinical issues related to the use of these three markers were discussed.

Conclusion—Few plasma tumor markers have been used effectively for the early detection of cancer, mainly due to their limited sensitivity and/or specificity. Multiple approaches have been developed to improve the clinical performance of tumor markers for the early detection of cancer. Metrological traceability and antibody specificity are important issues to ensure comparability of immunoassays for the measurement of plasma tumor markers.

Keywords
plasma; tumor marker; early detection; cancer

1. Introduction

Currently, one in four deaths in the United States is due to cancer[1]. Despite significant funding in cancer research, poor survival is common for advanced disease due to the lack of effective treatment options[2]. The 5-year relative survival rates among patients who are diagnosed with either advanced lung, colorectal, or breast cancer are only 3%, 10%, and 27%, respectively [1]. By contrast, survival is much better when cancers are diagnosed at an early stage. The 5-year relative survival rates among patients diagnosed with localized lung, colorectal or breast cancers are significantly higher at 50%, 90%, and 98%, respectively[1]. Based on these
method has been developed to identify autoantibody-based serum biomarkers for the early diagnosis of ovarian cancer[65].

Whether antigens or autoantibodies are used, a multiple marker strategy combines the merits of single markers and could result in both improved sensitivity and specificity over a single marker. Unfortunately, most early studies using multiple markers have improved sensitivity at the expense of a marked decrease in specificity. Recently, using appropriate statistical or bioinformatic methods, multiple marker strategies have improved sensitivity while maintaining specificity. One study by Zhang et al. showed the combination of four serum markers CA125II, CA72-4, CA15-3, and macrophage colony stimulating factor (M-CSF) through an Artificial Neural Network (ANN) model improved the overall accuracy to discern healthy women from patients with early stage ovarian cancer. At a fixed specificity of 98%, the sensitivities for ANN and CA125II alone were 71% (37/52) and 46% (24/52) (p=0.047), respectively, for detecting early stage epithelial ovarian cancer, and 71% (30/42) and 43% (18/42) (p=0.040), respectively, for detecting invasive early stage epithelial ovarian cancer [66]. In another study to improve the detection of early stage ovarian cancer, three proteomic biomarkers were identified as apolipoprotein A1 (down-regulated in cancer), a truncated form of transthyretin (down-regulated), and a cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4 (up-regulated). The sensitivity of a multivariate model combining the three biomarkers and CA125 was 74%, higher than that of CA125 alone of 65% at a matched specificity of 97%. When compared at a fixed sensitivity of 83%, the specificity of the model was significantly better than that of CA125 alone (94% versus 52%)[67].

6. Conclusion

Despite issues with sensitivity and/or specificity, PSA, CA125, and AFP have been used clinically for the early detection of prostate, ovarian, and liver cancer, respectively. Many strategies have been used to improve the sensitivity or specificity of these markers, including calculation of their changes over time, measurement of subfractions of these markers that are more cancer-specific, and combinations with other markers or imaging modalities. Immunoassays for these plasma tumor markers are commercially available. The results from these assays, however, are not interchangeable due to two fundamental principles of immunoassays: metrological traceability and antibody specificity. Therefore, standardization of these immunoassays will help to make the results more comparable. Identification of autoantibodies to tumor antigens and combinations of independent plasma tumor antigens are two promising future directions for the early detection of cancer.

7. Expert Opinion

Immunoassay of plasma tumor markers is important for two reasons. First, for the markers that have established clinical utility, immunoassays provide quantitative analysis of these markers in plasma and thus provide clinicians information for making medical decisions. Second, for candidate markers that need further validation, development of immunoassays is essential for establishing clinical performance of these markers. In fact, one limiting factor for many validation approaches is the lack of well-characterized, high-quality antibodies. Realizing this obstacle, an emerging partnership has been developed between the public and private sectors for development of high quality antibodies toward human proteins. Examples are the Human Antibody Initiative by the Human Proteome Organization (HUPO) and the Clinical Proteomic Technologies for Cancer program (CPTAC) by the U.S. National Cancer Institute (NCI). These noteworthy efforts will hopefully speed up the validation process and lead to more immunoassays that are potentially useful for early detection of cancer.
Three Biomarkers Identified from Serum Proteomic Analysis for the Detection of Early Stage Ovarian Cancer

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Abstract
Early detection remains the most promising approach to improve long-term survival of patients with ovarian cancer. In a five-center case-control study, serum proteomic expressions were analyzed on 153 patients with invasive epithelial ovarian cancer, 42 with other ovarian cancers, 166 with benign pelvic masses, and 142 healthy women. Data from patients with early stage ovarian cancer and healthy women at two centers were analyzed independently and the results cross-validated to discover potential biomarkers. The results were validated using the samples from two of the remaining centers. After protein identification, biomarkers for which an immunoassay was available were tested on samples from the fifth center, which included 41 healthy women, 41 patients with ovarian cancer, and 20 each with breast, colon, and prostate cancers. Three biomarkers were identified as follows: (a) apolipoprotein A1 (down-regulated in cancer); (b) a truncated form of transthyretin (down-regulated); and (c) a cleavage fragment of inter-alpha-trypsins inhibitor heavy chain H4 (up-regulated). In independent validation to detect early stage invasive epithelial ovarian cancer, from healthy controls, the sensitivity of a multivariate model combining the three biomarkers and CA125 [94% (95% CI, 52–90%)] was higher than that of CA125 alone [65% (95% CI, 43–84%)]) at a matched specificity of 97% (95% CI, 89–100%). When compared at a fixed sensitivity of 83% (95% CI, 61–95%), the specificity of the model [94% (95% CI, 85–98%)] was significantly better than that of CA125 alone [52% (95% CI, 39–65%)]. These biomarkers demonstrated the potential to improve the detection of early stage ovarian cancer.

Introduction
Despite progress in cancer therapy, ovarian cancer mortality has remained virtually unchanged over the past two decades (1). Annually in the United States alone, ~23,000 women are diagnosed with the disease and almost 14,000 women die from it (1). Given our knowledge about the steep survival gradient relative to the stage at which the disease is diagnosed, it is reasonable to suggest that early detection remains the most promising approach to improve the long-term survival of ovarian cancer patients.

The relatively low prevalence (40 out of 100,000) of ovarian cancer among postmenopausal women in the general population, the lack of a clearly defined precursor lesion, and the high cost and possible complications associated with surgical confirmatory procedures have placed stringent requirements on any test intended for general population screening. Currently, none of the existing serum markers, such as CA125, CA 72–4, or macrophage colony-stimulating factor, can be used individually for screening (2). Longitudinal studies are under way in Europe, Japan, and the United States to evaluate screening strategies using CA125 and/or transvaginal sonography (3–5) and their impact on overall cancer mortality (6). Preliminary results have shown encouraging evidence of a survival benefit among patients diagnosed through a screening regimen (3).

Reports from retrospective studies have shown that multivariate predictive models combining existing tumor markers improve cancer detection (7, 8). Recent advances in genomic and proteomic profiling technology have made it possible to apply computational methods to detect changes in protein expressions and their association to disease conditions, thereby hastening the identification of novel markers that may contribute to multimarker combinations with better diagnostic performance (9–13).

In this study, we hypothesized that comparison of protein expressions of serum specimens from patients with early stage ovarian cancer with those from healthy women could lead to the discovery of candidate biomarkers for the detection of early stage ovarian cancer. To ensure that the discovered biomarkers are truly associated with ovarian cancer rather than the result of biases in samples, profiling data of specimens from multiple institutions were used for cross-comparison and independent validation. We additionally determined the protein identities of the discovered biomarkers to allow for additional validation with independent methods and as a first step toward understanding the pathways in which they may function.

Materials and Methods
Samples. The study involved a retrospective sample of 645 serum specimens. All were collected with institutional approval. Proteomic profiles were obtained from 503 specimens collected at four medical centers (M. D. Anderson Cancer Center, Duke University Medical Center, Groningen University Hospital, the Netherlands, and the Royal Hospital for Women, Australia). Among them, the cancer group consisted of 65 patients with stages I/II invasive epithelial ovarian cancer, 88 patients with stages III/IV invasive epithelial ovarian cancer, 28 patients with borderline tumors, and 14 patients with recurrent disease, all optimally staged by pathologists based on the Federation Internationale des Gynaecologistes et Obstetrices criteria. Among the stages I/II invasive cases, 20 were serous, 17 were mucinous, 15 were endometroid, 8 were clear cell, 1 was carcinosarcoma, and 4 were mixed epithelial carcinoma. The samples also included 166 patients with benign pelvic masses and 142 healthy donors as controls. All of the samples were collected before the day of surgery or treatment, stored at −70°C, and thawed immediately before assay. CA125 levels had been obtained previously using a CA125 radioimmunoassay (Centocor). The clinical characteristics and age distribution of the proteomic profiling study population are summarized in Table 1.

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Three Biomarkers Identified from Serum Proteomic Analysis for the Detection of Early Stage Ovarian Cancer

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ABSTRACT

Early detection remains the most promising approach to improve long-term survival of patients with ovarian cancer. In a five-center case-control study, serum proteomic expressions were analyzed on 153 patients with invasive epithelial ovarian cancer, 42 with other ovarian cancers, 166 with benign pelvic masses, and 142 healthy women. Data from patients with early stage ovarian cancer and healthy women in two centers were analyzed independently and the results cross-validated to discover potential biomarkers. The results were validated using the samples from two of the remaining centers. After protein identification, biomarkers for which an immunoassay was available were tested on samples from the fifth center, which included 41 healthy women, 41 patients with ovarian cancer, and 20 each with breast, colon, and prostate cancers. Three biomarkers were identified as follows: (a) apolipoprotein A1 (down-regulated in cancer); (b) a truncated form of transthyretin (down-regulated); and (c) a cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4 (up-regulated). In independent validation to detect early stage invasive epithelial ovarian cancer from healthy controls, the sensitivity of a multivariate model combining the three biomarkers and CA125 [94% (95% CI, 85–98%)] was higher than that of CA125 alone [52% (95% CI, 39–65%)] at a matched specificity of 97% (95% CI, 89–100%). When compared at a fixed sensitivity of 83% (95% CI, 61–95%), the specificity of the model [94% (95% CI, 85–98%)] was significantly better than that of CA125 alone [52% (95% CI, 39–65%)]. These biomarkers demonstrated the potential to improve the detection of early stage ovarian cancer.

INTRODUCTION

Despite progress in cancer therapy, ovarian cancer mortality has remained virtually unchanged over the past two decades (1). Annually in the United States alone, ~23,000 women are diagnosed with the disease and almost 14,000 women die from it (1). Given our knowledge about the steep survival gradient relative to the stage at which the disease is diagnosed, it is reasonable to suggest that early detection remains the most promising approach to improve the long-term survival of ovarian cancer patients.

The relatively low prevalence (40 out of 100,000) of ovarian cancer among postmenopausal women in the general population, the lack of a clearly defined precursor lesion, and the high cost and possible complications associated with surgical confirmatory procedures have placed stringent requirements on any test intended for general population screening. Currently, none of the existing serum markers, such as CA125, CA 72–4, or macrophage colony-stimulating factor, can be used individually for screening (2). Longitudinal studies are under way in Europe, Japan, and the United States to evaluate screening strategies using CA125 and/or transvaginal sonography (3–5) and their impact on overall cancer mortality (6). Preliminary results have shown encouraging evidence of a survival benefit among patients diagnosed through a screening regimen (3).

Reports from retrospective studies have shown that multivariate predictive models combining existing tumor markers improve cancer detection (7, 8). Recent advances in genomic and proteomic profiling technology have made it possible to apply computational methods to detect changes in protein expressions and their association to disease conditions, thereby hastening the identification of novel markers that may contribute to multimarker combinations with better diagnostic performance (9–13).

In this study, we hypothesized that comparison of protein expressions of serum specimens from patients with early stage ovarian cancer with those from healthy women could lead to the discovery of candidate biomarkers for the detection of early stage ovarian cancer. To ensure that the discovered biomarkers are truly associated with ovarian cancer rather than the result of biases in samples, profiling data of specimens from multiple institutions were used for cross-comparison and independent validation. We additionally determined the protein identities of the discovered biomarkers to allow for additional validation with independent methods and as a first step toward understanding the pathways in which they may function.

MATERIALS AND METHODS

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Validation of serum biomarkers for detection of early-stage ovarian cancer

Vladimir Nosov, MD; Feng Su, MD; Malaika Amneus, MD; Michael Birrer, MD, PhD; Terry Robins, PhD; Jenny Kotlerman, MS; Srinivasa Reddy, PhD; Robin Farias-Eisner, MD, PhD

OBJECTIVE: Ovarian cancer has the highest mortality of all the gynecologic malignancies with most patients diagnosed at late stages. Serum CA-125 is elevated in only half of patients with stages I-II. We identified 3 serum proteins (apolipoprotein A-1, transthyretin, and transferrin) for the detection of ovarian cancer and reported them combined with CA-125 to effectively detect early-stage mucinous tumors. The objectives of this study were to assess the effectiveness of the panel in detection of early-stage serous and endometrioid ovarian cancers.

STUDY DESIGN: In all, 358 serum samples (control, benign adnexal masses, and early-stage and late-stage ovarian cancer) were obtained from the National Cancer Institute. The level of each marker was measured. Multiple logistic regression models were built to calculate sensitivity and specificity.

RESULTS: When combined with CA-125, the panel detected early-stage cancer with a sensitivity of 96%. The highest sensitivity was seen for detection of endometrioid subtype of early-stage carcinomas (98%).

CONCLUSION: A panel of 4 serum biomarkers effectively detected early-stage ovarian cancers with the highest reported overall sensitivity of 96%. Endometrioid tumors were detected at early stages with a sensitivity of 98%. Prospective clinical analysis of the panel is needed to validate it as an effective screening tool for early-stage ovarian cancer.

Key words: biomarker, early stage, ovarian cancer

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It appears that even a combination of serial ultrasounds and serum CA-125 measurements do not achieve sensitivity acceptable for widespread screening. The identification of more sensitive and specific biomarkers or biomarker panels for the early detection of ovarian cancer would be immediately beneficial.

We have previously analyzed protein profiles using surface enhanced laser desorption and ionization time-of-flight mass spectroscopy and identified 3 differentially expressed serum proteins for the detection of ovarian cancer: apolipoprotein A-1 (ApoA-1), transthyretin (TTR), and transferrin (TF). In a previous publication, these biomarkers, in combination with CA-125, were tested in the serum of women with serous, endometrioid and mucinous ovarian cancer, and ovarian tumors of low malignant potential (LMP), and in women with benign ovarian pathology and in women with normal ovaries. The biomarker panel distinguished normal samples from tumors of LMP with 91% sensitivity, and normal samples from early-stage ovarian cancer with a sensitivity of 89%. Collectively, ApoA-1, TTR, and TF (excluding CA-125) distin-
ferences in marker levels were also presented graphically with box plots.

Setting specificity and sensitivity as equal, MLRM was performed for all 358 interpretable specimens. Sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) were calculated. Prediction cut-offs for all 4 categories of specimens were established.

The MLRM prediction was then used to demonstrate marker level differences between external validation and ovarian cancer groups. To determine statistical significance, we assumed a 5% type I error rate and did not control for multiple comparisons. All tests were performed using a statistical software package (SAS 9.1; SAS Institute, Inc, Cary, NC).

**Results**

Table 1 summarizes patient characteristics in each of 4 groups analyzed. To determine whether representative serum marker values could be obtained from diluted serum samples, we performed dilutions on a subset of 20 serum samples (not included in the 358 test samples described in Table 1) at 1:10 and 1:5. The 1:10 dilution showed significant loss of quantification when compared with undiluted samples (data not shown), however, 1:5 dilution did not result in any significant loss of percent recovery (correlations for: ApoA-1 = 0.9651, TTR = 0.9664, TF = 0.9591). These high correlations of > 0.95 indicate that distribution of the data is not affected by the dilution (Table 2). We, therefore, used the 1:5 dilution (see “Materials and Methods”) on all the test samples to quantify the markers. The means procedure resulted in the data as shown in Table 3 and Figure.

Multiple logistic regression analysis of individual serum markers in each of 4 groups was performed. Comparing odds ratios in normal vs benign groups, only TF and CA-125 had significantly different values. When testing normal vs early-stage cancer groups, TF and CA-125 were significantly different. When comparing the levels of the markers in normal serum to those from the serum of late-stage group, ApoA-1, TF, and CA-125 were statistically significantly different (Table 4).

To determine sensitivity and specificity of the panel of the markers 2 separate analyses were performed. First, sensitivity, specificity, and AUC were determined for ApoA-1, TTR, and TF. Sensitivity and specificity for detection of early-stage ovarian cancer were 86% and 94% for the detection of late-stage ovarian cancer. Second, to assess the contribution of CA-125 to the biomarker

### Table 3

<table>
<thead>
<tr>
<th>Cancer group</th>
<th>Mean value (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-125 (U/mL)</td>
<td>ApoA-1 (mg/dL)</td>
</tr>
<tr>
<td>N (n = 93)</td>
<td>11.81 (6.22)</td>
</tr>
<tr>
<td>B (n = 79)</td>
<td>49.93 (144.71)</td>
</tr>
<tr>
<td>E (n = 90)</td>
<td>537.42 (733.97)</td>
</tr>
<tr>
<td>L (n = 96)</td>
<td>912.25 (1324.85)</td>
</tr>
</tbody>
</table>

ApoA-1, apolipoprotein A-1; B, benign; E, early stage; L, late stage; N, normal; SD, standard deviation; TF, transferrin; TTR, transthyretin.


### Figure

**Biomarker values in normal and ovarian cancer samples**

Individual marker values in normal samples (1), benign samples (2), early-stage cancers (3), and late-stage cancers (4). Apolipoprotein A-1 (ApoA-1), transthyretin (TTR), and transferrin (TF) in mg/dL; CA-125 in U/mL.

As indicated in Table 5, a separate analysis was performed to include CA-125 in addition to the 3 markers. As indicated in Table 5, inclusion of all 4 biomarkers brought the sensitivity and specificity of the panel to 96% (AUC, 99%) when comparing normal to early-stage cancer. This increased to a sensitivity and specificity of 98% (AUC, 100%) when comparing normal to late-stage ovarian cancer.

The early-stage ovarian cancer group was further subdivided into histologic categories. Each histologic subtype was analyzed separately to determine the sensitivity and specificity of the biomarker panel for detection of different subtypes of ovarian carcinoma (Table 6), excluding mucinous, which was reported previously. Sensitivity of the panel of 3 and 4 markers was the highest in endometrioid group: 88% and 98%, respectively. Sensitivity of detection of serous early-stage cancer with 4 markers was 94% (AUC, 99%).

**Comment**

The absolute majority of patients with ovarian carcinomas are diagnosed at late stages.

---

**Table 4**

<table>
<thead>
<tr>
<th>Comparison group</th>
<th>Marker</th>
<th>OR (CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs benign</td>
<td>ApoA-1</td>
<td>1.01 (0.99-1.02)</td>
<td>.19</td>
</tr>
<tr>
<td></td>
<td>TTR</td>
<td>1.06 (0.95-1.17)</td>
<td>.30</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>1.04 (1.02-1.05)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td></td>
<td>CA-125</td>
<td>0.94 (0.89-0.98)</td>
<td>.004</td>
</tr>
<tr>
<td>Normal vs early</td>
<td>ApoA-1</td>
<td>0.97 (0.93-1.01)</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>TTR</td>
<td>1.21 (0.93-1.57)</td>
<td>.15</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>0.93 (0.89-0.98)</td>
<td>.005</td>
</tr>
<tr>
<td></td>
<td>CA-125</td>
<td>1.12 (1.03-1.23)</td>
<td>.01</td>
</tr>
<tr>
<td>Normal vs late</td>
<td>ApoA-1</td>
<td>0.95 (0.90-1.00)</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td>TTR</td>
<td>1.07 (0.81-1.42)</td>
<td>.62</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>0.95 (0.91-0.99)</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>CA-125</td>
<td>1.14 (1.03-1.27)</td>
<td>.02</td>
</tr>
</tbody>
</table>

---

**Table 5**

<table>
<thead>
<tr>
<th>Markers used</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-1, TTR, TF</td>
<td>Normal vs benign</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Normal vs early stage</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Normal vs late stage</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>ApoA-1, TTR, TF + CA-125</td>
<td>Normal vs benign</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Normal vs early stage</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Normal vs late stage</td>
<td>0.98</td>
<td>0.98</td>
</tr>
</tbody>
</table>

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ApoA-1, apolipoprotein A-1; CI, confidence interval; OR, odds ratio; TF, transferrin; TTR, transthyretin.

Serum protein markers for early detection of ovarian cancer

Gil Mor*,†, Irene Visintin*,†, Yinglei Lai*, Hongyu Zhao†, Peter Schwartz*, Thomas Rutherford*, Luo Yue†, Patricia Bray-Ward†,§ and David C. Ward†,‡,‡

Departments of *Obstetrics and Gynecology, †Epidemiology and Public Health, and ‡Genetics, Yale University School of Medicine, New Haven, CT 06510; §Department of Statistics, The George Washington University, Washington, DC 20052; and Nevada Cancer Institute, Las Vegas, NV 89135

Contributed by David C. Ward, March 16, 2005

Early diagnosis of epithelial ovarian cancer (EOC) would significantly decrease the morbidity and mortality from this disease but is difficult in the absence of physical symptoms. Here, we report a blood test, based on the simultaneous quantitation of four analytes (leptin, prolactin, osteopontin, and insulin-like growth factor-II), that can discriminate between disease-free and EOC patients, including patients diagnosed with stage I and II disease, with high efficiency (95%). Microarray analysis was used initially to determine the levels of 169 proteins in serum from 28 healthy women, 18 women newly diagnosed with EOC, and 40 women with recurrent disease. Evaluation of proteins that showed significant differences in expression between controls and cancer patients by ELISA assays yielded the four analytes. These four proteins then were evaluated in a blind cross-validation study by using an additional 106 healthy females and 100 patients with EOC (24 stage I/II and 76 stage III/IV). Upon sample decoding, the results were analyzed by using three different classification algorithms and a binary code methodology. The four-analyte test was further validated in a blind binary code study by using 40 additional serum samples from normal and EOC cancer patients. No single protein could completely distinguish the cancer group from the healthy controls. However, the combination of the four analytes exhibited the following: sensitivity 95%, positive predictive value (PPV) 95%, specificity 95%, and negative predictive value (NPV) 94%, a considerable improvement on current methodology.

Materials and Methods

Sample Collection. Ten milliliters of blood was collected from each individual and centrifuged at 800 × g for 10 min and the serum fraction was separated, aliquotted, and stored at −80°C in the OB/GYN Tissue bank at Yale University School of Medicine.

Abbreviations: PPV, positive predictive value; EOC, epithelial ovarian cancer; RCA, rolling circle amplification; MIF-1, macrophage inhibitory factor-1; OPN, osteopontin; IGF-II, insulin-like growth factor-II; OVCA, ovarian cancer.

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PNAS, 2005, Vol. 102, No. 21, pp. 7677–7682
Screening for ovarian cancer in the general population

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Keywords:
ovarian cancer
general population
screening
CA125
tumour markers
ultrasound

Interrigation

Ovarian cancer accounts for 4% of cancers diagnosed in women, with over 225,000 new cases diagnosed worldwide each year. Incidence rates are highest in the USA and Northern Europe and lowest in Africa and Asia. In most developed countries, it is the most common genital tract malignancy, with women having a 1–2% lifetime risk of developing the disease. It is also associated with the highest mortality rates. Around 85% of cases occur over the age of 50 years, and 80–85% of cancers are epithelial in origin. The most common histological subtype of epithelial ovarian cancer (EOC) is serous ovarian cancer, which presents at advanced stages and has the poorest outcomes.

Sixty per cent of women are diagnosed at advanced stage, which has a 5-year survival as low as 10%. When the disease is caught early, 5-year survival is in excess of 90%. This forms the rationale for

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8.6 years showed that elevated CA125 levels in women without ovarian cancer had a flat or static profile or decreased with time, whereas levels associated with malignancy tended to rise. These data were used to construct a computerised algorithm that used an individual's age-specific incidence of ovarian cancer and CA125 profile to estimate a woman's risk of ovarian cancer (ROC). The closer the CA125 profile to the CA125 behaviour of known cases of ovarian cancer, the greater the ROC. The final result is presented as the individual's estimated risk of having ovarian cancer so that a ROC of 2% implies a risk of 1 in 50. Women are triaged into low, intermediate and elevated-risk based on their ROC result. The women at intermediate risk have repeat CA125, whereas those with elevated risk are referred for a CA125 and transvaginal scanning. If either are abnormal, the women are then referred for clinical assessment with a gynaecological oncologist with a view to surgery. The full screening algorithm has been described in detail elsewhere. The ROC algorithm increases the sensitivity of CA125 compared with a single cut-off value because women with normal but rising levels are identified as being at increased risk. At the same time, specificity is improved, as women with static but elevated levels are classified as low risk. For a target specificity of 98% for preclinical detection of ovarian cancer, the ROC calculation achieved a sensitivity of 86%.35

Prospective evaluation of the ROC algorithm in a randomised-controlled trial of 13,582 postmenopausal women aged over 50 years, showed a high specificity (99.8%; 95% CI 99.7 to 99.9) and PPV (19%; 95% CI 4.1 to 45.6) for primary invasive epithelial ovarian cancer. More recently, encouraging results have been reported by UK and US groups. In the prevalence screen of the ongoing randomised-controlled UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) (Fig. 1), multimodal screening using the ROC algorithm achieved a sensitivity of 89.5% (95% CI 75.2 to 97.1%), specificity of 99.8% (95% CI, 99.8 to 99.8%) and PPV of 35.1% (95% CI, 25.6 to 45.4%) with 47.1% of primary invasive epithelial cancers detected in early stage. In a smaller study of 3238 women by Lu et al., ROC algorithm followed by transvaginal scanning had a specificity of 99.7% (95% CI 99.5 to 99.9%) and PPV of 37.5% (95% CI 8.5 to 75.5%).

The ROC algorithm is also being evaluated prospectively in OCS trials in women at increased risk of familial ovarian cancer under the auspices of the Cancer Genetics Network and Gynaecology Oncology Group in the USA and the UK Familial Ovarian Cancer Screening Study in the UK. The ROC algorithm relies on modelling the behavior of a biomarker from disease onset to clinical presentation, and data for this may take years to accumulate. A computationally simpler longitudinal algorithm using data obtainable in a short period of time has been proposed for use in cancer screening using a new biomarker, especially when pre-clinical behaviour of the disease, biomarker, or both is uncertain.

Additional markers

Massive efforts have been made in the past decade to identify either a better marker or a panel of markers that would improve the performance of CA125. Nearly all the studies have used clinical samples, so their findings are more relevant to differential diagnosis of benign from malignant masses, avoiding unnecessary operations in women with benign lesions and ensuring that surgery, where there is high suspicion of ovarian cancer, is undertaken by trained gynaecological oncologists in tertiary-care centres. Here, serum CA125 using a cut-off of 35 kU/L in combination with imaging has been shown to achieve a sensitivity of 94% and specificity of 90% for ovarian cancer. The performance of various other markers reported in the past 5 years are shown in Table 1. Limited sensitivities and specificities constrain their use for screening purposes. The most promising marker has been serum human epididymis protein 4 (HE4).

Recently, the performance of 49 ovarian cancer biomarkers were assessed in pre-diagnostic specimens in asymptomatic women compared with clinical specimens obtained at diagnosis from a different set of individuals. For 'standard' tumour markers, such as CA125, HE4, CA72-4, and CA15-3, the performance in prediagnostic samples drawn within 6 months of cancer diagnosis was comparable to that in clinical samples. In contrast, for markers such as prolactin, transthyretin or apolipoprotein A1, which may be derived from the individual's response to the cancer, performance was poorer in prediagnostic specimens. Serum CA125 remained the single best biomarker for ovarian cancer, with sensitivity of 86% (95% CI 0.76 to 0.97) in cases where blood was drawn within 6 months of diagnosis, with the second best marker being HE4, with sensitivity of 73% (95% CI 0.60 to 0.86). For all markers,
A Framework for Evaluating Biomarkers for Early Detection: Validation of Biomarker Panels for Ovarian Cancer

Claire S. Zhu1, Paul F. Pinsky1, Daniel W. Cramer2, David F. Ransohoff3, Patricia Hartge4, Ruth M. Pfeiffer4, Nicole Urban5, Gil Mor6, Robert C. Bast Jr.7, Lee E. Moore4, Anna E. Lokshin8, Martin W. McIntosh5, Steven J. Skates9, Allison Viltonis2, Zhen Zhang10, David C. Ward11, James T. Symanowski12, Aleksy Lomakin13, Eric T. Fung14, Patrick M. Sluss9, Nathalie Scholler15, Karen H. Lu7, Adele M. Marrangoni8, Christos Patriotis1, Sudhir Srivastava1, Saundra S. Buys16, and Christine D. Berg1 for the PLCO Project Team

Abstract

A panel of biomarkers may improve predictive performance over individual markers. Although many biomarker panels have been described for ovarian cancer, few studies used prediagnostic samples to assess the potential of the panels for early detection. We conducted a multisite systematic evaluation of biomarker panels using prediagnostic serum samples from the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) screening trial.

Using a nested case–control design, levels of 28 biomarkers were measured laboratory-blinded in 118 serum samples obtained before cancer diagnosis and 951 serum samples from matched controls. Five predictive models, each containing 6 to 8 biomarkers, were evaluated according to a predetermined analysis plan. Three sequential analyses were conducted: blinded validation of previously established models (step 1); simultaneous split-sample discovery and validation of models (step 2); and exploratory discovery of new models (step 3). Sensitivity, specificity, sensitivity at 98% specificity, and AUC were computed for the models and CA125 alone among 67 cases diagnosed within one year of blood draw and 476 matched controls. In step 1, one model showed comparable performance to CA125, with sensitivity, specificity, sensitivity at 98% specificity, and AUC at 69.2%, 96.6%, and 0.892, respectively. Remaining models had poorer performance than CA125 alone. In step 2, we observed a similar pattern. In step 3, a model derived from all 28 markers failed to show improvement over CA125.

Thus, biomarker panels discovered in diagnostic samples may not validate in prediagnostic samples; utilizing prediagnostic samples for discovery may be helpful in developing validated early detection panels.

Cancer Prev Res; 4(3); 375–83. ©2011 AACR.

Introduction

Ovarian cancer is the fifth leading cause of cancer death among women in the US. Although early detection might reduce ovarian cancer mortality, there is currently no proven effective early detection tool for the disease.

In the last decade, many serum biomarkers or panels of biomarkers have been reported to detect ovarian cancer
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In the last decade, many serum biomarkers or panels of biomarkers have been reported to detect ovarian cancer.
with higher sensitivity and specificity than the best marker currently available, CA125 (1–4). With one exception (5), such studies utilized serum samples collected at the time of diagnosis, and generally included a high proportion of cases with advanced stage disease. Further, few of these biomarkers or panels have been evaluated in a rigorous validation study. Thus, their utility for screening, which requires detection at an asymptomatic phase, cannot be determined. This general scenario is not limited to ovarian cancer—for virtually all of the major cancers, many promising predictive biomarkers have been identified, but few have been tested rigorously in prediagnostic specimens (specimens collected before clinical manifestation of the disease from asymptomatic subjects).

This report is the second of 2 companion reports, both of which can be found in this issue, describing a multisite, simultaneous, coordinated effort to systematically evaluate the performance of biomarkers for early detection of ovarian cancer using a nested case–control design and stored, prediagnostic serum samples obtained from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. The first report details the developmental process for selecting the best biomarkers from phase II (diagnostic) and phase III (prediagnostic) specimens to be included in a final panel of biomarkers from a larger pool of candidate markers (6). This report proposes a novel, systematic approach for unbiased evaluation of classification models combining multiple biomarkers and presents the performance results in prediagnostic samples of 5 predictive models derived from the first report.

Materials and Methods

PLCO biorepository

The prediagnostic serum samples used in the current study were from the PLCO biorepository. PLCO is a randomized controlled cancer screening trial evaluating various screening tests for the 4 PLCO cancers. Over 150,000 healthy subjects ages 55 to 74 from across the United States were randomized to a screening or usual care arm at 10 screening sites from 1993 to 2001. The primary outcome of the trial is to assess whether routine screening can reduce cancer-specific mortality (7, 8). The overall screening protocol has been described elsewhere (8). For ovarian cancer screening, women with at least one ovary at baseline received a CA125 blood test at each of 6 annual screenings, and a transvaginal ultrasound (TVU) at the first 4 screenings (9). Subjects who tested positive for either CA125 or TVU were referred to their local physicians who determined the diagnostic workup procedures. Any diagnosis of cancer and its stage, grade, and initial treatment, were obtained. Subjects with positive tests but no cancer diagnosis continued to undergo annual screenings. Cancers diagnosed in between screenings, or after the screening period ended, were identified through annual surveys of cancer and vital status. Data on demographics, risk factors, and dietary information were collected through multiple questionnaires administered at baseline and during the follow-up period.

Blood samples were collected from intervention arm subjects at each of the 6 annual screens (10). Therefore, up to 6 serial bloods may be available for a given subject. The collection of biospecimens was approved by the NCI Special Studies Institutional Review Board (OH97-CN041) and by the local Institutional Review Board for each of the screening sites. Informed consent was obtained from all subjects who provided blood samples to be stored for future research. Blood samples were processed in several different ways to obtain serum, plasma, buffy coat, red blood cells, or cryopreserved whole blood.

Study coordination

Six investigator groups participated in this study; each group’s proposal was approved by the PLCO Etiologic and Early Marker Studies (EEMS) Review Panel, on the basis of scientific merits, to use PLCO prediagnostic specimens to evaluate a panel of biomarkers for early detection of ovarian cancer. The specific markers included in each panel are shown in Table 1. The rationale for selecting these markers is detailed in the companion report (6). Most of these markers had been previously shown to differentiate clinical cases from control subjects with high sensitivity and specificity (2–5, 11–13).

The NCI PLCO leadership assumed overall coordination of these studies, with the investigators’ consent, input, and collaboration, to standardize sampling, statistical methods, and data interpretation across the studies.

Common sampling plan. Figure 1 shows the subject selection criteria. Among 24,650 eligible subjects, 118 cases of pathologically confirmed (through May 2006) invasive ovarian, primary peritoneal, and fallopian tube cancers with appropriate consents and available samples were identified. Both screen-detected cases (identified from diagnostic workup subsequent to a positive CA125 or TVU test), and clinically diagnosed cases were included. For each case, 8 controls were randomly selected from 24,473 healthy subjects without cancer: 4 general population controls, 2 controls with a family history of breast or ovarian cancers, and 2 controls with elevated CA125. These special controls were included to assess the performance of the models in high-risk populations but were not included in primary analyses. Controls were frequency-matched by age and calendar year of blood draw. For each study subject, a single serum sample closest and prior to diagnosis (proximate sample) was selected for laboratory analysis.

Common data analysis plan. The common data analysis plan was formulated to clearly distinguish between validation and discovery, both of which were to be accommodated in the overall analysis strategy. In this study, validation refers to “hypothesis testing”, that is,
The current study is significant in several ways. First, it provides the first example of a coordinated, systematic approach to biomarker validation using prediagnostic samples. Second, the findings raise a question about the current paradigm for biomarker development, namely, using diagnostic samples for discovery and validating them in prediagnostic samples. It is possible that markers discovered in diagnostic samples are significantly differentially expressed only when the tumor becomes large, or clinically apparent. Such markers may have little value for early detection.

### Table 4. Results of models from steps 1 to 3

<table>
<thead>
<tr>
<th>Model</th>
<th>Sensitivity&lt;sup&gt;a&lt;/sup&gt; ≤12 mo (%) (95% CI)</th>
<th>Sensitivity&lt;sup&gt;a&lt;/sup&gt; 13–24 mo (%) (95% CI)</th>
<th>Specificity&lt;sup&gt;a&lt;/sup&gt; (%) (95% CI)</th>
<th>ROC&lt;sup&gt;**&lt;/sup&gt; Area (%) (95% CI)</th>
<th>Sensitivity at 98% Specificity&lt;sup&gt;b&lt;/sup&gt; (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>n = 67</td>
<td>n = 26</td>
<td>n = 476</td>
<td>n = 67</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>34.3 (23–46)</td>
<td>7.7 (1–25)</td>
<td>96.8 (95.2–98.4)</td>
<td>0.721 (0.64–0.80)</td>
<td>32.8 (22–44)</td>
</tr>
<tr>
<td>B1</td>
<td>69.2 (58–80)</td>
<td>12.5 (3–31)</td>
<td>96.6 (94.9–98.3)</td>
<td>0.892 (0.84–0.95)</td>
<td>64.6 (53–76)</td>
</tr>
<tr>
<td>C1</td>
<td>34.3 (23–46)</td>
<td>11.5 (2–30)</td>
<td>95.1 (93.1–97.1)</td>
<td>0.712 (0.63–0.79)</td>
<td>25.4 (15–36)</td>
</tr>
<tr>
<td>D1</td>
<td>95.4 (90–99)</td>
<td>76.0 (59–93)</td>
<td>32.2 (27.4–36.5)</td>
<td>0.858 (0.80–0.92)</td>
<td>52.3 (40–64)</td>
</tr>
<tr>
<td>E1</td>
<td>37.9 (26–50)</td>
<td>3.9 (0–20)</td>
<td>89.8 (87.0–92.6)</td>
<td>N/A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CA125&lt;sup&gt;d&lt;/sup&gt;</td>
<td>63.1 (51–75)</td>
<td>0.0 (0–13)</td>
<td>98.5 (97.4–99.6)</td>
<td>0.890 (0.84–0.94)</td>
<td>64.6 (53–76)</td>
</tr>
<tr>
<td>Step 2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>n = 30</td>
<td>n = 15</td>
<td>n = 237</td>
<td>n = 30</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>53.3 (35–71)</td>
<td>6.7 (0–32)</td>
<td>96.6 (94.3–98.8)</td>
<td>0.852 (0.77–0.94)</td>
<td>36.7 (20–54)</td>
</tr>
<tr>
<td>B2</td>
<td>80.0 (66–94)</td>
<td>21.4 (5–50)</td>
<td>92.2 (88.7–95.7)</td>
<td>N/A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2</td>
<td>70.0 (54–86)</td>
<td>6.7 (0–32)</td>
<td>91.9 (88.4–95.4)</td>
<td>0.848 (0.76–0.94)</td>
<td>46.7 (29–64)</td>
</tr>
<tr>
<td>D2</td>
<td>55.2 (37–73)</td>
<td>0.0 (0–22)</td>
<td>86.9 (82.5–91.3)</td>
<td>0.810 (0.72–0.90)</td>
<td>51.7 (34–69)</td>
</tr>
<tr>
<td>E2</td>
<td>30.0 (14–46)</td>
<td>13.3 (2–40)</td>
<td>96.2 (93.7–98.7)</td>
<td>0.590 (0.46–0.72)</td>
<td>23.3 (8–38)</td>
</tr>
<tr>
<td>CA125&lt;sup&gt;d&lt;/sup&gt;</td>
<td>72.4 (56–89)</td>
<td>0.0 (0–22)</td>
<td>97.9 (96.0–99.8)</td>
<td>0.898 (0.82–0.98)</td>
<td>72.4 (56–89)</td>
</tr>
<tr>
<td>Step 3 (Pan-site)</td>
<td>N/A&lt;sup&gt;f&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;f&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.911 (0.86–0.96)</td>
<td>68.2 (57–80)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated based on cutoff specified by the model. Note that we included all general population controls in the calculation of specificity and ROC; these as a whole were comparable to the 1-year cases with respect to the matching variables of age and calendar year of blood draw.

<sup>b</sup>Calculated based on the 67 cases diagnosed ≤12 months from blood draw.

<sup>c</sup>This model did not produce a propensity score, thus the measure cannot be calculated.

<sup>d</sup>Using data previously obtained in PLCO for the same subject and study year as the samples in the current study, and a cutoff of ≥35 U/mL.

<sup>e</sup>For step 2 model, data from the validation set is shown.

<sup>f</sup>The pan-site model did not have a cutoff, therefore no sensitivity or specificity can be calculated.

### Figure 2. ROC curves for step 1 models (a) and Step 2 models (b), compared to that of CA125 and the step 3 (pan-site) model in the within-one-year cases. Black solid, CA125 alone; blue solid, step 3 model; blue dotted, Panel D; blue dashed, Panel C; red solid, Panel B; red dotted, Panel A; red dashed, Panel E. Note: Figure 2b curves are based on validation set only.
Review
An overview of biomarkers for the ovarian cancer diagnosis

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A R T I C L E  I N F O

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A B S T R A C T

Even though there are a lot of options in treating gynecological malignancies, ovarian cancer still remains a leading cause of death. Diagnosis at an early stage is the most important determinant of survival. Current diagnostic tools applied at clinics have had very limited success in early detection. Discovery of new diagnostic biomarkers/panels for early diagnosis of ovarian cancer is one of the main challenges of modern medicine. With the progress of techniques in genomics and proteomics, numerous molecular biomarkers/panels were identified and showed promise for ovarian cancer diagnosis, but still need further validation. This article summarizes various types of markers investigated by different strategies/technologies for the ovarian cancer diagnosis at present, including gene-, protein-based and emerging ovarian cancer indicators (such as microRNA-, metabolite-based). Before biomarker tests are translated for routine use, more researches, such as retrospective and prospective clinical trials, are needed to evaluate the overall clinical utility of the tests.

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1. Introduction

Ovarian cancer is the most lethal of all common gynecologic malignancies, with more than 204,000 new cases and 125,000 deaths each year, accounting for 4% of all cancer cases and 4.2% of all cancer deaths in women around the world [1]. In Switzerland, recent statistical data from the Swiss Association of Cancer Registries (www.nicer.org) showed that ovarian cancer is the seventh most common cancer and the fifth cause of death from cancer in Swiss women.

Contributing to the poor prognosis of ovarian cancer is the lack of symptoms in the early stages of the disease. More than 70% of the women are diagnosed with late stage disease [International Federation of Gynecology and Obstetrics (FIGO) stage III or IV], after distant metastasis has occurred. The 5-year survival rate for women diagnosed with late stage disease is less than 20% even with extensive surgery and chemotherapy, compared to up to 90% for women diagnosed with early stage disease [2]. Therefore, detection of ovarian cancer at an early stage is critical for curative treatment interventions. Unfortunately, current diagnosis methods for the detection of early stage ovarian cancer are inadequate.
the capacity of detecting the expression of novel transcripts allowing for the identification of previously uncharacterized genes, thus providing a unique advantage over the traditional microarray-based approach for expression profiling. In ovarian cancer, several known and novel genes whose expressions are elevated have been identified by SAGE technology. These genes included claudin 3 (CLDN3) [9], WAP four-disulfide core domain 2 (WFDCC2, also known as HE4) [9], folate receptor 1 (FOLR1) [9], collagen type XVIII a1 (COL18A1) [9], cyclin D1 (CCND1) [9], FLJ12988 [9].

3. Protein-based ovarian cancer biomarkers

Although gene-based biomarkers are known to have potential for ovarian cancer, there is still no novel cancer specific biomarker in clinic. This is due to the fact that gene levels are not always linked directly to levels of proteins, the molecules that biologically do functions. Proteomics has emerged as a powerful technology to decipher biological processes. It means large-scale characterization of proteins including more complicated features like isoforms, modifications, interactions and functional structures. One of the main goals of proteomics is the identification of biomarkers for diseases from tissues and body fluids. The major proteomics technique that fundamentally supported the discovery of cancer biomarkers is MS which can determine precise mass and charge of protein, thus identity of the actual precursor proteins or protein profiles. Among several different MS-based proteomics approaches, currently, matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) and surface-enhanced laser desorption and ionization time-of-flight (SELDI-TOF) are two of the most frequently used methods for new biomarker discovery [10].

Proteomic applications to ovarian cancer diagnosis have followed two paths [11]: one, called “proteomic pattern diagnostics” or “serum proteomic profiling”, is based on complex mass spectrometric differences between proteomic patterns of samples with and without cancer identified by bioinformatics. Many previously published studies showed that proteomic pattern analysis in ovarian cancer has the potential to be a novel, highly sensitive diagnostic tool for detection at an early stage [12]. However, with the impressive results in terms of specificity and sensitivity in ovarian cancer detection, some criticism regarding instrument reproducibility, quality control and standard operating procedures for sample collection, handling and shipping have been raised. Recently researchers have emphasized more and more on the importance of reliability and reproducibility of a MS technology in protein profiling.

An alternative or integrative proteomic approach to ovarian cancer biomarkers is its use for the identification of single, novel biomarkers and the subsequent development of new assays [11]. In recent years many promising biomarkers discovered by proteomic analysis for ovarian cancer diagnosis were published [13–15]. Among the markers identified by proteomic analysis, some biomarkers, such as cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4 [13], have often been normal serum proteins that have undergone posttranslational modification by proteases and reflect the protease profiles of particular cancers. Some biomarkers such as transferrin [14], are acute phase proteins and have been associated with systemic inflammation as well as other non-cancer conditions. Other biomarkers, such as the vitamin E-binding plasma protein Aftamin, had decreased serum concentrations in ovarian cancer patients and could contribute independent diagnostic information to CA-125 [15]. Thus establishing their potential as an adjunct marker to CA-125 [15]. However, they are still not cancer specific markers and derived directly from the ovarian cancer. So for proteomics-based biomarkers, their significance and degree of specificity for ovarian cancer remain to be explored. Recently, there are many proteins that have been studied in the search for EOC biomarkers. Of these proteins, mesothelin, osteopontin, and HE4 have been selected by the SPORE (Specialized Program of Research Excellence) committee for their high level of sensitivity and specificity in differentiating EOC from normal ovarian epithelium [16]. But the fact is that to date no single test or modality has met the criteria (positive predictive value of 10%) for early diagnosis of ovarian cancer [2].

4. Emerging ovarian cancer biomarkers

Following biomarker discovery on gene and protein level, recently two new fields are receiving increased attention in biomarker research of cancer, including ovarian cancer: analysis of the miRNAome and of the metabolome.

4.1. MicroRNA-based ovarian cancer biomarkers

MicroRNAs (miRNAs) are approximately 22 nt non-coding RNAs, which regulate gene expression in a sequence-specific manner via translational inhibition or messenger RNA (mRNA) degradation, and thus regulate diverse biological processes including development, cell proliferation, differentiation and apoptosis. About 3% of human genes encode for miRNAs, and up to 30% of human protein coding genes may be regulated by miRNAs, unique to each cell type and to the development and differentiation stage of the cell. Accumulating evidence has revealed aberrant expression of miRNAs in cancer including ovarian cancer, suggesting that they may act as a novel class of oncogenes or tumor-suppressor genes. Given the critical pathogenic roles of miRNAs in cancer progression, characterizing the regulation of miRNAs will provide novel opportunities for the development of cancer biomarkers and/or the identification of new therapeutic targets in the foreseeable future. Recently, development of dedicated microarrays has made it possible to analyze miRNA expression profiles in different oncotypes. Because miRNA expression profiles parallel the developmental origins of tissues, and because relatively few miRNAs can be used to effectively type tissues, they are potentially superior markers than messenger RNAs for cancer diagnosis and classification [17]. In the last 5 years several miRNA expression profiles of EOC have been published, reporting a decreased expression of a substantial proportion of miRNAs as compared to normal counterpart [18]. Recently, by using a custom microarray platform to compare miRNA profiles between 69 EOC surgical specimens and 15 normal ovaries, 29 differentially expressed miRNAs were found. Among them, miR-200a, miR-200b, miR-200c and miR-141 have been shown to be overexpressed. On the other hand, miR-199a, miR-140, miR-145 and miR-125b1 were among the most down-modulated miRNAs. In addition, it is believed that miRNA signatures of ovarian tumors may also distinguish these tumors based on their histologic subtypes and low- and high-grade malignancies [18].

One aspect of miRNA biogenesis that makes them particularly attractive as a biomarker is the fact that they are maintained in a protected state in serum and plasma, thus allowing the detection of miRNA expression patterns directly from serum. Recent work found that the miRNA profiles of circulating tumor exosomes from EOC patients closely related with miRNA expression in primary tumors and could be used to distinguish cancer patients from patients with benign ovarian disease and from normal controls, thus having potential to be diagnostic markers of ovarian cancer. In this work, circulating tumor exosomes were isolated from serum using magnetic beads and an antiEpiCAM antibody, and then miRNAs were extracted, labeled and detected by microarray. The results indicated that eight diagnostic miRNAs, including miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214 were up-regulated in ovarian cancer exosomes [19].
the capacity of detecting the expression of novel transcripts allowing for the identification of previously uncharacterized genes, thus providing a unique advantage over the traditional microarray-based approach for expression profiling. In ovarian cancer, several known and novel genes whose expressions are elevated have been identified by SAGE technology. These genes included Claudin 3 (CLDN3) [9], WAP four-disulfide core domain 2 (WFDC2, also known as HE4) [9], folate receptor 1 (FOLR1) [9], collagen type XVIII a1 (COL18A1) [9], cyclin D1 (CCND1) [9], FLJ12988 [9].

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An alternative or integrative proteomic approach to ovarian cancer biomarkers is its use for the identification of single, novel biomarkers and the subsequent development of new assays [11]. In recent years many promising biomarkers discovered by proteomic analysis for ovarian cancer diagnosis were published [13–15]. Among the markers identified by proteomic analysis, some biomarkers, such as cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4 [13], have often been normal serum proteins that have undergone posttranslational modification by proteases and reflect the protease profiles of particular cancers. Some biomarkers such as transferrin [14], are acute phase proteins and have been associated with systemic inflammation as well as other non-cancer conditions. Other biomarkers, such as the vitamin E-binding plasma protein A1am, has decreased serum concentrations in ovarian cancer patients and could contribute independent diagnostic information to CA-125 [15]. Thus establishing their potential as an adjunct marker to CA-125 [15]. However, they are not cancer-specific markers and derived directly from the ovarian cancers. So for proteomics-based biomarkers, their significance and degree of specificity for ovarian cancer remain to be explored. Recently, there are many proteins that have been studied in the search for EOC biomarkers. Of these proteins, mesothelin, osteopontin, and HE4 have been selected by the SPORE (Specialized Program of Research Excellence) committee for their high level of sensitivity and specificity in differentiating EOC from normal ovarian epithelium [16]. But the fact is that to date no single test or modality has met the criteria (positive predictive value of 10%) for early diagnosis of ovarian cancer [2].

4. Emerging ovarian cancer biomarkers

Following biomarker discovery on gene and protein level, recently two new fields are receiving increased attention in biomarker research of cancer, including ovarian cancer: analysis of the miRNAome and of the metabolome.

4.1. MicroRNA-based ovarian cancer biomarkers

MicroRNAs (miRNAs) are approximately 22 nt non-coding RNAs, which regulate gene expression in a sequence-specific manner via translational inhibition or messenger RNA (miRNA) degradation, and thus regulate diverse biological processes including development, cell proliferation, differentiation and apoptosis. About 3% of human genes encode for miRNAs, and up to 30% of human protein coding genes may be regulated by miRNAs, unique to each cell type and to the development and differentiation stage of the cell. Accumulating evidence has revealed aberrant expression of miRNAs in cancer including ovarian cancer, suggesting that they may act as a novel class of oncogenes or tumor-suppressor genes. Given the critical pathogenic roles of miRNAs in cancer progression, characterizing the regulation of miRNAs will provide novel opportunities for the development of cancer biomarkers and/or the identification of new therapeutic targets in the foreseeable future. Recently, development of dedicated microarrays has made it possible to analyze miRNA expression profiles in different oncotypes. Because miRNA expression profiles parallel the developmental origins of tissues, and because relatively few miRNAs can be used to effectively type tissues, they are potentially superior markers than messenger RNAs for cancer diagnosis and classification [17]. In the last 5 years several miRNA expression profiles of EOC have been published, reporting a decreased expression of a substantial proportion of miRNAs as compared to normal counterpart [18]. Recently, by using a custom microarray platform to compare miRNA profiles between 69 EOC surgical specimens and 15 normal ovaries, 29 differentially expressed miRNAs were found. Among them, miR-200a, miR-200b, miR-200c and miR-141 have been shown to be overexpressed. On the other hand, miR-199a, miR-140, miR-145 and miR-125b1 were among the most down-modulated miRNAs. In addition, it is believed that mRNA signatures of ovarian tumors may also distinguish these tumors based on their histologic subtypes and low- and high-grade malignancies [18].

One aspect of miRNA biogenesis that makes them particularly attractive as a biomarker is the fact that they are maintained in a protected state in serum and plasma, thus allowing the detection of miRNA expression patterns directly from serum. Recent work found that the miRNA profiles of circulating tumor exosomes from EOC patients closely related with miRNA expression in primary tumors and could be used to distinguish cancer patients from patients with benign ovarian disease and from normal controls, thus having potential to be diagnostic markers of ovarian cancer. In this work, circulating tumor exosomes were isolated from serum using magnetic beads and an antiEpcam antibody, and then miRNAs were extracted, labeled and detected by microarray. The results indicated that eight diagnostic miRNAs, including miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214 were up-regulated in ovarian cancer exosomes [19].
More recently, a novel real-time PCR platform was used to detect serum miRNA, founding miR-21, miR-92, miR-93, miR-126, and miR-29a were up-regulated, while miR-155, miR-127, and miR-99b were down-regulated in serum collected from ovarian carcinoma patients compared to healthy controls. Up-regulation of miR-21, miR-92, and miR-93 in the serum of three cancer patients with normal CA-125 level suggests that miRNA may be complementary to current detection approaches [20].

Overall, supported by a growing number of findings, it has become clear that miRNAs play key roles in both normal and pathologic ovarian activities by targeting the expression of specific genes. However, until now a clear consensus on miRNA signatures associated to diagnosis, prognosis or prediction of response to therapy has not yet been reached in the case of EOC. A greater understanding of the role of miRNAs in ovarian cancer is needed and will allow for improved interventions against this devastating malignancy.

4.2. Metabolite-based ovarian cancer biomarkers

Metabolomics, an omic science in systems biology, is the global quantitative assessment of endogenous metabolites within a biological system. Metabolites result from the interaction of the system’s genome with its environment; they are not merely the end product of gene expression, but form part of the regulatory system in an integrated manner. Either individually or grouped as a metabolomic profile, detection of metabolites is usually carried out in cells, tissues, or biofluids by either nuclear magnetic resonance (NMR) spectroscopy or MS. With the development of metabolic and molecular imaging technologies which enable the discrimination of metabolic markers noninvasively in vivo, metabolomics, as a translational research tool, can provide a link between the laboratory and clinic. It is also possible for the metabolome to have a multitude of uses in oncology, including the early detection and diagnosis of cancer, monitoring drug treatment response and drug toxicity [21]. In the area of ovarian cancer diagnosis, 1H NMR spectroscopy was done on serum specimens of 38 preoperative EOC patients, 12 patients with benign ovarian cysts and 51 healthy women including 32 postmenopausal and 19 pre-menopausal. The results showed that 100% sensitivity and 100% specificity for the detection of EOC at the 1H NMR regions 2.77 and 2.04 parts per million (ppm) from the origin. These findings indicated that 1H NMR metabonomic analysis of serum achieves complete separation of EOC patients from healthy controls and deserves further evaluation as a potential novel strategy for the early detection of EOC [22]. In another study, gas chromatography/time-of-flight mass spectrometry (GC–TOF MS) was used to analyze metabolite profiling of fresh frozen tumor samples from 66 invasive ovarian carcinomas and 9 borderline tumors of the ovary, showing that a statistically significant differentiation between borderline tumors and carcinomas as reflected by differences in 51 metabolites. This study indicated there is a consistent and significant change in primary metabolism of ovarian tumors, which can be detected using large-scale metabolic profiling [23].

These limited available data are encouraging and show that the potential utility of metabolomics in ovarian cancer diagnosis, but metabolomics is still in its infancy. For the future development and application of metabolomics, it will be important to prompt a full integration of metabolomics into the context of cancer research for entire analyses of molecular changes in malignant tumors.

5. Ovarian cancer biomarker panels

Given the complexity and heterogeneity of ovarian cancer, it is unlikely that a single biomarker will be able to detect all subtypes and stages of the disease with a high specificity and a high sensitivity. Many current studies show that combining several biomarkers dramatically improves sensitivity of CA-125 in ovarian cancer patients [24]. Markers have generally been analyzed only 2 or 3 at a time. The increased sensitivity achieved with markers in combination has generally been associated with a marked decrease in specificity [3]. A couple of biomarker panels have been published with adaptable sensitivity and specificity range, which might hold great potential for the detection of ovarian cancer [4]. For example, recently, a novel multiplex assay that used a panel of six serum biomarkers: leptin, prolactin, osteopontin, insulin-like growth factor II (IGF-II), macrophage inhibitory factor (MIF) and CA-125 and was studied on 362 healthy controls and 156 patients with newly diagnosed ovarian cancer (including 13 stage I cases), yielded 95.3% sensitivity and 99.4% specificity [25]. However, these data generated much controversy about experimental design and statistical analysis.
Most of impressive sensitivities and specificities for biomarker panels arose from relatively small numbers of samples (especially few cases of stage I diseases) without an independent validation study. So before biomarker tests are translated for routine use, more researches, such as retrospective and prospective clinical trials, are needed to evaluate the overall clinical utility of the tests. In the future, it will still be crucial to further develop panels of biomarkers not only for early detection but also for treatment guidance of ovarian cancer.

6. Conclusion

During the last decade, with the development of high-throughput technologies in genomics and proteomics, a number of biomarkers, some part of which were listed in Table 1, have shown promises across a variety of ovarian cancer studies and also provided new insights into ovarian cancer diagnosis, but few have turned out to be useful in clinic. It remains unclear, whether a single biomarker, a panel of biomarkers, or multiplexed information will yield the most accurate approaches to ovarian cancer detection. The strategies or technologies mentioned in this review hold significant promise in discovering more robust biomarkers for diagnosis, prognosis or prediction of therapy in ovarian cancer. At present, the research on ovarian cancer biomarkers is still under way in three main aspects: One is further validation and the ongoing clinical trials of available or potential biomarkers. Another is investigation of novel more specific and sensitive ovarian cancer biomarkers with further improved technologies on different biological levels. The third is development of multiple biomarkers for generating panels to maximize the sensitivity and specificity of detection. In the future, through effective integration of various more advanced technologies and help of bioinformatics, more useful biomarkers for ovarian cancer diagnosis are likely to emerge. Furthermore, sharing of information among the scientific community will quicken the pace in the field of biomarker research from different angles.

Conflict of interest statement

No potential conflicts of interest were disclosed

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References

SMRP and HE4 as biomarkers for ovarian carcinoma when used alone and in combination with CA-125 and/or each other

by Ingegerd Hellstrom and Karl Erik Hellstrom

1. There is a Need for Biomarkers to Detect Ovarian Carcinoma by Assaying Serum and/or Other Body Fluids

Assays measuring tumor antigens in serum have the advantage that they are noninvasive, quick, and relatively inexpensive. Early detection as well as monitoring of disease in treated patients requires high specificity and sensitivity and constant levels of circulating marker unless there is a change in the patient's clinical status. CA-125 is the present "gold standard" for diagnosis of ovarian carcinoma using serum samples (1-4). However, it is elevated in several nonmalignant conditions, which can lead to false-positive results (5). There is a need for additional markers to improve sensitivity with retained or better specificity, and many new biomarkers have been introduced and continue to be evaluated. Our group has focused on soluble mesothelin-related proteins (SMRP) and on HE4, a protease that is secreted into serum. In immunohistological studies of ovarian cancer samples with little or no detectable CA 125 expression, mesothelin and HE4 stood out as the most promising markers, when reactivity with normal tissues was taken into account (6). Other biomarkers in this study included HK4, HK6, OPN, claudin 3, DF3, VEGF, MUC I, and CA19-9.

2. SMRP as Marker for Diagnostic Assays of Serum and Urine

With the goal to obtain monoclonal antibodies (MAbs) for therapy, our group immunized mice with human ovarian carcinoma cells in the mid-1990s. This work resulted in MAb569, which reacts with ovarian carcinomas and has low reactivity with normal tissues except for the mesothelium. N-terminal amino acid sequencing of the antigen recognized by MAb 569 showed identity with the sequence of mesothelin, a tumor marker first described by Pastan's group (7), except for the lack of a 24 bp insert. By following our standard procedures for characterizing antigens detected by MAbs (8), we found the MAb569-defined antigen in supernatants of antigen-positive tumor cells and subsequently in malignant effusions, suggesting that it may be a marker for serum-based diagnosis. This finding was surprising because studies by Pastan's group had indicated that mesothelin is stably expressed at the cell surface and not released in to tumor culture supernatants or body fluids from cancer patients (9).
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