Biomarkers in Ovarian Cancer Screening

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The author has no conflicts of interest to disclose.

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Abstract

There are no effective early-detection modalities for epithelial ovarian cancer (EOC), the fifth leading cause of cancer death in women. However, the 5-year survival rate for women diagnosed with stage I disease is 90%, demonstrating the need for improved early-detection methods. Biomarkers are used in conjunction with clinical assessment, for screening and detecting cancer occurrence, and for determining response and recurrence. Currently, two biomarkers (CA-125 and HE4) are used for monitoring and identification of recurrence of EOC; a third marker (OVA1) is used as the differential in a preoperative setting. In addition to serum markers, researchers are looking at an ovarian cancer symptom index, which may improve specificity to malignancy detection. Ultimately, the clinical benefit of a marker depends on its behavior preceding the months and years prior to symptoms, while the malignancy is developing. The best screening test will detect the early-stage and noninvasive cancers.

J Adv Pract Oncol 2010;1:97–105

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer death in women, with an estimated 15,520 dying of the disease in 2010 (American Cancer Society [ACS], 2010). There are no effective early-detection modalities, and the majority of women with ovarian cancer will be diagnosed in late stage, with the 5-year survival rate less than 50% (National Cancer Institute [NCI], 2010a). However, for the 25% of women diagnosed with stage I disease, the 5-year survival rate increases to 90%, reinforcing the need for improved early-detection methods, including the use of serum biomarkers. Women at high risk for EOC include those with the *BRCA1* and *BRCA2* gene mutation, who have an estimated 39% and 22% risk, respectively, of being diagnosed with EOC by age 70 (Chen et al., 2006). Although this is the ideal population to study for risk assessment and to aid development of early-detection modalities, this subset accounts for less than 10% of the women diagnosed with EOC.

Tumor markers (or biomarkers) are molecules occurring in blood, urine, or tissue. They are used in conjunction with clinical assessment, for

screening and detecting cancer occurrence as well as for determining response and recurrence (ACS, 2010). Tumor markers are often detected by monoclonal antibodies and are frequently produced by the body in response to cancer or certain benign conditions (Hussain et al., 2010).

For a tumor marker to be effective in overall utility, and impact long-term survival, it must be highly sensitive for early-stage cancer detection (Hensley, 2010). Additionally, the test requires specificity for the target disease, thereby reducing unnecessary and expensive procedures, such as imaging and surgery (Yurkovetsky et al., 2010). A tumor marker should also add value to decision making with regard to diagnosis, treatment response, and recurrence of disease. Because of the low prevalence of ovarian cancer (average risk, 40/100,000), a high specificity is essential to achieve an acceptable positive predictive value (PPV; Andersen et al., 2010). PPV reflects the probability of a positive test detecting the underlying condition being tested, with the result indicative of the disease prevalence. For an ovarian cancer tumor marker to be successful as a screening strategy, it should demonstrate a sensitivity of at least 75% and a specificity of > 99.6%, giving it a PPV of 10%. A PPV of 10% translates into one in ten surgical interventions confirming an ovarian cancer diagnosis (Kulasingam, Pavlou, & Diamandis, 2010).

Currently, two US Food and Drug Administration (FDA)-approved tumor markers—CA-125 (cancer antigen 125) and HE4 (human epididymis protein 4; Fujirebio Diagnostics, Inc.)—are used for monitoring and identification of recurrence of EOC (Karst & Drapkin, 2010). A third marker, OVA1 (ovarian tumor triage test; Vermillion, Inc., 2010; Fremont, CA), is also used as the differential in a preoperative setting, as a basis for referral to a gynecologist-oncologist for a suspected malignancy for appropriate surgery (Quest Diagnostics Inc., 2010).

This article is the first in a series about biomarkers (also known as tumor markers). Look for future issues of the *Journal of the Advanced Practitioner in Oncology* for articles on biomarkers in other clinical settings. Article series will be a feature of the journal, and we welcome your suggestions for future installments. Please e-mail your ideas to editor@advancedpractitioner.com.

CA-125

Since its discovery in 1981, CA-125 is the most frequently used biomarker for EOC (Bast et al., 1981). CA-125 is an antigen expressed by fetal and amniotic and coelomic epithelium (mesothelial cells of the pleura, pericardium, and peritoneum). In the adult female, the antigen is derived from the celomic epithelium and mullerian epithelium (tubal, endometrial, and endocervical). CA-125 is present in low levels in healthy adults, in 50% of women with stage 1 ovarian cancer, and in up to 90% of women with advanced ovarian cancer (Karst & Drapkin, 2010). Because the serum CA-125 level may be elevated in a variety of benign conditions and nongynecologic malignancies, such as endometriosis; ovarian cysts; or cancers of the endometrium, breasts, and lungs, its specificity as a tumor marker is low (Fritsche & Bast, 1998; Sjovall, Nilsson, & Einhorn, 2002).

Serum CA-125 has a sensitivity of 50% to 60% in early-stage disease, where specificity is set at 99% in postmenopausal women (Yurkovetsky et al., 2010). A serum CA-125 level less than 35 U/mL is considered normal; however, in a premenopausal woman, the marker has less sensitivity and specificity. In a postmenopausal woman with a pelvic mass and ascites, a serum CA-125 level > 65 U/mL is cause for concern. Currently, there are no effective screening strategies for early detection of EOC, although the use of CA-125 with multimodalities such a bimanual pelvic examination and transvaginal ultrasonography (TVU) may increase early detection of EOC (Menon et al., 2005). Currently, the multimodality of TVU screening added to CA-125 measurement has not significantly improved the PPV in studies (Olivier, Lubsen-Brandsma, Verhoef, & van Beurden, 2006).

The National Institutes of Health (NIH) is conducting the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) screening trial for evaluation of early detection of these diseases (Buys et al., 2005). In the ovarian segment, the objective is to evaluate mortality as an outcome in women who are randomized to undergo both CA-125 screening and TVU. In the study, 78,237 healthy women, between 55 and 74 years of age, were randomly assigned to screening with annual CA-125 and TVU or to a control group (no intervention).

Table 1. Ovarian cancer screening trials: design parameters

	UKCTOCS	PLCO
Centers	12	10
Arms	3	2
Study population	50–74 years of age Post menopause	55–74 years of age Post menopause
Endpoints	Ovarian cancer mortality	Cause-specific mortality
Size	200,000 total 50,000 in each of two intervention arms and 100,000 in control arm	74,000 total 37,000 in each arm
Enrollment period	3 years	3 years
Screening length	6 screens, 1 year apart	4 screens, 1 year apart
Follow-up duration	Minimum of 7 years post randomization	Minimum of 10 years post randomization
Screening protocols	Annual TVU (n = 50,000) MMS using ROCA (n = 50,000)	Annual TVU, CA-125, bimanual pelvic exam

Note: UKCTOCS = UK Collaborative Trial of Ovarian Cancer Screening; PLCO = Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; TVU = transvaginal ultrasonography; MMS = multimodal strategy; ROCA = risk of ovarian cancer algorithm. Source: Menon et al., 2009; Buys, 2005.

From baseline data of 28,816 women screened, 436 women (1.5%) had an abnormal CA-125 level and a PPV for invasive cancer of 3.7 % (Buys et al., 2005). With 4-year follow-up, the PPV was 2.6% (Olivier et al., 2006). These studies reveal that the combination of CA-125 with TVU has not shown adequate sensitivity to warrant use in the general population.

However, the largest ongoing randomized trial, the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), has randomly assigned 202,638 postmenopausal women to one of three arms: no screening, annual TVU, or multimodal screening (MMS; Table 1). The intervention arm had better results. The sensitivity, specificity, and PPV for all primary ovarian and tubal cancers were 89.4%, 99.8%, and 43.3% for MMS and 84.9%, 98.2%, and 5.3% for TVU, respectively. The sensitivity of the MMS and TVU is higher than that reported in the PLCO trial, perhaps due to the trial design. The results of ongoing screening are still being obtained, so the effect of screening on mortality has yet to be determined (Menon et al., 2009).

Another approach to achieve improved sensitivity is to look at longitudinal measurements of CA-125, under the assumption that CA-125 levels will remain stable in benign disease but increase over time if ovarian cancer is present. A Bayesian algorithm is then used to plot serial CA-125 measurements over a period of years and to calculate the "probability" of ovarian cancer. Another algorithm is the risk of ovarian cancer algorithm, or ROCA (Figure 1; Skates et al., 2003). Theoretical challenges with biomarker sensitivity may be due to the heterogeneous nature of ovarian cancer. Bast (2003) reports that serum CA-125 antigen levels may increase exponentially over 10 to 21 months before diagnosis. Therefore, specificity would be improved with combination CA-125, TVU, and sequential monitoring of CA-125 levels over time.

However, discussion surrounds the biology of ovarian cancer and whether tumors arise from a single clone of cells in the ovaries, rather than multiple sites throughout the peritoneal cavity (Karst & Drapkin, 2010). The question arises whether stage I cancers might exhibit a different pattern of genetic abnormality, which permits growth and invasion but no metastasis. Or, in advanced disease, tumor cells could develop on the surface of the epithelium of the ovaries, then detach, implant, and spread without invading the ovaries. This pattern may demonstrate different biology than a stage 1 tumor that persists and invades over time (Bast, 2003).

Ovarian cancer has several distinct and mixed subtypes. Serous carcinomas tend to be the most common, most likely high grade, and aggressive. Elevation of CA-125 levels is mostly seen with serous carcinoma and is rarely noted with mucinous cell tumors (Høgdall et al., 2007). Due to the complexity and uniqueness of the disease,

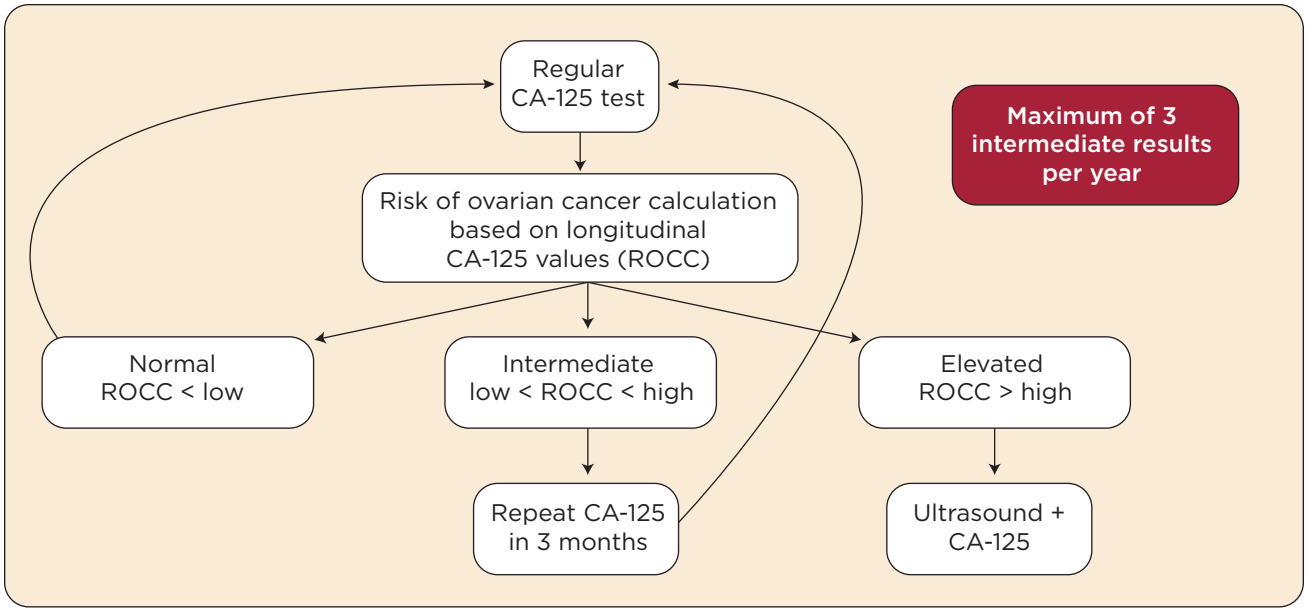

Figure 1. Risk of ovarian cancer algorithm (ROCA). Adapted from Skates et al., 2003.

consultation with a gynecologic oncologist preoperatively should be strongly recommended for any woman undergoing surgery for a pelvic mass suspicious for a gynecologic malignancy (Earle et al., 2006; NCCN, 2010a).

The primary use of CA-125 is to monitor the disease status of women with ovarian cancer. If the CA-125 level is elevated when a diagnosis of EOC is confirmed by pathology, the tumor marker becomes useful in assessing the patient's response to chemotherapy and remission status, or perhaps detecting early recurrence (Markman et al., 2006). Women with early-stage EOC (stages I–II) have improved survival when levels of CA-125 normalize after the first cycle of chemotherapy.

In a study of 427 women, normalization of CA-125 levels after one cycle of chemotherapy was associated with a recurrence-free survival of 87% and an overall survival of 92%, compared with 68% and 77%, respectively, in women whose CA-125 levels remained elevated (Chan, 2010). A favorable prognosis and response are reported to result in a median progression-free survival of 24 months when the CA-125 nadir is less than or equal to 10 U/mL following adjuvant chemotherapy (Chan, 2010; Markman et al., 2006).

HE4

HE4, a product of the *WFDCR* gene, is a new protein marker that although not disease specific, is shown to be overexpressed in ovarian cancer but not in benign conditions. Thus, HE4 is different from CA-125. HE4 is made up of two whey acidic protein domains and a four-disulfide core. It is an approved tumor marker for monitoring ovarian recurrence and disease progression as an adjunct to CA-125 levels (Quest Diagnostics, Inc., 2010). The reference range for the immunoassay is < 150 pM (Table 2). HE4 is not indicated as a screening marker for EOC, although it is currently under review as a diagnostic marker.

In a pilot study of 11 markers, the combination of CA-125 and HE4 had the highest predictive value of the combination markers (Moore et al., 2009). As a single marker, HE4 had a sensitivity of 72.9% when specificity was set at 95%.

Combination CA-125 and HE4 was tested on 225 women with ovarian and endometrial cancers, women with endometriosis, and healthy patients. The dual tests demonstrated a sensitivity of 92.9% at 95% specificity compared with a 78.6% sensitivity for either CA-125 or HE4 (Huhtinen et al., 2009). HE4 levels were elevated in both patients with endometrial and ovarian cancers, but unlike CA-125, it was not elevated in women with endometriosis. Similar to CA-125, mucinous and germ cell histologies rarely elevate HE4 levels (Quest Diagnostics, Inc., 2010). However, even with the promising results of the combination test using CA-125 and HE4, improvements are needed for a sensitivity

Table 2. Distribution of HE4 assay values

	Number of Subjects	Percent of subjects			
		< 150 pM	150.1–300 pM	300.1–500 pM	> 500 pM
Apparently healthy women					
Premenopausal	76	95	4	0	1
Postmenopausal	103	94	5	0	1
Malignant conditions					
Ovarian cancer	127	21	14	16	48
Breast cancer	46	87	9	4	0
Lung cancer	50	58	30	12	0
Endometrial cancer	116	74	13	3	9
Gastrointestinal cancer	56	84	14	0	2
Nonmalignant conditions					
Pregnancy	22	95	5	0	0
Benign gynecologic disease	347	93	5	0	1
Other benign disease	108	76	7	6	10
Hypertension/congestive heart failure	96	78	17	2	3

Note: 1,147 serum specimens; 94% of healthy female patients had an HE4 assay level ≤ 150 pM. Source: Quest Diagnostics, Inc., 2010

of 75% and a specificity of 99.7% to achieve the target PPV of 10%.

Symptom Index

In addition to serum markers, researchers are looking at an ovarian cancer symptom index (SI), which may improve specificity to malignancy detection (Goff, Mandel, Melancon, & Muntz, 2004). Sensitivity of the SI was 56.7% for women with early-stage disease and 79.5% for women with advanced-stage disease. For women younger than age 50, the specificity was 86%, and for women older than age 50, it was 90% (Goff et al., 2007). Characteristics of a positive SI in women with ovarian cancer include the presence of pelvic or abdominal pain, bloating, increased abdominal girth, and early satiety (occurring more than 12 times in a month) over the course of a year or less (Goff et al., 2004a).

Goff et al. (2010) presented results of a prospective case-control study of 74 women with ovarian cancer and 137 controls. A three-marker decision rule using SI, CA-125, and HE4 was used to predict ovarian cancer. When the SI was used alone, sensitivity was 64% and specificity was 88%. The CA-125 level had the highest overall sensitivity at 95% and a specificity of 81% (67.7% and 78.6% for early-stage disease and high-risk women, respectively). The HE4 level had the best overall sensitivity of 95% and specificity of 100% in high-risk groups. When all three tests were used together, the sensitivity was 84% (67.7% and 100% for early-stage disease and high-risk women, respectively), and the specificity was 98.5% (Andersen et al., 2010). The authors concluded that the combination of the three-marker tests together had an increased sensitivity and warranted a three-step strategy prior to imaging (Goff et al., 2010). More research needs to determine whether this three-test approach will become an effective annual first-line screening method.

OVA1

OVA1 is the first blood test approved by the FDA to determine the likelihood of malignancy in the presence of an ovarian mass. OVA1 is not a biomarker per se but a protein-based in vitro diagnostic mulitvariate index assay, used as an algorithm in evaluating the need for a referral to a gynecologist-oncologist (Quest Diagnostics, Inc., 2010). Several nonrandomized studies (Earle et al., 2006; Bristow, Tomacruz, Armstrong, Trimble, & Montz, 2002) suggest improved survival for women with EOC if surgery is performed by

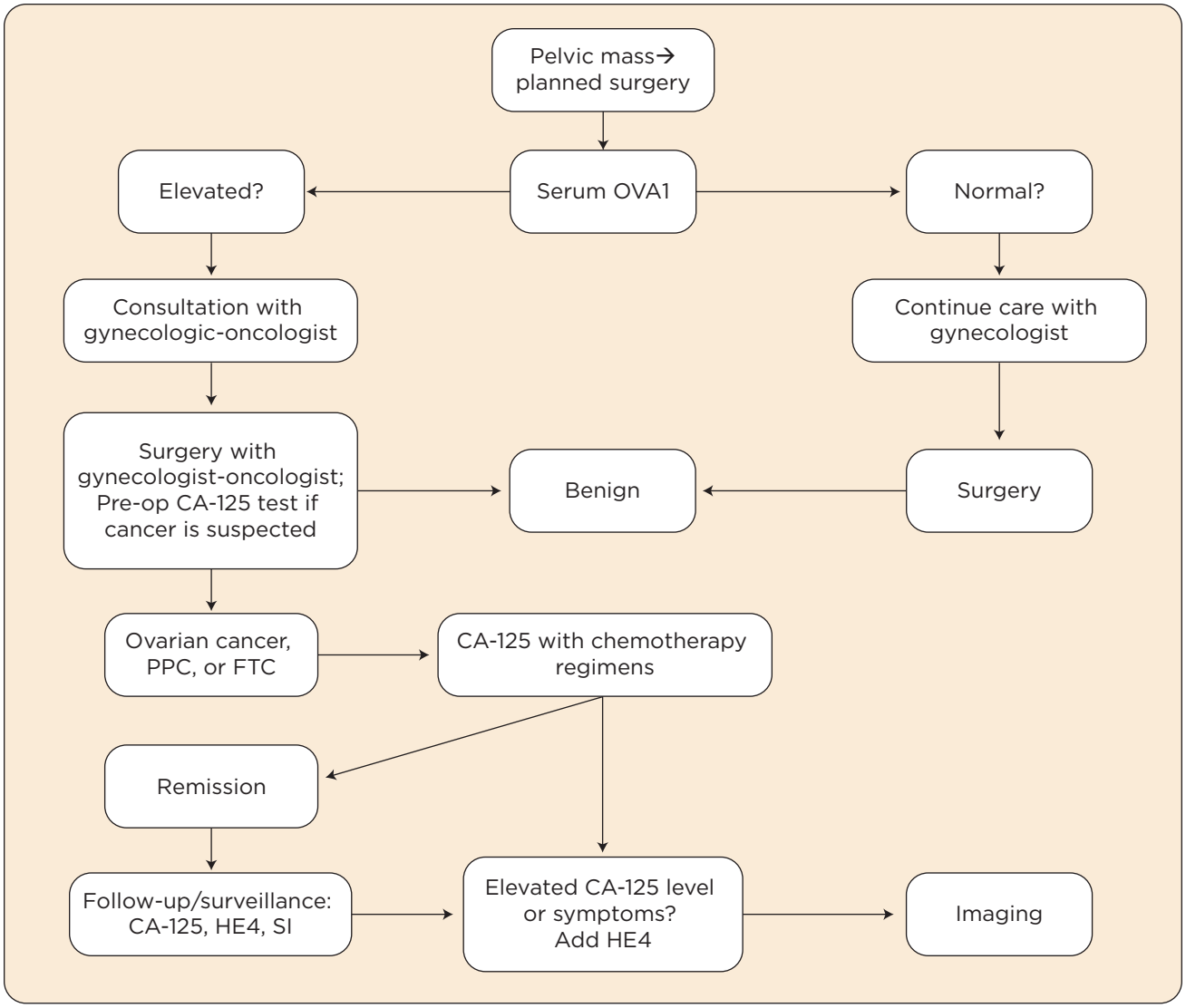

Figure 2. Algorithm for OVA1, CA-125, and HE4 markers. SI = symptom index (presence of pelvic or abdominal pain, bloating, increased abdominal girth, and early satiety over the course of a year or less); PPC = primary peritoneal cancer; FTC = fallopian tube cancer.

a gynecologist-oncologist. The OVA1 provides a measure of five protein biomarkers (transthyretin, apolipoprotein A1, beta-2 microglobulin, transferrin, and CA-125) in serum blood to determine the likelihood of cancer.

In the pivotal OvaSure-1 trials, there were 524 evaluable women between the ages of 18 and 92 years. All patients had a documented ovarian mass and a planned surgical intervention. The group was divided into two subsets: 284 from the University of Kentucky and another from 27 demographically diverse sites (Ueland, Zhang, Crutcher, & Fung, 2009). Samples were also stratified by menopausal at-risk status and by low- versus high-risk groups. The study outcome was identification of women who had a higher likelihood of malignancy. The algorithm allowed for detection of 90% of stage I EOCs and 100% of stages II–IV cancers, with a greater than 90% negative predictive value in both premenopausal and postmenopausal women (Ueland et al., 2009). The test is approved for women older than age 18 with an ovarian adnexal mass and scheduled surgery, prior to a gynecologic-oncologist consultation (Figure 2). The OVA1 is not a screening test for ovarian cancer (Quest Diagnostics, Inc., 2010).

In 2002, Petricoin and colleagues published their findings of serum protein patterns used to detect ovarian cancer. They reported that the proteomic analysis performed with a sensitivity of

100% and a specificity of 95%, identifying 50 of 50 cancers and 63 of 66 noncancer samples. The platform used mass spectroscopy, a technique used to separate proteins and molecules and to distinguish a unique pattern, or fingerprint, among thousands of proteins, to differentiate ovarian cancer samples from women with noncancerous conditions. However, concerns were raised that the results were not reproducible, with no evidence for technology validation and a lack of peer-to-peer publications on the sensitivity and specificity of the test using the new technology. Although the results were impressive, a 5% false-positive rate remained high, risking the potential of unnecessary tests and surgery (NCI, 2010b). Further testing was recommended prior to marketing this test.

Proteomics appears to have a favorable future as a more sensitive and specific tumor marker. Identification of protein patterns and characteristics is performed through surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) and matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) technology. SELDI-TOF has shown to discriminate proteomic spectra patterns that differentiate serum proteins of patients with ovarian cancer from those of patients with nonmalignant conditions (Tinelli et al., 2007). Larger, population-based studies are currently being conducted. Researchers from NCI and the FDA Clinical Proteomics Program are working with Correlogic Systems, Inc., to approve OvaCheck, a blood test for the early detection of ovarian cancer.

Theoretically, a panel of combined biomarkers could increase the sensitivity and specificity of ovarian cancer, enabling early detection prior to the presentation of symptoms. A study published by Visintin et al (2008) initially reported a six-panel biomarker using a multiplex, bead-based immunoassay system, which resulted in a 95.3% sensitivity and a 99.4% specificity for the detection of ovarian cancer. The authors indicated that the PPV for the general population was above the suggested 10% necessary to be used as a screening test. Based on these results, LabCorp announced the availability of this test under OvaSure (LabCorp, 2008). However, because it did not have FDA approval to market the test, and the data were not provided to support the manufacturer's claims, the OvaSure test was removed from the market. The authors subsequently provided correction to the original results and retracted the recommendation for general screening (Visintin et al., 2008).

Multimarker Assay

Yurkovetsky and colleagues (2010) analyzed 96 biomarker combinations in women with early-stage ovarian cancer; benign gynecologic tumors; and cancer of the breasts, colorectum, and lungs in addition to healthy controls. The objective was to distinguish early-stage ovarian cancer from advanced-stage ovarian cancer. Using a panel of CA-125, HE4, CEA (carcinoembryonic antigen), and VCAM-1 (vascular cell adhesion molecule) and a sample of 44 patients with early-stage ovarian cancer, 124 with advanced-stage cancer, and 929 healthy women, the authors reported an 86% and 95% sensitivity in early-stage and late-stage cancers, respectively. Specificity was 98% for advanced-stage disease. The study was blinded to 210 patients with breast cancer, 31 patients with colorectal cancer, and 74 patients with lung cancer. The biomarker panel correctly identified 94% of breast cancers, 100% of colorectal cancers, and 64% of lung cancers and nonovarian cancer. More research will be ongoing to determine whether a multimarker assay could be used in a first- or second-line setting or with the addition of a two-step strategy such as TVU. Other markers being studied include the combination of CA-125, mesothelin, and HE4 (Hussain et al., 2010).

Conclusion

Currently, monitoring CA-125 levels in combination with TVU is only recommended for high-risk women. This group includes, but is not limited to, women with first- and second-degree relatives with breast and/or ovarian cancer or a personal history of *BRCA1/2* mutation (NCCN, 2010b). The potential benefit associated with screening is the ability to detect ovarian cancer in an early, and potentially curable, stage, resulting in a reduction in mortality. Unless a biomarker is sufficiently specific and sensitive to detect early-stage ovarian cancer, there will not only be an emotional burden but an economic one, resulting in unnecessary imaging and surgical intervention. The use of CA-125 and HE4 levels in the recurrent ovarian cancer setting may identify earlier-onset recurrences. However, the clinical benefit of a marker depends on its behavior preceding the months and years prior to symptoms, while the malignancy is developing.

The best screening test will detect the early-stage and noninvasive cancers. Until the results of the PLCO and the UKCTOCS trials are available, the ability to reduce mortality remains unknown. The current convergence of advances in molecular biology and advanced technologies will assist scientists as they unravel the complexity of genes, proteins, and the utilization of biomarkers for early detection of ovarian cancer.

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