

COVER STORY: TRI-POINT SERIES

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Key

To



Women with ovarian cancer are often diagnosed too late—80% of these tumors are found when the disease has already metastasized. However, due to discoveries of markers such as CA125 and HE4, and improved early identification of symptoms, women with this disease may face a better prognosis: Diagnosis in the earliest stages brings a 5-year survival rate of 65%–93%, according to the American Cancer Society. In this article, a clinician, a basic scientist, and a clinical researcher share their thoughts on the latest ovarian cancer biomarkers.



Ovarian Cancer Detection

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PERSPECTIVES

Clinical Practitioner Perspective



► By Nita K. Lee, M.D., M.P.H.

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Highlights

- Ovarian cancer produces symptoms that can facilitate detection and treatment.
- When symptoms and an exam are suspicious for pelvic masses, patients should have a pelvic exam, targeted imaging, and appropriate referral to gynecology or gynecologic oncology.
- Routine CA125 or US screening of asymptomatic, low-risk women with normal clinical exams is not recommended.
- Patients with hereditary breast and ovarian cancer syndromes or a strong family history of breast and/or ovarian cancers should strongly consider screening with transvaginal US, CA125, and regular clinical pelvic examinations.
- Future screening tools will likely encompass multimodal techniques, including biomarker panels, US, novel radiologic techniques, and possible symptom indices.

Epidemiology and Ovarian Cancer Survival

Epithelial ovarian cancer remains the leading cause of death in women with gynecologic cancers in the United States. The American Cancer Society estimated 21,550 new cases and 14,600 deaths due to ovarian cancer in 2009.¹ More than two-thirds of cases present with stage III–IV disease, indicating disseminated intraperitoneal spread or distant metastasis. Despite strides in treatments with surgery and chemotherapy, the prognosis is grave in advanced stages, with an overall survival of 16%–40%. In contrast, survival is 65%–90% for early stage disease. Much effort is being focused on earlier detection of ovarian cancer and population-based screening, in the hope that this will translate into improved survival.

Good Screening Test Criteria

An ideal screening test should have high sensitivity and high specificity. Because invasive surgery is currently necessary for definitive diagnosis of ovarian cancer after a positive screen, avoiding false positives and maintaining an acceptable positive predictive value (PPV) is important. Development of screening tests is limited by low disease prevalence in the general population and the lack of a clear precursor lesion for invasive ovarian cancer.

Ultrasound as a Screening Tool

Transvaginal ultrasound (US) remains a tolerable, low-risk technique. Parameters such as ovarian volume and tumor complexity have been associated with malignancy. In a study of 25,327 asymptomatic women screened annually with transvaginal US, 364 (1.4%) had surgery, which identified 44 ovarian cancers and 7 metastatic cancers.² Screening had a sensitivity of 85%, a specificity of 98.7%, and a PPV of 14%. About 60% of patients had a family history of ovarian or breast cancer, making generalizations to low-risk populations difficult.

Newer techniques in US imaging may improve performance, but cost-effectiveness is likely limited in the general population.

CA125 and Novel Biomarkers

CA125 is a glycoprotein that can be overexpressed in ovarian cancers. Serum CA125 is often elevated in these cancers and is used to follow treatment response. As a screening biomarker, CA125 is limited due to false positives stemming from a number of other conditions, such as endometriosis, benign ovarian cysts, pelvic inflammatory disease, pregnancy, and even gastrointestinal pathology. Close to 50% of patients with early stage cancers could have normal CA125 values. CA125 is not recommended as a screening test in asymptomatic women, but can be used to evaluate postmenopausal women with adnexal masses. A newer approach to CA125 employs mathematical models to monitor an individual patient's CA125 levels over time. This Risk of Ovarian Cancer Algorithm (ROCA) suggests that CA125 trends in an individual might better predict ovarian cancer development than isolated single values, and could be used as part of a screening program.³

Human epididymis protein 4 (HE4) is elevated in the serum of ovarian cancer patients, and is the only other serum biomarker available clinically and approved by the U.S. Food and Drug Administration (FDA) for monitoring recurrent disease. It is not recommended for use as a screening test in asymptomatic women. Researchers are investigating other serum and panel biomarkers.

Multimodality Screening

Multimodality screening employing US and serum markers have shown more promise in both the general and high-risk populations. Two large-scale, prospective, randomized trials in the United States and the United Kingdom are under way to determine this screening's impact on mortality.

Symptom Recognition and Improved Triage

In contrast to the popular belief that ovarian cancer is asymptomatic, many studies have established that patients have specific symptoms and often seek care in the months preceding their diagnosis.⁴ Better understanding and education of patients and providers about what these symptoms are should help raise suspicion levels and lead to further work-up as needed. Earlier detection and referral could influence overall survival by finding cancers at an earlier stage and potentially lowering disease volume at the time of diagnosis and initial surgery.

Goff and colleagues developed a symptom index, which is positive if women report symptoms of bloating or increased abdominal size, abdominal or pelvic pain, difficulty eating, or early satiety. Symptoms must occur more frequently than 12 times per month and within the last 12 months. The authors have investigated the symptom index combined with CA125 and other serum markers and reported improved performance.⁵

Patients with such persistent and suspicious symptoms should undergo a thorough pelvic exam and US imaging or a CT scan. They can then be referred for surgical management with general gynecology or gynecologic oncology, as appropriate.

General Low-Risk Population Screening

Screening asymptomatic women who are at low risk for ovarian cancer is currently not recommended due to several limitations in current modalities⁶ and because no studies have shown a clear improvement in overall survival in this screened population.

High-Risk Population Screening

Approximately 10% of ovarian cancers are hereditary. Patients with hereditary breast and ovarian cancer syndrome (*BRCA1* or *BRCA2* mutations) have a lifetime risk of ovarian cancer as large as 47% and 25%, respectively. Given their high disease rates, these patients have been targeted for screening programs. Women who do not undergo prophylactic surgery to remove fallopian tubes and ovaries should undergo multimodality screening, with concurrent transvaginal US and CA125 testing, every 6 months.⁷

Basic Researcher Perspective

► By Kimberly K. Leslie, M.D., and David Bender, M.D.

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Highlights

- Targeting three molecular pathways—PARP in *BRCA*-null tumors, VEGF, and mTOR—has shown clinical activity in advanced ovarian cancer; these pathways are central to ovarian cancer progression.
- Serous ovarian cancer is one of three tumor types to be analyzed through the National Cancer Institute's "The Cancer Genome Atlas" (TCGA) project.
- Effective, large-scale population screening for ovarian cancer remains elusive.

The Challenge Ahead

Ovarian malignancies of epithelial origin constitute the most lethal gynecologic tumors. About 70% of patients present with advanced disease involving the upper abdomen. To make a major impact on clinical outcomes, widespread population-based screening for detecting earlier stage disease, better first-line chemotherapy, and individualized molecular therapy must become available. These goals cannot be reached until we have achieved a more complete understanding of the biologic underpinnings of ovarian carcinogenesis.

Understanding the Biology of Epithelial Ovarian Cancer

Ovarian cancer is a complex disease on the molecular level. In early phase clinical trials, the recent success of new targeted agents in prolonging progression-free survival transformed our understanding of ovarian cancer biology. Clinically active therapies against poly (ADP-ribose)-polymerase (PARP), the vascular endothelial growth factor (VEGF) pathway and other angiogenesis networks, and PI3 kinase/AKT/mTOR provide insight into the mechanisms that drive ovarian cancer growth and progression.¹ The recent observation that targeting one form of DNA repair with PARP inhibitors is especially effective in tumors with *BRCA* mutations or gene silencing through methylation is revealing; these tumors, having lost their ability to repair single-strand DNA breaks by homologous recombination due to nonfunctional *BRCA*, rely on non-homologous end-joining that is mediated by PARP. Inhibiting PARP significantly prolonged survival in a phase I study of ovarian cancer patients with *BRCA* mutations who had previously failed chemotherapy—some were resistant to platinum.²

To further our understanding of molecular ovarian carcinogenesis, ovarian serous carcinoma—the most common ovarian epithelial tumor type—has been chosen as one of three cancers to undergo extensive molecular characterization by the National Cancer Institute's (NCI) "The Cancer Genome Atlas" (TCGA) project. Data are just beginning to become available through the NCI TCGA portal; no publications are yet listed describing findings from this dataset. However, one preliminary report covering methods of TCGA data analysis suggests that mutations in *AKT*, *KRAS*, *PTEN*, *TP53*, and *BRCA* are likely to be important in this disease.³ Mutations in these pathways are more likely to be "drivers" of epithelial ovarian malignancy and metastasis than "passenger" mutations, which are not central to tumor growth and progression. The challenge will be to apply TCGA findings in clinical practice. To date, the biologic profiles of ovarian tumors have not been sufficiently incorporated into treatment plans and options.

More recent pathologic and genomic data (*p53* signatures) suggests that serous ovarian cancers may originate from the fimbriated end of the fallopian tube and not the ovarian epithelium. In addition to the similarity between the epithelium of the fallopian tube and ovarian cancer at the genomic level, an intense pathologic evaluation of fallopian tube segments identified serous intraepithelial carcinomas in

women with ovarian cancer, which suggests a tubal origin for the disease.⁴ A better understanding of the origin of these high-grade serous carcinomas will have future implications for disease screening, prevention, and treatment.

Elusive Early Detection Biomarkers

Early detection of asymptomatic women with ovarian cancer by reliable population screening is required to improve outcome. Detecting these patients by proteomic mass spectrometry serum screening remains but a dream, despite reports of early success that engendered enthusiasm.^{5,6} The prevalence of ovarian cancer in the postmenopausal population is 1 in 2,500. This low prevalence makes population-based screening a challenge. A high sensitivity of more than 75% for early stage disease testing is needed.⁷ Specificity must be very high (> 99.6%) to give a PPV of 10%—the lower limit of acceptability—meaning that 10 surgeries must be performed to diagnose 1 ovarian cancer case. Serum CA125 measurement is currently the most widely employed ovarian cancer biomarker. CA125 is a high-molecular-weight mucinous glycoprotein (MUC16). Although up to 80% of ovarian cancers are associated with elevated serum CA125 levels (> 35 U/ml), only 50% of patients with early stage disease will have a high level. Hence, CA125 as a biomarker is often not useful for identifying early ovarian malignancies. Another proposed early marker is the serum protein HE4, which is overexpressed in epithelial ovarian cancers and is FDA-approved to monitor patients with epithelial ovarian cancer.⁸ In combination, HE4 and CA125 may be more useful than either test alone. Employing both markers as a dual test to distinguish benign from malignant ovarian masses is currently under investigation in large, multi-center trials, and other markers are also undergoing validation.⁹

In conclusion, research on the mechanisms of ovarian carcinogenesis and its early detection has been challenging. However, with TCGA on the horizon and the ongoing identification of new screening biomarkers, the future appears to be brightening.

OVARIAN CANCER IS A COMPLEX DISEASE ON THE MOLECULAR LEVEL. IN EARLY PHASE CLINICAL TRIALS, THE RECENT SUCCESS OF NEW TARGETED AGENTS IN PROLONGING PROGRESSION-FREE SURVIVAL TRANSFORMED OUR UNDERSTANDING OF OVARIAN CANCER BIOLOGY.

Clinical Researcher Perspective

► By David A. Fishman, M.D., and Sonia Dutta, B.S.



Ms. Dutta is a fourth-year medical student and Howard Hughes Medical Institute Research Fellow working with Dr. Fishman, who is professor and director of Gynecologic Oncology Research and the National Ovarian Cancer Early Detection Program at Mount Sinai School of Medicine, New York City.

Highlights

- At initial diagnosis, most women have advanced stage disease; if early stage epithelial ovarian carcinoma is detected, not only is treatment less radical, but survival and quality of life are much improved.
- Current approaches to ovarian cancer screening do not have adequate sensitivity and specificity to precisely detect early stage disease.
- Among the hundreds of new biomarkers identified, none has clinical validation to act as a sole biomarker of early stage ovarian cancer.
- Novel imaging techniques, such as contrast ultrasonography, may have clinical value as adjuncts to newly developed serum biomarkers for detecting early stage disease.

Although all cancer patients can potentially benefit from early detection, a suitable screening method for epithelial ovarian carcinoma (EOC) has yet to be developed. Evidence suggests that developing effective strategies for early detection will require design tools based on the molecular, genetic, and biochemical events that regulate carcinogenesis, invasion, and metastatic dissemination. Major impediments include the molecular heterogeneity of tumors, the frequency of benign diseases that reduce biomarker specificity for cancer, and low biomarker concentrations, especially for early stage disease. The pathophysiologic events shared between cancer and prevalent non-cancer conditions confound biomarker specificity. Finally, early stage disease occurs in a small volume of tissue, limiting the serum/plasma/urine biomarker concentration to a level below the detection threshold of most diagnostic platforms.

In the hope of discovering the ideal biomarker, multiple groups have used technologies such as cDNA microarrays, mRNA arrays, and proteomics to uncover various proteins associated with the tumor microenvironment. Research has identified hundreds of molecules, with several of recent interest:

1. **Lysophosphatidic acid** is a phospholipid secreted by ovarian tumors, with known functions in cell proliferation, invasion, and angiogenesis. Since 1998, this acid

has shown potential as a biomarker, given its accurate detection of early stage disease and its relatively high sensitivity of 95% and specificity of 89%.¹ Unfortunately, its utility as a biomarker remains weak because it is also elevated in many benign conditions.

2. **HE4**, a protease inhibitor, is overexpressed in EOC and has an increased sensitivity for detecting cancer at stage I. It is as sensitive as, yet more specific than, CA125.² Additionally, multiple groups have studied HE4 in combination with CA125, with promising results. The highest sensitivity obtained has been 96.3% and 92.9% specificity.³
3. **Osteopontin** is a glycoprotein involved in cell adhesion, inflammation, and tumorigenesis. Elevated osteopontin is associated with the progression of multiple cancers, including ovarian.⁴ The sensitivity and specificity of this protein are inferior to CA125. However, when combined, sensitivity rises to 93.5%, whereas the specificity decreases considerably.⁴ Recently, osteopontin has been used in multiplex assays with other proteins (prolactin, leptin, IGF-II), showing potential as an early detection tool.⁵
4. **Kallikrein (KLK)** is the general name for a group of serine proteases that regulate proteolytic cascades functioning in cell growth, angiogenesis, and invasion. Most of the 16 family members are overexpressed in cancer and show value as prognostic markers. KLK8 is associated with early disease, whereas KLKs 5, 6, 10, and 13 achieve better sensitivities and specificities when combined with CA125.^{6, 7}
5. **Patient-Derived Tumor-Reactive Antibodies:** Tumor antibodies are found in circulation soon after tumor development. Compared with other biomarkers, they are stable and less sensitive to confounding factors such as stress and sample manipulation.⁸ Recently, researchers discovered unique antibodies associated with early and late stage disease. The combination of 4 antibodies—nucleophosmin, cathepsin D, GRP78, and SSX—appears to be specific for stage I EOC.⁸

Currently, it does not seem that a single biomarker test will achieve sensitivity and specificity adequate for accurately detecting early stage ovarian cancer. Consequently, other tools must be employed to increase the precision with which we detect early stage EOC, specifically emerging diagnostic imaging techniques such as contrast-enhanced ultrasonography.

CURRENTLY, IT DOES NOT SEEM THAT A SINGLE BIOMARKER TEST WILL ACHIEVE SENSITIVITY AND SPECIFICITY ADEQUATE FOR ACCURATELY DETECTING EARLY STAGE OVARIAN CANCER.

In collaboration with Arthur Fleischer's group, our group has been investigating contrast-enhanced US as a tool to detect early stage ovarian cancer. Although microscopic stage I disease is notoriously resistant to US detection, recent sonographic developments involving harmonics, pulse inversion, and contrast agents justify the hope that depiction of the aberrant tumor microvasculature associated with early stage disease can have clinical value. In vitro contrast agents consist of small, stabilized microbubbles, on the order of 1–10 μm in diameter, which allow visualization of tumor neovascularization by delineating leaky vascular channels unique to malignancy.⁹ This enhanced ability to quantify and visualize vascular changes specific to early stage disease may facilitate detecting this cancer stage with greater accuracy.⁹ Our group has reported that quantification of contrast-enhancement kinetics has 100% sensitivity, 96.2% specificity, and the potential to accurately differentiate benign from malignant tumors.⁹

Today, we are in an era in which it is possible to decrease the morbidity and mortality associated with ovarian cancer. Advancements in proteomic technologies are facilitating continuous discovery of hundreds of interesting and hopefully clinically relevant proteins. As these biomarkers are further studied and their sensitivities and specificities determined, we hope that a clinically validated panel of multiple analyses will be developed which, in combination with new diagnostic imaging techniques, can accurately detect early stage EOC. ■

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