**The Biology Behind**

**Ovarian Cancer Biomarkers in Urine**

Commentary on Ye et al., p. 432

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In this issue of *Clinical Cancer Research*, Ye et al. (1) have taken steps to address the pressing need for the identification of biomarkers for use in detecting ovarian cancer.

For the past 30 years, the statistics have remained the same. The majority of women who develop ovarian cancer will die of this disease. It is the fifth leading cause of all female cancer-related deaths in North America, and the most prevalent and lethal of the gynecologic cancers (2, 3). The disease carries a 1:70 lifetime risk, and >60% of the women who develop ovarian cancer will die from their disease. Despite improved knowledge of the etiology of the disease, aggressive cytoreductive surgery, and modern combination chemotherapy, there has been little change in mortality. Poor outcomes have been attributed to two notable deficiencies in the ability to manage this disease. Lack of an adequate screening test for early disease detection, in combination with only subtle presentation of symptoms at this stage, result in the diagnosis frequently being made only after progression to later stages, at which point the peritoneal dissemination of the cancer limits effective treatment. Improvement in the 5-year survival rate of patients with ovarian cancer has also been prevented by the frequent development of resistance to standard chemotherapeutic strategies. Resistance to cell death is a fundamental characteristic of cancer cells, and a primary cause of treatment failure in recurrent ovarian cancers (4, 5).

The potential effect of early detection of ovarian cancer on patient outcome is strongly implied by the differential survival rates of women diagnosed at different stages of disease progression. The overall 5-year survival of women with ovarian cancer is <30%, whereas up to 90% of women diagnosed at stage I are likely to be alive after 5 years (6). This success is, at least in part, due to the ability to optimally debulk tumor tissue when it is confined to the ovaries, which is a significant prognostic factor for ovarian cancer (7, 8). Figure 1 shows graphically the dramatic differences in survival for women with ovarian cancer that is detected when it is localized, compared with disease that has spread regionally or to distant sites. The excellent survival rates for women with early stage disease provide a strong rationale for research efforts to develop strategies to screen for early stage ovarian cancer.

Reasoning that the protein profile in urine may be less complex than that in blood, and that proteins in urine may be more stable than those in blood, as well as recognizing the convenience that a urinary test would have when compared with a more invasive blood test, Ye et al. (1) assessed the protein/peptide profiles in the urine of women with ovarian cancer. The goal of this work was to identify urinary proteins or peptides that might be evaluated as candidates for use as a screening tool for ovarian cancer. A two-step proteomics approach was taken. First, pooled urine samples were assessed using mass spectrometry and two-dimensional gel electrophoresis, in order to identify changes common to patients with ovarian cancer but not found in samples from non–cancer patients. Thus, the candidate markers identified were then evaluated for further testing, by obtaining antibody reagents and ELISAs. The second phase then involved using these assays to screen urine samples from women with ovarian cancer, compared with several control populations (cancers other than ovarian, benign conditions, and healthy controls). These analyses have identified two potential urinary biomarkers that seem to be elevated in ovarian cancer (1).

The first of the markers identified in this way was a glycosylated form of eosinophil-derived neurotoxin—also known as eosinophil protein X. Glycosylated forms of eosinophil-derived neurotoxin were found to be elevated by ~2× in patients with ovarian cancer compared to those with benign conditions, whereas nonglycosylated eosinophil-derived neurotoxin levels were similar in both groups. The second of the markers identified by Ye et al. (1) is a series of several COOH-terminal fragments of the secreted protein osteopontin. A cluster of osteopontin fragments, revealed by two-dimensional electrophoresis, was detected in patients with ovarian cancer but not in controls. When these two markers were used in combination, the sensitivity at detecting ovarian cancer was found to be 72% in the samples used, with 95% specificity versus specificity of 47% and 63% for osteopontin fragments or glycosylated eosinophil-derived neurotoxins, respectively, when used alone.

Neither osteopontin nor eosinophil-derived neurotoxin are cancer-specific markers. Both fragments have been associated with systemic inflammation as well as other non–cancer conditions. Eosinophil-derived neurotoxin in urine, for example, has been shown to be elevated in various eosinophilic conditions (9), and to change over the course of pregnancy (10). Interestingly, eosinophil-derived neurotoxin has been shown to be one of several proteins isolated from urine that have antitumor and antiangiogenic activity via induction of apoptosis of endothelial cells (11), although the relationship—if
any—of this finding to the study of Ye et al. (1) is not understood.

Osteopontin has also been shown to be elevated in inflammatory conditions (e.g., ref. 12). There is also a growing literature documenting increased osteopontin levels in the tumors and blood of patients with cancer. Elevated osteopontin in tumor tissue has been shown in a wide variety of cancers (e.g., refs. 13, 14). Elevated osteopontin plasma or serum levels have been found in patients with ovarian cancer (15), as well as multiple other tumor types, including metastatic breast cancer (16, 17), hormone refractory prostate cancer (18) and bladder cancer (19), and in some cases, these levels have been associated with poor patient prognosis. For both osteopontin and eosinophil-derived neurotoxin, however, the significance and degree of specificity for cancer of the glycosylated form (eosinophil-derived neurotoxin) or COOH-terminal cleavage fragments (of osteopontin) described by Ye et al. (1) remain to be explored. This information will be important in determining the specificity of the measurement of these fragments to detect ovarian cancer, especially in a screening setting.

Osteopontin fragments in urine could be generated by proteinases present in the urine (20). Proteinases have been detected in the urine of patients with cancer (e.g., refs. 21, 22), and proteinase inhibitors are also present in urine (23). Thus, care will need to be taken to ensure that urine collection and storage are uniform in trials assessing the usefulness of the urinary biomarkers described by Ye et al. (1), and that osteopontin fragments are not created following the collection of samples. Interestingly, urine matrix metalloproteinases have been proposed as a potential screening test for gynecologic malignancies, including ovarian cancer (24).

It is interesting to speculate on the source of the osteopontin fragments detected by Ye et al. (1). In Fig. 2, the COOH-terminal osteopontin fragments described by Ye et al. (1) are compared with fragments that would be generated by the cleavage of osteopontin by matrix metalloproteinases MMP-3 and MMP-7 as well as thrombin. It is not known if the osteopontin fragments identified (1) arise due to cleavage by specific proteases present in the urine of patients with ovarian cancer, or are reflective of some other cancer-associated state. It also will be of interest to determine which enzymes are responsible for the fragments, if they are indeed proteolytically generated, and to determine if the osteopontin fragments play a functional role. It has been suggested that osteopontin fragments are important in regulating access of the protein to various integrins to which osteopontin can bind (Fig. 2). The significance of osteopontin fragments of various sizes, including those described by Ye et al. (1), remains poorly understood, but this information might provide insights into the biology of osteopontin in ovarian cancer.

As noted above, there is a pressing need for early detection of ovarian cancer, in order to identify more patients at earlier stages of the disease, where survival is considerably improved (Fig. 1). There are, however, numerous challenges associated with screening for ovarian cancer. The inaccessibility of the ovaries and the absence of a confirmed premalignant condition make screening for preclinical disease particularly difficult. Because ovarian cancer is relatively rare, any useful screening method must be highly specific for ovarian cancer.

![Survival for women with ovarian cancer](Fig. 1) Survival for women with ovarian cancer is directly related to stage of the disease at presentation. Ten-year survival is nearly 90% when the disease is localized to the ovaries at the time of diagnosis, dropping to just under 60% for disease that has spread regionally, and only ~20% if the disease has spread to distant sites at the time of diagnosis. Data from the Surveillance, Epidemiology, and End Results database (40).
malignancy, and it has been estimated that a specificity of 99.7% is needed to achieve a positive predictive value of 10%, with a sensitivity of 67% in postmenopausal women (25). Optimal screening also requires sufficient sensitivity to detect early, preclinical disease to enable interventions that will improve patient outcomes.

Advances in technology during the last decade have stimulated increased research efforts in ovarian cancer detection by screening for preclinical, early stage disease using imaging techniques and serum biomarkers. The most thoroughly investigated biomarker in ovarian cancer screening is CA125, a mucin-type glycoprotein that is elevated (i.e., ≥35 units/mL) in 83% of patients with ovarian cancer. Unfortunately, levels are elevated in only 50% of patients with stage I disease, and can be elevated with other cancers as well as benign diseases of the ovaries and reproductive tract (26), thus, CA125 has inadequate sensitivity and specificity for detecting preclinical disease (Table 1). Use of a mathematical algorithm and serial CA125 measurements improves the sensitivity of screening asymptomatic postmenopausal women (27). The assessment of complex ovarian morphology by transvaginal ultrasound has been reported to increase the sensitivity and positive predictive value in multimodal screening (28) to a level sufficient to warrant large-scale clinical trials of ovarian cancer screening. Overall specificity and screening sensitivity of ultrasound after an elevated CA125 exceeded 99.6% and 70%, respectively, thereby yielding a positive predictive value exceeding 10%. However, sensitivity for early-stage disease was only 40%.

For women at higher risk of ovarian cancer due to strong family history or the presence of BRCA1 or BRCA2 gene mutations, the choices are limited: careful monitoring or prophylactic oophorectomy. The inability to reliably detect early ovarian cancer is a severe limitation to careful monitoring alone. This is especially true in light of recent evidence that annual surveillance by serum CA125 measurement and transvaginal ultrasound scanning in women at increased familial risk of ovarian cancer is ineffective in detecting tumors at a sufficiently early stage to influence prognosis (29).

Table 1. Serum biomarkers evaluated for their ability to detect ovarian cancer

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>No. of ovarian cancers tested</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>CA125</td>
<td>75000</td>
<td>65</td>
<td>97.99</td>
<td>4.6</td>
<td>(25, 32, 39)</td>
</tr>
<tr>
<td>CA125 and soluble IL-2Rα</td>
<td>39</td>
<td>88.5</td>
<td>27.1</td>
<td>(36)</td>
<td></td>
</tr>
<tr>
<td>CA125 and prostatin</td>
<td>37</td>
<td>92</td>
<td>94</td>
<td>(37)</td>
<td></td>
</tr>
<tr>
<td>Serum proteomic pattern</td>
<td>50</td>
<td>100</td>
<td>95</td>
<td>94</td>
<td>(30)</td>
</tr>
<tr>
<td>CA125 and apolipoprotein A1, transthyretin, and inter-α-trypsin inhibitor heavy chain H4</td>
<td>41</td>
<td>74</td>
<td>97</td>
<td>(32)</td>
<td></td>
</tr>
<tr>
<td>Leptin, prolactin, osteopontin, and insulin-like growth factor-II</td>
<td>100</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>(15)</td>
</tr>
<tr>
<td>Urinary eosinophil-derived neurotoxin and osteopontin COOH-terminal fragments</td>
<td>128</td>
<td>72</td>
<td>93</td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>CA125 and transvaginal sonography</td>
<td>22,000</td>
<td>70</td>
<td>99.9</td>
<td>26.8</td>
<td>(28, 38)</td>
</tr>
</tbody>
</table>

NOTE: The ability of the serum test to identify the presence of ovarian cancer. For comparison, results from the urinary biomarker study of Ye et al. (1) and a large-scale prospective study of CA125 with transvaginal sonography are also included in the table. Sensitivity, the probability of correctly identifying that ovarian cancer is present. Specificity, the probability of correctly identifying that ovarian cancer is not present. Positive predictive value, the probability that ovarian cancer is present given a positive test result.
Currently available screening methods, including CA125, additional biomarkers, and transvaginal ultrasound lack the necessary sensitivity and specificity to provide accurate and cost-efficient screening for the general population or even the population at high risk. These limitations, in combination with advances in proteomic technology, have launched ovarian cancer diagnostics into the forefront of cancer research. An initial proteomics study in 2002 reported the detection of discriminatory proteomic patterns in the serum of women with ovarian cancer, enabling the correct identification of all 50 ovarian cancer cases, including all 18 stage I cancers, and 63 of the 66 non–cancer cases (30). More recent studies have identified some of the specific serum proteins that are elevated in ovarian cancer, and there are several reports of the evaluation of multiple biomarkers in combination (15, 31, 32), including one in which a combination of four biomarkers correctly identified cancer in 26 of 27 patients with stage I and II ovarian cancer (15). Although candidate biomarkers are now being reported with increasing frequency, and with varying sensitivity and specificity (Table 1), there remain many challenges in translating these findings into clinical practice (reviewed in ref. 33). Ye et al. (1) have offered the first report of potential biomarkers of ovarian cancer in urine. Although the identification of two candidate biomarkers is promising, and the advantages of analyzing urine versus serum samples are appealing, the specificity of the biomarkers identified in this study is not greater than previously reported biomarkers or combinations thereof, and would be insufficient for screening purposes.

The past 3 years have seen a significant growth in the research efforts devoted to the detection of early ovarian cancer, although none of the methods identified thus far will likely be sufficient to result in the accurate and reliable screening test that is needed. It is likely that a combination of tests will be necessary to ensure sufficient sensitivity and specificity. Although progress in the development of methods for early detection of ovarian cancer has historically been very slow, the recent advances in technology and the associated enthusiasm for investigation in this field have ensured that the development of a reliable and sensitive screening test for ovarian cancer is one giant step closer. The ability to use readily accessible urine samples to detect ovarian cancer clearly would be advantageous compared with more invasive samples such as blood or more complex clinical procedures such as ultrasound (34). As with any biomarker approach, there are multiple hurdles to be overcome (35), only some of which are discussed above. It will be especially important to test these markers prospectively in a group of women in whom the presence of ovarian cancer is not known. However, in spite of these caveats, and recognizing that much more needs to be done to determine if the markers identified here will be promising, the study presented by Ye et al. (1) is a first step in assessing the utility of urine samples in the detection of ovarian cancer and offers biomarkers that can be further assessed for their clinical utility in identifying this difficult disease.

References

35. Ludwig JA, Weinstein JN. Biomarkers in cancer
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