Can Molecular Diagnostics Usher in a New Era for Screening, Diagnosis, and Treatment of Ovarian Cancer?

The authors of “Detection of Somatic TP53 Mutations in Tampons of Patients with High-Grade Serous Ovarian Cancer,” published in this issue of Obstetrics & Gynecology (see page 881) used DNA from the cells obtained from tampons inserted before diagnostic surgery for women with pelvic masses to assess ovarian cancer risk.1 Tampons were inserted before and removed at diagnostic surgery, and DNA was sequenced from cells in the vaginal secretions. TP53 mutations were identified in three of five women with ovarian cancer. These cells were verified as being from the existing ovarian cancer using deep sequencing technology. The authors are to be congratulated on their unique conception and use of this technology in the quest for early detection and better treatment of patients with ovarian cancer.

We have known for some time that ovarian cancer cells can be detected in vaginal or cervical cytology obtained from vaginal secretions. This was described first by Ruth Graham, George Papanicolaou’s cytology technician in the 1950s.2 The current study demonstrates that we have technology that is able to go well beyond simple cytologic detection, and this technique described by the authors could have far-reaching consequences for screening, diagnosis, and treatment of ovarian cancer.

For instance, high-grade papillary serous cancers are believed to be the result of malignant transformation of the fallopian tube epithelium.3 If this is the case, early cancer detection or even detection in the premalignant phase may be within reach. Therefore, this technology could have a great effect on screening for and the survival of a large subset of ovarian cancer patients. In addition, the cells obtained preoperatively could help in assessing risk of malignancy in an ovarian mass before surgery or even help in treatment planning as we enter into the era of molecular diagnostics and can assess cancers for prognosis and features of treatment response. Are there patients who might benefit from different neoadjuvant therapies based on the molecular profile of the cancer?

A real novelty of this test is not the small amount of input DNA, but the very low limit of detection needed for this test. Most molecular tests of cancer specimens need at least 10% or more (usually 30%) of the cells to be cancer cells. In the study by Erickson et al, only a tiny fraction of the cells were cancer cells.1 As a result, a very small fraction of the DNA in the tampon showed the TP53 variants that the authors were seeking. When attempting to detect variants at extremely low allele frequencies (ie, variants that are very rare among the total DNA in the sample), one needs to sequence to extremely high depth (“ultra-deep sequencing”), and even then, variant calls are based on a very small number of sequence reads. The probability that a variant represents a sequencing error then becomes
high. This would be like announcing Spring based on seeing a single robin. You are probably right but not definitely. The authors have used a special technology to prevent sequencing artifacts, and it looks as if it worked, because the three mutations found in tampons were also found in the corresponding tumors.

However, if this assay is brought into clinical practice, there will not be paired tumor tissue, and therefore clinicians will not be able to tell true somatic variants from spurious variants introduced by sequencing errors. Therefore, the assay will need to be validated to show what fraction of screen positives are sequencing artifacts on a large patient population. Also, if the variant allele fraction in a sample is as low as 0.01%, one has to be extremely concerned about sample-to-sample contamination. There is no evidence of contamination here, and the tampon mutations matched the tumor mutations, but we know that low-level DNA contamination is extremely common in molecular diagnostics. There is no laboratory clean enough to prevent contamination 100% of the time. Even in the absence of physical sample contamination, index swapping on the sequencer—a phenomenon whose cause is not well-understood—can result in a few reads from one patient being ascribed to another patient. This small number of mutant TP53 reads would be enough to result in a false-positive call. The authors do not specify whether there was any evidence of sample contamination, such as reads showing TP53 p.Y220C (patient 4’s mutation) in sequence data for patient 5. These reads might have been present but below the limit of detection for the assay, although this limit seems to have been set spectacularly low. The limit of detection is set in many molecular diagnostic laboratories at 5%. If it is set lower, one starts to obtain a very large number of false-positive results.

One issue that can help militate against contamination worries is that screen positives should be rare if this assay ever comes to market. The probability of having two positive samples on the same run will always be low. The probability of having two positive samples showing the same mutation will be essentially zero.

In terms of clinical utility, the sensitivity of this test may be around 60% in patients with intact tubes and with clinically obvious cancer, but we do not know what it will be in patients with less-advanced disease. It also could play a role similar to CA 125 or other blood tests in predicting the nature of a clinically obvious pelvic mass before going to surgery.

Its most powerful use could be in screening for ovarian cancer. Screening for ovarian cancer is the Holy Grail among gynecologic oncologists. Ovarian cancer is the most lethal of all the gynecologic cancers. It is estimated that, in 2013, more than 14,000 women in the United States died from ovarian cancer and more than 22,000 women were newly diagnosed with the disease.4 Women with early-stage disease often have no or only vague symptoms such as bloating, back pain, and fatigue, leaving most women undiagnosed until later stages of the disease. Standard treatment of ovarian cancer consists of surgical resection of disease followed by taxane- and platinum-based chemotherapy, which yields a partial response rate of greater than 80% and a complete response rate of 40–60% in patients with advanced disease. Survival has improved significantly with more aggressive cytoreductive efforts, better chemotherapy, and better routes of chemotherapy administration such that median survival in some patient populations is more than 10 years. However, treating the disease once it is present is both morbid and expensive. Early detection of this disease or even detection in a premalignant phase would be a real breakthrough.

This technology has great promise, but we must be careful to fully validate it before it is ready for prime time general use. Its most useful potential is in the area of screening. However, the barrier to ovarian cancer screening is the fact that the prevalence of the disease is so low in the general population that any screening test must have an unrealistic sensitivity and specificity. The positive predictive value of any ovarian cancer screening is to raise the positive predictive values to a level that makes intervention safe for patients. This dilemma is illustrated in Table 1.

A good example of this problem was demonstrated in the Petricoin serum screen using mass spectrometry.5 In this study, the authors detected 100% of the cancers in their population. Unfortunately the study population was composed of 50% individuals without cancer and 50% cancer patients.

Table 1. Effect of Disease Prevalence on the Positive Predictive Value of a Screening Test

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Data are %.
Assume a sensitivity of 100%.
Numbers in bold are positive predictive values.
Hence, the studied patient population was not reality, because the prevalence of the disease was 50%, not 1/2,500. The significance of this can be seen in Table 1. Other types of screening using conventional technology, such as serial ultrasonography and serum markers, have failed to demonstrate a significant change in cancer incidence or even a decrease in the stage of disease at the time of diagnosis in a screened population.

This technology shows great promise, and the authors are to be congratulated on a great effort on many levels. The technology represented here has the potential to do what other screening tests have not. Other technologies also show promise, such as harvesting circulating DNA or cancer cells from peripheral blood. This technology also has the potential to distinguish a malignant from a benign process. We need to embrace this technology and continue to evaluate it. We must be careful not to endorse it until its usefulness is fully validated. Clearly, more investigation of this or like technology is needed in the quest for screening, early diagnosis, and treatment of ovarian cancer, but we are making progress.

REFERENCES