Why do universal HRD testing in ovarian cancer?

The rationale, and the test options for homologous recombination deficiency

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September 2020—Genetic testing in ovarian cancer has a therapeutic implication that will aid in developing a treatment plan, and it is pathologists who should take the lead in creating the testing protocol, said Samuel Caughron, MD, pathologist, president, and CEO of MAWD Pathology Group, in a recent CAP TODAY webinar.

Dr. Caughron explained the rationale for universal homologous recombination deficiency testing in patients with advanced ovarian cancer. The webinar, made possible by a medical sponsorship from AstraZeneca, is at www.captodayonline.com.

Dr. Caughron

Homologous recombination deficiency, or HRD, is comparable to microsatellite instability, said Dr. Caughron, who is also chair of pathology and medical director of the clinical laboratory at AdventHealth Kansas City. Homologous recombination repair, or HRR, is comparable to mismatch repair. “But it’s fixing a different kind of damage,” he said. Mismatch repair fixes single base mismatches. HRR fixes double-strand breaks. In individuals with HRD, the HRR pathway is compromised.

While *BRCA1* and *BRCA2* are the most prominent molecules involved in HRR, others—*RAD51, ATR/ATM*, and *MRN* complex—“combine to give you homologous recombination repair.” When that functionality is lost, there are two possible outcomes: The double-strand breaks persist and cause a complete fracturing of DNA at that site, or the breaks may be repaired by non-homologous end joining, a non-high-fidelity repair pathway that introduces inversions and other genomic aberrations.

Both outcomes result in genomic instability, manifested as a cell’s ability to survive despite DNA damage. “This is the profile of tumors that have *BRCA1* and/or *BRCA2* proteins that are defective due to mutations, as well as mutations in the other HRR proteins.”

HRD can be caused by a range of specific genomic mutations or alterations in the HRR genes, including ones in *BRCA1/2*. But altered HRR gene expression, such as promoter methylation, can also cause HRD. And in a percentage of HRD cases the causes are unknown.

There are two core testing strategies for identifying HRD. The first is to test for the presence of specific mutations within *BRCA1/2* and other HRR genes by looking at a single gene or panel of genes. “This is a complex problem,” Dr. Caughron said, because *BRCA1* and *BRCA2* are large genes with thousands of documented mutations, and identifying and interpreting them isn’t straightforward.

The other strategy is to test for the phenotypic effects of HRD within the genome of the tumor. One is loss of heterozygosity, or the presence of a single allele, a cross-chromosomal event that results in the loss of entire genes and the surrounding chromosomal region. In LOH, “There’s a stretch of DNA where, when we look at the genomic analysis, we see only one set of sequences.”

Another is telomeric allelic imbalance, or the accumulation of a discrepancy in the 1:1 allele ratio in the telomere of the chromosome. TAI, which is caused by reciprocal translocations, is “a signature you find in tumors that have genomic instability or HRD.” And the third aberration is chromosomal rearrangements, such as large chromosomal breaks. These are transition points between regions of abnormal and normal DNA, or between two different regions of abnormality.

“By identifying these features, we can find tumors that have homologous recombination deficiency without having to identify specific gene mutations or determine whether the mutation is deleterious,” Dr. Caughron said. There are commercially available assays that can assess one or more of these HRD phenotypes, and other assays that can detect related genes. “And there’s value in looking for genes,” he noted, because of the familial implications.

About 70 percent of patients with epithelial ovarian cancer have high-grade serous histology, and more than half of all high-grade serous ovarian cancers have an HRD phenotype (Konstantinopoulos PA, et al. *Cancer Discov.* 2015;5[11]:1137–1154; Frey MK, et al. *Gynecol Oncol Res Pract.* 2017;4:4). A little less than a quarter of women with advanced ovarian cancer have *BRCA* mutations, the “largest single cause of that type of biology to the tumor.” But a significant portion of HRD tumor cases are due to promoter methylation, and another portion is caused by gene mutations associated with other HRR system proteins. And for another segment, “we know the patient has an HRD phenotype, but we can’t identify the specific cause.”

Thus, about one in four women with advanced ovarian cancer has a *BRCA* mutation, and about one in two has HRD. “And an important point here is women without a *BRCA* mutation may still have tumors with HRD,” Dr. Caughron said.

Historically, he said, pathologists have thought of *BRCA* as a germline gene, and 14 to 21 percent of women with ovarian cancer have a germline *BRCA* mutation. But up to an additional 12 percent of women with ovarian cancer have a somatic *BRCA* mutation (Sugino K, et al. *Sci Rep.* 2019;9[1]:17808; Pennington KP, et al. *Clin Cancer Res.* 2014;20[3]:764–775). This means 25 to 36 percent of women with ovarian cancer—rather than 14 to 21 percent—have HRD, “with 12 percent, or a significant chunk, of those being from somatic mutations.” To test for somatic mutations, “you have to test the tumor cells themselves,” which means testing for the gene mutations that cause HRD or identifying HRD phenotypes within the tumor.

“There’s a significant difference in what you’ll identify if you take different test strategies,” Dr. Caughron said. Germline testing for *BRCA1/2* on peripheral blood samples (“this is not a liquid biopsy,” he noted) detects mutations in about 14 percent of women (Pal T, et al. *Cancer.* 2005;104[12]:2807–2816). Germline and somatic testing on tumor samples identifies *BRCA* mutations in about 22 percent of women (Pennington KP, et al. *Clin Cancer Res.* 2014;20[3]:764–775; Pal T, et al. *Cancer.* 2005;104[12]:2807–2816). “This is a baseline that’s important to be aware of as pathologists,” Dr. Caughron said. “Almost a quarter of women with ovarian cancer are going to have a *BRCA1* or *BRCA2* mutation.”

The HRRm gene panel, which identifies mutations in one or more of the 13 HRR genes (*ATM, BRCA1, BRCA2, BARD1, BRIP1, CHEK1, CHEK2, FAM175A, MRE11A, NBN, PALB2, RAD51C,* and *RAD51D*), raises to 31 the percentage of ovarian cancer patients found to have HRD. And the HRD genomic instability test, which can identify loss of heterozygosity and sometimes other HRD phenotypes, and includes *BRCA1/2* mutations, increases the percentage to almost 50.

“The strategy we have taken at AdventHealth Kansas City, where we have one of the busiest gynecologic oncology services in the region and see a lot of ovarian cancer patients, is to do *BRCA1/2* tumor testing at the time of diagnosis,” Dr. Caughron said. If no mutations are detected, the oncologist has a subsequent conversation with the patient about doing additional HRD genomic instability testing on the tumor. “We pick up a significant percentage of them doing just *BRCA1/2* mutation testing as initiated by the pathologist, but we are exploring moving straight to genomic instability testing,” rather than waiting for the oncologist to talk to the patient, “because of the importance of getting this information as quickly as possible.”

BRCA1/2 and HRD testing have implications for ovarian cancer treatment because patients with HRD tumors may

be treated with PARP inhibitors. PARP, or poly (ADP-ribose) polymerase, enzymes play a key role in base excision repair, he said. “They’re really the quarterback in initiating a pathway for single-strand break repair.” They bind to single-strand breaks and recruit repair proteins. When PARP enzymes are inhibited, single-strand breaks persist, or are potentially repaired by non-homologous end joining. And in cells with HRD, double-strand breaks can no longer be repaired efficiently.

“We find two concepts here,” he said. Both PARP molecules and homologous recombination are important DNA repair mechanisms, and the combination of two separate nonlethal defects—HRD and PARP-inhibited cells—can become lethal, leading to cell death. “Cells that are both PARP inhibited and have HRD will accumulate mistakes at such a high rate that they’re going to end in apoptosis,” or “synthetic lethality.” Patients who have an HRD tumor are treated with PARP inhibitors, Dr. Caughron said, “to combine the inherent HRD defect with an extrinsic shutting down of PARP-dependent repair, accelerating cell death and death of the tumor.”

Testing guidelines have begun to incorporate the recognition that *BRCA* testing and HRD testing are significant not only for germline considerations but also for tumor testing, Dr. Caughron said. The ASCO guideline advises performing somatic tumor testing for *BRCA1/2* in women who do not carry a germline *BRCA1/2* variant. “Germline certainly has a significant role, as it always has, but now there is an additional critical role for tumor testing to understand what treatment implications might exist for the tumor.”

The NCCN guidelines recognize that germline and somatic *BRCA1/2* status informs both treatment and maintenance therapy, and they advise germline and somatic tumor testing for all women with ovarian cancer. In the absence of a *BRCA1/2* mutation, the NCCN guidelines say, HRD status may provide information on the magnitude of benefit of PARP inhibitor maintenance therapy after first-line chemotherapy (category 2B). Finally, testing for the presence of high-penetrance ovarian cancer susceptibility genes is indicated for anyone with a personal history of ovarian cancer. “The guidelines today interweave the genetic value with the value of somatic testing,” Dr. Caughron said.

Dr. Caughron presented a case (illustrative only) of newly diagnosed stage IIIC ovarian cancer. A 49-year-old female with no family history of breast or ovarian cancer presents with bloating and abdominal pain, and ECOG Performance Status 1. Her abdominal and pelvic CT scans identify two pelvic masses of 8 cm and 6 cm in the left ovary. She has peritoneal carcinomatosis, and her CA-125 levels are “extremely high” at 1195 U/mL. The diagnosis is stage III HGSOC. She has surgery and completes cytoreductive therapy, and platinum-based chemotherapy is begun.

The patient receives genetic counseling, and germline *BRCA* testing is ordered after chemotherapy is initiated. The test result is negative: no g*BRCA* mutations detected. The next step is to test the tumor. “Tumor testing did confirm the presence of HRD in this patient,” he said. “At the completion of the therapy, that’s going to have significant implications for how this patient will be managed.”

The significant development today, Dr. Caughron said, is that genetic testing in ovarian cancer is no longer only for familial risk assessment, but has therapeutic implications. “It’s going to aid in the development of a comprehensive treatment plan,” because homologous recombination mutational status and HRD status are associated with increased sensitivity to platinum chemotherapy or PARP inhibition. And pathologists, he said, “are uniquely positioned to make sure patients get HRD and/or homologous recombination repair gene testing,” and to develop and lead a precision medicine protocol for testing.

“We have taken a position of advocating for this, and I can tell you that at least in our organization, and in our region, oncologists have been extremely appreciative of the pathologists taking a leadership role and being active in making sure this testing can happen on the tumor to help them manage these patients.”

There are multiple options for HRD testing, Dr. Caughron said, and all have advantages and limitations:

- Tumor testing for *BRCA*m identifies up to 50 percent more women with

mutations than germline testing alone (Frey MK, et al. *Gynecol Oncol Res Pract.* 2017;4:4). The limitations: It does not determine familial risk, although patients found to have a somatic mutation can be referred to clinical genetics for germline testing. In addition, it can detect germline mutations but cannot differentiate between germline and somatic mutations. And it may miss some patients with large genomic rearrangements or intronic mutations (Capoluongo E, et al. *Oncotarget.* 2018;9[28]:19463–19468).

- Germline blood or saliva testing determines familial risk and is minimally invasive, and results are highly accurate and reproducible. However, it cannot identify patients with only somatic mutations.
- Liquid biopsy looks strictly for circulating tumor DNA and has the potential to identify cancer biomarkers at specific time points over the course of the disease. It doesn't determine familial risk—it can detect germline mutations but cannot differentiate between germline and somatic. The mutation profile is dependent on tumor shedding. "And as we all know about liquid biopsies," Dr. Caughron said, "we can have confidence in a positive result but we have to put a negative result in the appropriate patient context to understand if it is a false-negative, which happens more commonly with liquid biopsies than other testing."

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