Cervical Cancer, Pap Smears, and HPV in the Molecular Era

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Carcinoma of the cervix is the second leading cause of cancer related deaths in women worldwide with a total of over 300,000 deaths per year. In 2009, the estimated number of new cases in the United States was 11,270, with 4,070 deaths (www.cancer.gov). There are two main types of cervical cancer: squamous cell carcinoma (most common) and adenocarcinoma. Years prior to the development of cancer, pre-cancerous or dysplastic cells appear in the cervix, and these can gradually progress to cancer. Detection of these pre-cancerous cells is the goal of the screening test commonly known as the Pap test.

In the Pap test, cells are scraped from the cervix and either smeared on a slide (conventional Pap test) or placed in a liquid preservative (liquid based Pap test). The slides or vials are then sent to a pathology laboratory where they are analyzed by a cytotechnologist specifically trained in this area and/or a pathologist. The results are reported using standardized criteria and a standardized reporting system (Bethesda system). The main categories for diagnosis would be (1) Negative/reactive, (2) ASC-US (atypical squamous cells of undetermined significance, (3) ASC-H (atypical squamous cells, cannot rule out high grade dysplasia), (4) LSIL (low grade squamous intraepithelial lesion (mild dysplasia), (5) HSIL (high grade squamous intraepithelial lesion (moderate to severe dysplasia), (6) AGUS (atypical glandular cells), (7) Carcinoma. All but the first would be considered an abnormal smear requiring varying degrees of follow-up or treatment.

Since its introduction, the Pap test can be considered to be the most successful screening test ever devised with incidence rates of cervical cancer decreasing from 14.8 per 100,000 persons in 1975 to 6.5 per 100,000 persons in 2006. Significant advances have also been made since its inception. The first is the use of liquid based methods. In a conventional Pap smear, cells are smeared directly onto a slide resulting in areas that are many layers of cells thick. The liquid based methods use a machine to smear a single layer of cells on the slide making it much easier for the cytotech or pathologist to detect abnormal cells. Computer assisted screening is also available where the slide is scanned by a machine and cells that may be atypical based on several criteria are flagged for further review. The third major advance has been in the area of Human Papillomavirus (HPV) testing.

HPV is the most common sexually transmitted infectious agent worldwide and has been found in 27% of women between ages 14–59 in the US. There are over 100 different types with at least 40 known to infect the genital tract. Different types are known to cause papillomas/warts at various body sites, but it is also a cancer causing virus that has been found in 99% of cervical cancers as well as other cancers in the genital region, including penile cancers in men. With regard to cervical cancers, HPV types can be divided into high risk (22 known types), low risk (20 known types) and unknown risk. (It should be noted that these high risk and low risk HPV types are not synonymous with the high grade and low grade dysplasias referred to in Pap smears). High risk HPV types are most significantly associated with dysplasia (both low and high grade) as well as cervical cancer. HPV type 16 has consistently been the most common high risk HPV type identified worldwide and in combination with HPV type 18, account for approximately 70% of all cervical cancers. Low risk types are associated with genital warts, and HPV types 6 and 11 are the most common, accounting for 90% of cases. It is for this reason that the HPV vaccine, Gardasil, approved by the FDA for both men and women ages 9-26, is targeted against these four major subtypes (16, 18, 6, and 11). The other FDA approved vaccine, Cervarix, approved for females of ages 10-25, protects against HPV types 16 and 18.

Many factors affect a woman's risk for developing cervical cancer, but the presence of high risk HPV is one of the most important. However, only very few women who are infected with HPV develop dysplasia or carcinoma. Like most other infections, HPV can be cleared by the body and 50% of infections are cleared within 1 year and 90% in 2 years. It is those high risk types that are not cleared but remain for long periods (persistence of infection), that put persons at greater risk for high grade dysplasia and carcinoma. It is for these reasons that HPV testing now plays a very central role in cervical cancer screening, and is used both as an additional test in patients with an abnormal pap smear or as a screening tool in patients with normal smears. Most patients with a Pap smear diagnosis of ASC-US (which essentially means the cells are abnormal but not obviously dysplastic or pre-cancerous), are tested for HPV. Those that are positive for high risk HPV are treated differently from those that are negative. Similarly, patients with Pap smears that are normal under the microscope, but who are positive for high risk HPV, especially types 16 and 18, would be at greater risk of developing dysplasia than those with negative HPV tests. Again it must be emphasized that a positive high risk HPV test does not equate to high grade dysplasia, and there is a long and complex path from one to the other and only in a small number of persons. The goal of the Pap smear and HPV testing is to detect early changes so that dysplasia/pre-cancer can be detected and treated before it becomes cancer.

There are various tests on the market for HPV, two of which are approved by the FDA. The first, the Hybrid-Capture II (HCII) test is considered a pooled test and looks for 13 different high risk types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). The second test (Cervista) is also predominantly a pooled test in that it looks for 14 high risk types, adding type 66 to those tested by HCII. These tests are reported as positive or negative for high risk HPV depending on the detection of one of these 13 or 14 types. Additionally however, Cervista can also specifically identify types 16 and 18. Another testing method developed by Access Genetics and utilized by us at Pennsylvania Specialty Pathology, is based on PCR (generally considered the

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gold standard for molecular testing). It identifies and creates millions of copies of any HPV DNA present within the cells. This allows us to specifically identify and subtype any HPV present and then assign a risk category. Over the two years that we have been providing this test methodology, we have performed over 1800 HPV tests and have identified 240 high risk, 129 low risk, and 58 unknown risk HPV positive cases. Based on the high risk HPV types that can be identified by HCII and Cervista, these latter tests would have been positive in only 68% and 81% of the high risk cases we identified, respectively, while the remaining 32% and 19% would have been reported as negative.

Apart from detection rate, other advantages to specifically typing the virus would be in determining persistence of infection as discussed above. Pooled tests that report only positive or negative results cannot distinguish between persistence of the same virus subtype and clearing of one type with re-infection by another. This phenomenon of clearing and re-infection may occur in up to 20% of patients with repeatedly positive HPV tests.

In the post vaccination era, as the population is protected against types 16 and 18, the significance of other high risk types is certain to increase, and the ability to identify all types will be useful. Another advantage of our PCR method is that we can use the same extracted DNA from the pap cells to test for other infectious diseases such as Chlamydia, Gonorrhea and Herpes, as well as inherited diseases such as cystic fibrosis and inherited thrombophilia.

The guidelines for Pap testing and HPV screening have changed over the years as we continuously gain more information on this disease. Most recently, in November 2009, the American College of Obstetricians and Gynecologists (ACOG) published revised guidelines for Pap smear screening. While every female at risk should be part of a regular screening program for cervical cancer, each person is unique and should consult with their doctor to determine the best approach to prevention of this disease.