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EDUCATIONAL COMMENTARY: DETECTION OF HUMAN PAPILLOMA VIRUS (HPV) IN THE DIAGNOSIS OF CERVICAL CANCER

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Learning Objectives

On completion of this exercise, the participant should be able to

- describe the human papilloma virus (HPV), including transmission and disease associations;
- discuss the HPV strains that carry a high risk for causing cervical cancer;
- discuss the role of the HPV vaccine in prevention of cervical cancer;
- compare the different methods for detection of HPV, providing examples of each; and
- explain the current recommendations and guidelines for screening of cervical cancer.

Introduction

Worldwide, cervical cancer is the fourth most common cancer among women, with approximately 570,000 diagnosed cases of invasive cervical carcinoma and 311,000 deaths from cervical cancer annually.\(^1\) The evidence linking human papilloma virus (HPV) to cervical cancer is extensive. Virtually all cases of cervical cancer are attributable to HPV infection with HPV 16 accounting for approximately 50% of cases and HPV 18 for 20%.\(^2\) Human papillomavirus types 31, 33, 45, 52, and 58 are estimated to cause an additional 19%.\(^3\)

Cervical cancer screening uses combinations of cervical cytology (Papanicolaou [Pap] test) and testing for HPV strains that carry a high risk for causing cervical cancer. In the United States, current practice for detection of cervical cancer includes routine screening using cervical cytology and high-risk HPV (hrHPV) testing for women aged 30 to 64 years. Testing protocols vary by age, previous test results, and guidelines that are available from various resources. Recommendations are available from organizations including the American Cancer Society, U.S. Preventive Services Task Force, and the American College of Obstetricians and Gynecologists. The introduction of new tests for the detection of hrHPV has led to changes in cervical cancer screening guidelines.
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Human Papillomavirus

Papillomaviruses are double-stranded DNA viruses that make up the *Papillomavirus* genus of the Papillomaviridae family. Human papillomaviruses infect only humans. Different genotypes of HPV infect different body sites and are thus associated with different diseases ([Table 1](#)). Certain HPV types have a predilection for the cutaneous epithelium and are found in plantar warts, common warts, and flat warts. Human papillomavirus types associated with plantar and common warts include types 1, 2, and 4. Flat warts are most often caused by HPV 3 and 10.4 Human papillomavirus types with a predilection for anogenital keratinized skin and mucous membrane infection result in infections at sites such as the penis, scrotum, perineum, anal canal, perianal region, lining of the vagina, vulva, rectum, and cervix. More than 40 mucosal HPV types can infect the genital tract, which can also infect the lining of the mouth and throat.5 The anogenital clinical presentations and disease associations differ by HPV type. Genital warts are benign warts caused most often by HPV types 6 and 11.6,7 Squamous intraepithelial lesions and/or carcinoma of the vagina, vulva, cervix, anus, or penis are associated with 15 different HPV types. These 15 HPV genotypes are associated with cancer and are known as high-risk, carcinogenic, or cancer-associated HPV. The common high-risk (oncogenic or cancer-associated) types of HPV include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, and 82.4,7-9 Human papillomavirus type 16 is the most common and is associated with the highest risk of progression to cancer. In addition, HPV type 16 can infect the oral mucosa and has been associated with squamous cell carcinoma of the oral cavity.6,10,11

Globally, anogenital HPV is the most common sexually transmitted infection. Genital and cervical HPV infections are primarily transmitted by genital-genital or anal-genital contact. The most consistent predictor of genital HPV infection is sexual activity. Both vaginal and anal intercourse are major risk factors for HPV infection.11 Peak prevalence of HPV infection typically occurs between the ages of 15 and 25 years. It is estimated that at least 80% of sexually active women and men are exposed to HPV once in their lifetime.8 In the United States, anogenital HPV infection is common in young, sexually active, women, with an estimated prevalence of 20 million infections and an annual incidence of 5.5 million infections.12-14 Most HPV infections, including those with carcinogenic HPV genotypes, typically resolve within 12 months in a person with a normal immune system.15,16 Cytologic abnormalities may be detectable in screening via Pap smear during productive cervical HPV infection but are usually transient.
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Table 1. Disease Associations with Human Papillomavirus Types.4,7,8 This table lists the more commonly reported HPV types associated with various conditions. The most prevalent HPV types associated with particular lesions can vary by geography and demographic characteristics of the population.

<table>
<thead>
<tr>
<th>Disease</th>
<th>HPV Types Most Frequently Associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous Warts</td>
<td></td>
</tr>
<tr>
<td>Common and plantar warts</td>
<td>1, 2, and 4</td>
</tr>
<tr>
<td>Flat wart</td>
<td>3, 10</td>
</tr>
<tr>
<td>Genital warts</td>
<td>6 and 11</td>
</tr>
<tr>
<td>Squamous intraepithelial lesionsa</td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>16, 31, 6, 11</td>
</tr>
<tr>
<td>High grade</td>
<td>16, 18, 31, 33, 45, 52, 58</td>
</tr>
<tr>
<td>Oropharyngeal cancer</td>
<td>16</td>
</tr>
<tr>
<td>Anal cancer</td>
<td>16</td>
</tr>
</tbody>
</table>

These include the most common HPV types associated with squamous intraepithelial lesions and cancers of the cervix, vagina, vulva, anus, and penis.

However, carcinogenic HPV infections that persist beyond 12 months increase the likelihood of precancerous or cancerous lesions. In the United States, the median age of cytologically detected precancerous cervical lesions is approximately 10 years after the median age of first sexual encounter.17

HPV Vaccine

In the United States, there is a 9-valent vaccine (Gardasil-9 by Merck & Co.) available that blocks the strains of HPV that cause 90% of cervical cancers, including HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58.3,9,17 Routine HPV immunization is recommended in many countries, including the United States, for adolescents and young adults. Many studies have reported declining prevalence and incidence of HPV infection, as well as HPV-related disease, following the introduction of HPV vaccination.18,19 As an example, in the United States, the prevalence of HPV 6, 11, 16, and 18 in cervical samples from female patients from the pre-vaccine (2003 to 2006) and post-vaccine (2011 to 2013) eras decreased by 71% (from 11.5% to 3.3%) among patients aged 14 to 19 years and by 61% (from 18.5% to 7.2%) among those aged 20 to 24 years.20 Despite these benefits, the HPV vaccines are underused, do not target all pertinent HPV types, and lack efficacy against preestablished infections.21
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Screening for Cervical Cancer

Although cervical cancer is common among women worldwide, most cases occur in developing countries, because developed countries have robust screening and HPV vaccination programs. Cervical cancer screening began with the development of the Pap smear test in the 1940s. In countries that adopted routine Pap test screening, the incidence and mortality of cervical cancer have decreased. In the United States, screening methods now include tests for high-risk strains of HPV, which are central to the pathogenesis of cervical cancer. Infection with high-risk strains of HPV and persistence of HPV infection are the most important determinants of progression to cervical cancer. The development of laboratory testing methods that can detect oncogenic HPV genotypic strains has led to a debate about which patients to screen, which testing methods are preferable (Pap test, hrHPV testing, or both), and how often to screen. Different recommendations have been released by several guiding bodies including the American Society for Colposcopy and Cervical Pathology (ASCCP), the American Society for Clinical Pathology (ASCP), the Society of Gynecologic Oncology (SGO), the American College of Obstetricians and Gynecologists (ACOG), and the US Preventive Services Task Force (USPSTF).

Methods for Cervical Cancer Screening

The available methods for screening of cervical cancer include the Pap test (cytology) and molecular hrHPV testing. These are used alone or in combination. Cell samples for cervical cytology and hrHPV testing are obtained during the speculum examination. With certain types of Pap tests, the same specimen can be used for both tests or separate samples can be obtained during the same patient examination.

The Pap smear consists of cells sampled from the cervix. It yields cytologic results; a skilled cytologist or pathologist examines cells via microscopy to look for abnormal cells from the ectocervix and endocervix, where cervical dysplasia and cancers arise. The Pap test is used to detect abnormalities in cells that can be caused by cancer or noncancerous conditions such as infections and inflammation.

HPV Laboratory Detection Methods

Molecular hrHPV testing is more sensitive than the Pap test for detection of precancerous conditions. There are several HPV testing systems available. These can be used for one or more types of testing: co-testing with cytology, reflex testing in response to a cervical cytology result of atypical squamous cells of
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undetermined significance, primary screening with HPV testing alone, or HPV genotyping. Human papillomavirus tests identify most, but not all, of the hrHPV types that cause cervical cancer. The common high-risk (oncogenic or cancer-associated) types of HPV include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, and 82. The common low-risk (nononcogenic types) include 6, 11, 40, 2, 43, 44, 54, 61, 72, and 81. The available types of HPV tests use several different methods for detection of HPV. Those tests used for primary screening rather than co-testing can specifically identify types 16 and 18 or pool results for all high-risk types. Other tests provide genotyping of types 16 and 18 (or a combined type 18/45 result) and can be performed as reflex testing for specimens with a positive high-risk pooled result but they are not used for initial screening.

All commercial DNA HPV tests use one of the two main techniques of testing for HPV DNA. Signal amplification uses hybridization in the liquid phase. The second technique, target amplification, uses gene amplification with polymerase chain reaction. Detection of specific genotypes requires amplification, followed by hybridization with specific probe types. Quantitative detection of viral HPV DNA can be used to assess viral load. A third approach is the detection of mRNA encoding proteins E6 and E7 of hrHPV subtypes.

Seven commercial HPV tests are approved by the US Food and Drug Administration (FDA). The specimens for these assays are collected from the endocervix using a spatula or cervical brush, which is then placed in a specific transport medium or a liquid-based cytology sampling medium. The systems only test for HPV types that have been associated with cancer. These include the Qiagen Digene Hybrid Capture 2 High-Risk HPV DNA assay, Hologic Cervista HPV HR assay, Hologic Cervista HPV 16/18, Roche Cobas HPV test, Hologic Aptima HPV, and Becton Dickerson (BD) Onclarity HPV Assay (Table 2). One of these tests, the Roche Cobas HPV test, has been approved by the FDA for primary HPV testing without a Pap test in women 25 years and older. The other tests are approved for use in co-testing or reflex testing with a Pap test.

Based on the methodology used for testing, reporting of results will differ. Tests that detect the presence or absence of any of the 13 to 14 hrHPV types that are associated with cancer will not report which of the individual subtypes is present, only that there was a detection of a high-risk subtype if present (pooled results). A negative test result means that no oncogenic or hrHPV genotypes or only HPV types that carry low oncogenic risk were detected. Tests for HPV genotyping will report the presence or absence of HPV 16, 18, or 18/45, which are the types most commonly associated with high-grade cervical intraepithelial
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If a test with a pooled result is positive, an additional method which will provide genotyping of types 16 and 18 (or a combined type 18/45 result) and can be performed as reflex testing.24–26

The clinical application of HPV detection is limited to testing of cervical specimens as part of cervical cancer screening. Although HPV testing of other specimen sites (vaginal, penile, and anal swabs) has been used for surveillance and research, it is not approved for routine use in the United States. Human papillomavirus testing to determine appropriateness of HPV vaccination is not warranted. In the United States, there are no FDA-approved tests available to detect HPV infection of oropharyngeal, anal, or male genital specimens. There are also no FDA-approved serologic or blood tests to detect HPV infection.

Guideline Recommendations

Guidelines published by the American College of Obstetricians and Gynecologists, American Society for Colposcopy and Cervical Pathology, and the US Preventive Services Task Force recommend initiating cervical cancer screening at 21 years of age.22,25,27,30–32 In women aged 21 to 29 years, with a normal immune system and previous screening results (if any) that are all normal, the guidelines suggest screening with the Pap test. If the Pap test result is abnormal, some clinicians will order reflex hrHPV testing. If the Pap test result is negative, screening should be repeated at intervals of 3 years. Screening women younger than 30 years with hrHPV testing alone or co-testing is not recommended27 because HPV infection may be transient and cervical dysplasia may regress spontaneously, particularly in young women; thus, the poor specificity and poor positive predictive value of HPV testing limits its usefulness as a screening modality in this age group.28

Human papillomavirus infection is more likely to be persistent in women 30 years and older than in women younger than 30 years old and it has a greater likelihood of clinical significance in this group than in younger age groups.27 There are several screening strategies for women 30 years or older. The strategies include co-testing (Pap test and hrHPV testing) every 5 years, primary hrHPV testing every 5 years, pap test with reflex hrHPV testing every 3 years, and pap test alone every 3 years.22,25,27,30–32 These frequencies are for patients whose previous test results are normal. Patients with abnormal results require further follow-up. There is not sufficient data to indicate that one screening strategy is superior to another. It is important to recognize the higher rate of detection of cervical disease with hrHPV testing (alone or with Pap testing) compared with Pap testing alone.
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Table 2. Commercial Human Papillomavirus (HPV) Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of HPV Strains Detected</th>
<th>HPV Types Identified</th>
<th>Technology</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen Digene Hybrid Capture 2 High-Risk HPV DNA Assay</td>
<td>14</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68</td>
<td>DNA test, nucleic acid hybridization</td>
<td>Pooled result</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Does not detect 66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hologic Cervista HPV HR Assay</td>
<td>14</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
<td>DNA test, signal amplification</td>
<td>Pooled result</td>
</tr>
<tr>
<td>Hologic Cervista HPV 16/18</td>
<td>2</td>
<td>16, 18</td>
<td>DNA test, signal amplification</td>
<td>Specific for 16 and 18</td>
</tr>
<tr>
<td>Roche Cobas HPV test</td>
<td>14</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
<td>DNA test; multiplex real-time PCR</td>
<td>Specific for 16 and 18 and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pooled result for other 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>types</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hologic Aptima HPV Assay</td>
<td>14</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
<td>RNA test; identifies E6 and E7 RNA</td>
<td>Pooled result</td>
</tr>
<tr>
<td>Hologic Aptima HPV 16/45</td>
<td>2</td>
<td>16, 18/45</td>
<td>RNA test; identifies E6 and E7 RNA</td>
<td>Specific for 16 and 18/45</td>
</tr>
<tr>
<td>Becton Dickerson (BD) Onclarity HPV Assay</td>
<td>14</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
<td>RNA test; identifies E6 and E7 RNA</td>
<td>Pooled result for:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(16, 18, 31, 45, 51 and 52); (33, 58); (35, 39, 68) and (56, 59, 66)</td>
</tr>
</tbody>
</table>

Abbreviation: HPV, human papillomavirus; PCR, polymerase chain reaction.
However, false-positive results in both HPV detection and colposcopies have been noted with HPV testing alone. The current recommendations from the US Preventive Services Task Force for women aged 30 to 65 is hrHPV testing alone every 5 years, Pap testing alone every 3 years, or co-testing every 5 years. They concluded that primary hrHPV testing alone, at an interval of 5 years, offers the best balance among the different cervical screening strategies.22,28 The hrHPV test detects known cancer-causing types of HPV but some gynecologists believe there may be unknown cancer-causing viruses so they continue to perform the Pap smear plus hrHPV testing.

If a woman has a cervical HPV infection, a Pap test should be performed. If the Pap test result is normal, the provider may choose to follow up with testing specifically for HPV16 and HPV18 or perform co-testing again in 12 months. If a woman has a cervical HPV infection and abnormal Pap test result, the health care provider will make informed patient management decisions and request additional testing. Precancerous cervical abnormalities and carcinoma require extended surveillance. Women with pathology of moderate to high-grade intraepithelial cervical neoplasia (grade 2 or 3) should receive continued screening until at least 20 years after the grade 2 or 3 result using co-testing, even after the age of 65 years.

In the United States, the American College of Gynecologists and the US Preventive Services Task Force guidelines recommend discontinuing screening after age 65 years for women who are up to date on testing with normal results. It is also recommended to discontinue cervical cancer screening for women who have had a hysterectomy with removal of the cervix and do not have a history of a high-grade cervical precancerous lesion or cervical cancer. Current standard screening recommendations should be followed for women who have received HPV vaccine until data from clinical trials is available. The optimal approach to cervical cancer screening in women who have received the HPV vaccine, or whose male partners have received HPV vaccine, remains uncertain because the HPV vaccine does not provide immunity against all types of HPV that are responsible for cervical cancers and some vaccine recipients may already have been infected with hrHPV at the time of vaccination.

Conclusion

Since the introduction of testing for hrHPV, cervical cancer screening guidelines have changed drastically over the past 10 years. Molecular hrHPV testing is rapidly being introduced into cervical cancer screening and follow-up of cytology-positive women because it can provide valuable information for the
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practitioner’s clinical evaluation of at-risk women. Current recommendations include that hrHPV testing be included in cervical screening based on patient age and history; the main choices include co-testing with the Pap test and hrHPV testing or primary hrHPV testing alone. The introduction of the HPV vaccine has been associated with declines in HPV prevalence and incidence. Nevertheless, current HPV vaccines do not offer protection against all types of hrHPV that cause cervical cancer and, therefore, cervical screening will continue to be recommended among vaccinated populations.

References


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