

LEARNING OBJECTIVES

- Describe the types of human papillomavirus (HPV) and their risk association with cervical cancer
- Discuss the pathogenesis of precancerous lesions of the cervix
- Review the methods for collecting cytology specimens and for detecting HPV DNA
- Summarize the current guidelines for cervical cancer screening

The role of human papillomavirus testing in cervical cancer screening

HPV infection is usually transient; however, lesions can progress to cervical cancer. Updated screening guidelines help to identify those at high risk for more serious disease.

Lauren Morasse, MS, PA; Adi Davidov, MD; Mario R. Castellanos, MD

Human papillomavirus (HPV) that infects the GU tract is the most common sexually transmitted infection in both men and women in the United States. Currently, an estimated 20 million Americans are infected with HPV; and each year, approximately 6.2 million people become newly infected.¹

HPV infection manifests as genital warts and is known to cause cervical cancer in women.² Cervical cytology screening programs are among the most remarkable successes achieved in the prevention of cancer and cancer-related deaths. The introduction of HPV testing has further improved screening protocols. HPV DNA can now be readily identified through commercially available clinical tests.³ However, an understanding of the current recommendations for ordering an HPV test as well as an awareness of its limitations and risks are essential.

Approximately 80% of women will be infected with HPV at some point during their lifetime;⁴ however, a premalignant cervical lesion will actually develop in very few. Most cases will be transient viral infections, but a major limitation of the HPV test is that it does not distinguish a benign viral infection from a true precancerous lesion. Although clinical trials have shown that HPV testing has a role in cervical cancer screening protocols, inappropriate use of this test can lead to unnecessary follow-ups or invasive diagnostic procedures performed on a patient with a transient infection. This article reviews the current cervical cancer screening guidelines, with a focus on the role of HPV testing.

Human papillomavirus infection More than 100 types of HPV have been identified.^{5,6} These viruses are grouped as

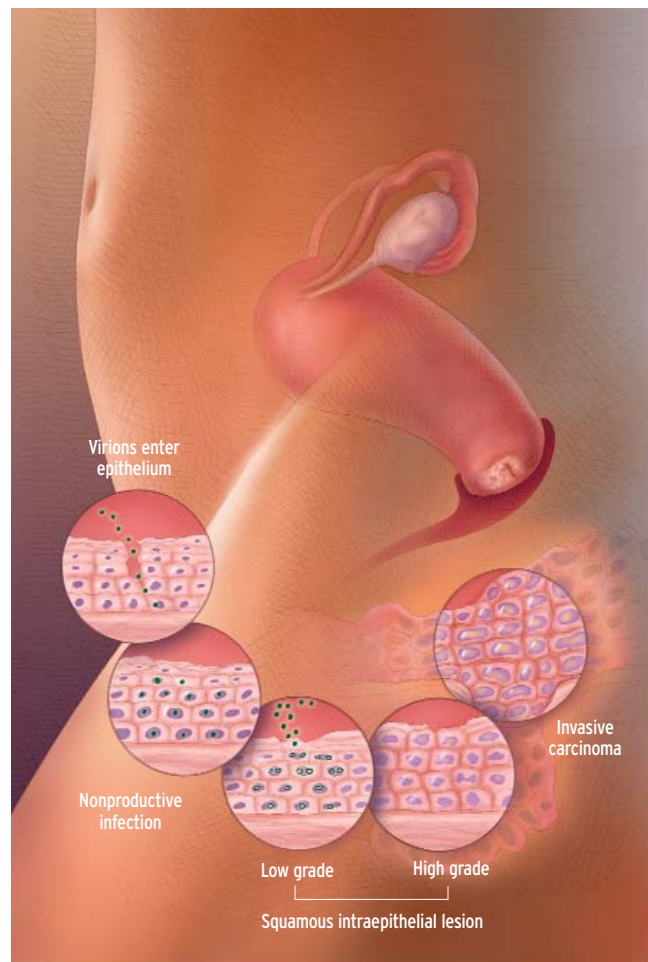


FIGURE 1. Progression of HPV infection to cervical cancer

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low- or high-risk based on their association with cervical cancer. Low-risk virus types (*nononcogenic* viruses) cause a spectrum of benign abnormalities. These viruses do not undergo a malignant transformation; instead the viruses cause *koilocytes*, cytologic changes consistent with HPV infection, or lead to *condyloma acuminatum*, a verrucous growth readily seen on physical examination of the cervix, vagina, perineum, or anus.³ The genital warts are usually caused by HPV types 6 and 11, the most commonly occurring low-risk HPV viruses. The high-risk virus types (*oncogenic* viruses) can also cause cytopathic changes seen on cytology as koilocytes; but unlike low-risk viruses, oncogenic viruses can cause changes that can be precursors to abnormal cellular development (*dysplasia*) that ultimately lead to malignant transformation and cervical cancer in susceptible patients.²

The most commonly occurring high-risk HPV types in the United States, HPV-16 and HPV-18, account for most cervical cancers. Other high-risk types that can be identified with FDA-approved assays are 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Both low- and high-risk viruses can cause condyloma. However, infections that progress to moderate or severe dysplasia are caused by the high-risk types.⁴ Although 80% of HPV infections are caused by high-risk types,⁴ most HPV infections do not require treatment. Approximately 70% of new HPV infections resolve within 1 year, and 90% of lesions regress spontaneously within 2 years.⁷ Persistent HPV infections can lead to cervical cancer 5 to 10 years after the onset of infection. Studies show that 10% of HPV infections progress to moderate or severe dysplasia within 2 years.⁸

CLASSIFICATION OF PRECANCEROUS LESIONS

Understanding the pathogenesis of precancerous lesions is essential to understanding the current cervical screening guidelines. Figure 1 illustrates the continuum of HPV infection as it develops into carcinoma in the cervical epithelium. The development of cervical cancer is a continuous disease process that progresses gradually through stages.^{2,4} HPV infects the cervical epithelium and causes pathognomonic changes, including cytoplasmic vacuolization, nuclear atypia (*koilocytosis*), and multinucleation. These changes can be seen on histology and cytology and are characteristic of condyloma acuminatum. As HPV infection progresses, dysplasia

develops in the squamous epithelium. The Bethesda System is used for reporting cytology findings. Its terminology correlates with the progression of lesions in the cervix.

Dysplasia seen on histology specimens is referred to as *cervical intraepithelial neoplasia* (CIN), and the lesions are classified as grades I (mild), II (moderate), or III (severe). CIN I represents a benign viral infection similar to condyloma acuminata; therefore, these two diagnoses are simply referred to as *low-grade lesions*. Dysplasia seen on cytology is referred to as a *squamous intraepithelial lesion* (SIL). Cells taken from low-grade lesions are called *low-grade SIL* (LSIL), and cells taken from high-grade dysplasia are referred to as *high-grade SIL* (HSIL). HSIL corresponds to CIN II or CIN III. High-grade dysplasia as a manifestation of HPV infection is a premalignant lesion and, if left untreated, will progress to carcinoma. HPV DNA in these high-grade lesions is incorporated into the host genome leading to cancer.²

Squamous cells that do not meet the diagnostic criteria for one of the Bethesda System categories are termed *atypical squamous cells* (ASC). Cytologists further classify these cells as *atypical squamous cells cannot exclude high grade* (ASC-H) if a high-grade lesion is suspected or as *atypical squamous cells of undetermined significance* (ASC-US) if a benign process is suspected. In the United States, ASC-US lesions are diagnosed in more than 2 million women and LSIL is diagnosed in more than 1 million women each year.⁹ The American Society for Colposcopy and Cervical Pathology (ASCCP) established guidelines for when to refer a patient for colposcopy and when only repeat cytology is appropriate. According to ASCCP guidelines, cytology that reveals LSIL, HSIL, or ASC-H should be followed-up with colposcopy to look for CIN.¹⁰

SCREENING METHODS

Cytology specimens are currently collected by one of two methods. A conventional Pap smear involves placing a sample of cervical cells directly on a slide and preserving it with a fixative. Liquid-based cytology, the preferred method, involves immersing the sample of cervical cells into a container of liquid fixative.¹⁰ The ability to place a thin monolayer onto a slide, making interpretation of the specimen easier, is an advantage of this system. In addition, the liquid-based media can be used for adjunctive testing, such as tests for *Neisseria*

KEY POINTS

- Approximately 80% of women will be infected with human papillomavirus (HPV) at some point during their lifetime; however, a premalignant cervical lesion will actually develop in very few. Most infections will be transient.
- Dysplasia seen on histology specimens is referred to as *cervical intraepithelial neoplasia* (CIN). Dysplasia seen on cytology is referred to as a *squamous intraepithelial lesion* (SIL). High-grade dysplasia as a manifestation of HPV infection will progress to carcinoma if left untreated.
- The American Society for Colposcopy and Cervical Pathology and the American College of Obstetricians and Gynecologists recommend that every woman should be screened for cervical cancer by Pap smear starting at age 21 years or within 3 years of initial sexual activity—whichever comes first. Women younger than 30 years should be screened annually. Screening frequency can be lowered to every 2 to 3 years in women older than 30 years provided that three consecutive cytology findings are negative.
- “Reflex” HPV testing is beneficial in triaging women with atypical squamous cells of undetermined significance seen on cervical cytology. Primary HPV testing as an adjunct to cervical cytology in women older than 30 years has increased sensitivity for detecting CIN II or CIN III.

gonorrhoeae, *Chlamydia*, and HPV. Two tests currently have FDA approval for liquid-based cervical cancer screening (ThinPrep Imaging System [Cytoc Corp, Boxborough, Massachusetts] and BD SurePath liquid-based Pap [BD Diagnostics-Tripath; Burlington, North Carolina]).

Several tests for detecting HPV DNA are available commercially; however, only the Digene Hybrid Capture 2 (HC2; Qiagen Inc, Valencia, California) is approved by the FDA for detecting HPV DNA. The HC2 HPV test has two probes. One probe is used to detect the high-risk HPV types, and the other detects the low-risk HPV types. Detection of low-risk subtypes has little clinical use. Positivity for high-risk HPV types has been shown to indicate a higher risk of an underlying high-grade dysplasia. The HPV test referred to in this article is the Digene High-Risk HPV HC2 DNA test.

“HPV testing as an adjunct to cervical cytology in women older than 30 years increases the sensitivity of detecting CIN.”

Study findings proved that HPV testing is beneficial in two scenarios.^{11,12} The first FDA-approved indication is when cytology diagnoses ASC-US. The ASC-US and LSIL Triage Study (ALTS), a large sentinel study sponsored by the National Cancer Institute,¹² demonstrated that patients with ASC-US usually do not have an underlying CIN lesion and only 10% to 15% of patients will have an associated high-grade dysplasia. The data from ALTS confirmed that “reflex” HPV testing was beneficial in triaging these patients.¹² Patients with ASC-US who are HPV-negative are not at risk for a high-grade CIN lesion and can be managed with repeat cytology in 1 year. Patients with ASC-US who are HPV-positive should be referred for colposcopy because these patients are at risk of having high-grade dysplasia.¹² The ALTS data also demonstrated that patients with ASC-H and LSIL should be referred for immediate colposcopy because most of these patients were found to be HPV-positive and at risk for high-grade dysplasia.¹²

ASCCP recommendations suggest using HPV testing as a primary cervical cancer screen in conjunction with cytology in women 30 years and older to improve the sensitivity of detecting CIN;¹³ this is the other FDA-approved use for the HC2 HPV test. Routine HPV testing is not recommended for women younger than 30 years because the prevalence of HPV infection is very high in this group. Routine testing would result in a high number of referrals for colposcopy in patients with a self-limiting viral infection; few of these patients would have a true precancerous lesion.¹⁰ Cervical cancer screening protocols for women younger than 30 years consists of cytology alone.

CURRENT GUIDELINES

ASCCP and the American College of Obstetricians and Gynecologists (ACOG) recommend that every woman should be screened starting at age 21 years or within 3 years of initial sexual activity, whichever comes first. Women younger than 30 years should be screened annually. Screening frequency can be lowered to every 2 to 3 years for women older than 30 years after three consecutive cytology findings are negative.³

Triage for women with ASC-US The most commonly diagnosed abnormality seen on cytology is ASC-US.¹⁴ The underlying risk for high-grade dysplasia is relatively low in these women; therefore, various strategies aimed at reducing the need for colposcopy have been studied. “Reflex” HPV testing is proven beneficial.¹⁰ HPV-negative women have a similar risk for an underlying high-grade dysplasia as do patients with negative cytology findings.¹⁵ HPV-positive patients are at increased risk for high-grade dysplasia; therefore, colposcopic examination is recommended. This strategy avoids colposcopy in approximately half of patients with ASC-US, thereby reducing cost and unnecessary invasive testing.

Primary HPV testing and cytology Most patients with a diagnosis of cervical cancer in the United States typically have had inadequate or no screening.¹⁶ However, a small proportion of patients will develop cervical cancer despite adequate screening with cervical cytology. This is a result of the inherent inaccuracies associated with sampling and interpretation difficulties. Use of primary HPV testing as an adjunct to cervical cytology in women older than 30 years is proven to increase the sensitivity of detecting CIN II or CIN III from 60% to 95%.¹⁰

Follow-up guidelines An abnormality seen on cytology is typically an indication for a colposcopic evaluation. The only exception is the patient with a diagnosis of ASC-US on cytology and negative HPV test results. The recommendations for patients who obtain primary cervical cancer screening with HPV testing and cytology are more complex because four possible scenarios can result.

- **Cytology, negative; HPV, negative** The risk of underlying CIN II or CIN III is extremely low; therefore, repeat testing can be delayed for 2 to 3 years.
- **Cytology, positive; HPV, positive** The patient should be referred for immediate colposcopic examination.
- **Cytology, positive; HPV, negative** The patient should be referred for colposcopy, unless cytology indicates ASC-US.
- **Cytology, negative; HPV, positive** This last scenario is the most challenging for clinicians. The literature has shown that approximately 6.5% of women older than 30 years will be HPV-positive and 58% of the women with HPV will have negative results on cytology.¹⁷ However, despite HPV-positivity, the risk for having an underlying CIN II or CIN III lesion is quite low in these women (2.4%-5.1%).¹⁶ Current ACOG and ASCCP recommendations are to repeat the cytology and HPV test in 1 year, and colposcopy is recommended if either test result is positive. If both cytology and HPV results are negative, repeat testing can be delayed for 2 to 3 years.

ADDITIONAL USES FOR HPV TESTING

HPV testing has FDA approval only for the two indications discussed above. However, the test has been used to manage treatment in other clinical scenarios.

Colposcopically confirmed CIN I Current ACOG and ASCCP guidelines do not recommend treating CIN I confirmed by colposcopy. However, the likelihood of progression to CIN II or CIN III in a subset of patients with persistent HPV infection increases each year HPV is present. Therefore, closely following patients with a CIN I lesion is imperative. Suggested strategies include repeat cytology every 6 months, with repeat colposcopy if the results are positive, and repeat HPV testing in 1 year, with repeat colposcopy if results are positive.

Posttreatment follow-up of CIN II or CIN III High-grade CIN will recur in 10% of patients treated for the disease.¹⁸ Furthermore, approximately 8 out of 1,000 patients treated for high-grade CIN will develop invasive cancer.¹⁹ Therefore, close follow-up is mandated for these patients. ACOG and ASCCP guidelines recommend cytology every 6 months, either with or without colposcopy, for these patients. Another option is HPV testing at 6 to 12 months with referral to colposcopy if the HPV test results are positive.

CONCLUSION

Cervical cancer was diagnosed in 493,243 women worldwide in 2002, and deaths totaled 273,505.²⁰ In the United States alone in 2006, cervical cancer was diagnosed in 9,710 women and nearly 3,700 women died from the disease.²¹ Cervical cancer is caused by HPV infection transmitted via sexual contact. The incidence of HPV infection can be limited by safe-sex practices or abstinence. The most effective way to prevent cervical cancer is to diagnose HPV infection in its early stages. Cervical cancer screening has achieved remarkable successes in recent years; however, like many other screening tests, it has limitations. A major limitation is that most women who acquire HPV have only a transient viral infection. Therefore, adhering to proven cervical cancer screening protocols is important in order to avoid unnecessary medical workups and invasive procedures.

A major advance in cervical cancer screening is HPV testing, which makes identification of HPV infections readily available within the clinical setting. Despite the expectation of incorporating this test into several cervical cancer screening algorithms, data from clinical trials demonstrate only two specific instances in which HPV testing is beneficial: Reflex HPV testing in women with ASC-US and as an adjunct to cytology in women older than 30 years. These two indications allow triaging of only women at high risk of developing cervical cancer to colposcopy. HPV testing of any another population of women during cervical cancer screening is not indicated because of the high incidence of transient HPV infection in women not likely to have an underlying high-grade CIN lesion.

Finally, even with the quadrivalent HPV vaccine (Gardasil) now in use, PAs are cautioned to continue proper patient education on cervical cancer screening. Counseling should focus

on regular screening practices. This is the primary preventive method for all types of premalignant cervical lesions, and failure to be screened is the number one reason for developing cervical cancer in the United States.¹⁶ Women who develop premalignant lesions while participating in regular cervical cancer screening programs reap the benefits of early diagnosis and treatment, thereby avoiding the potential progression to cervical cancer. **JAAPA**

Lauren Morasse is a recent graduate of the PA program at Wagner College, Staten Island, New York, and practices in cardiothoracic surgery at New York Methodist Hospital in Brooklyn. **Adi Davidov** is Director, Colposcopy Clinics, and Director of Gynecology, Staten Island University Hospital, Staten Island, New York. **Mario Castellanos** is Director, Medical Women's Health Division, and Clinical Director of Research, Department of Medicine, at the same facility. They have indicated no relationships to disclose relating to the content of this article.

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