

# Educational Case: Cervical Neoplasia: HPV and Its Link to Cancer

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*The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see <http://journals.sagepub.com/doi/10.1177/2374289517715040>.*

## Keywords

pathology competencies, disease mechanisms, HPV, human papillomavirus, cervical cancer, neoplasia, disease screening, PAP smear

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## Primary Objective

*Objective N3.1: Morphologic Features of Neoplasia:* Describe the essential morphologic features of neoplasms and indicate how these can be used to diagnose, classify, and predict biological behavior of cancers.

Competency 1: Disease Mechanisms and Processes; Topic N: Neoplasia; Learning Goal 3: Characteristics of Neoplasia.

## Secondary Objectives

*Objective CYP2.1 Screening:* Describe the principles of an effective screening test and the uses and limitations of cytology.

*Objective CYP2.2 Adjunct testing (HPV):* Describe how adjunct testing is used in conjunction with cytology examination.

*Objective CYP2.3 Cervical Screening:* Describe how to find and utilize current algorithms for management of cervical screening.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic CYP: Cytopathology; Learning Goal 2: Advantages of Cytopathology.

## Patient Presentation

A 45-year-old female presents to her new primary care physician to establish care after not visiting a doctor for 10 years.

She was recently referred for a follow-up visit after receiving an abnormal liquid Pap result from a free community outreach cervical cancer screening program. She denies any significant past medical or surgical history. The patient's last menstrual period was 2 weeks ago, and she is currently sexually active with multiple partners. She occasionally uses barrier methods of contraception. Physical examination and pelvic examination are within normal limits. Before discussing the results, the patient remembers having a history of abnormal pap smears that required further testing, but these were not pursued since she was lost to follow-up.

## Diagnostic Findings

Upon further review of the patient's history, the patient was 35 years old at the time of her last Pap test and pelvic examination. Cytology of her old Pap test showed a low-grade squamous intraepithelial lesion (LSIL). Human papillomavirus

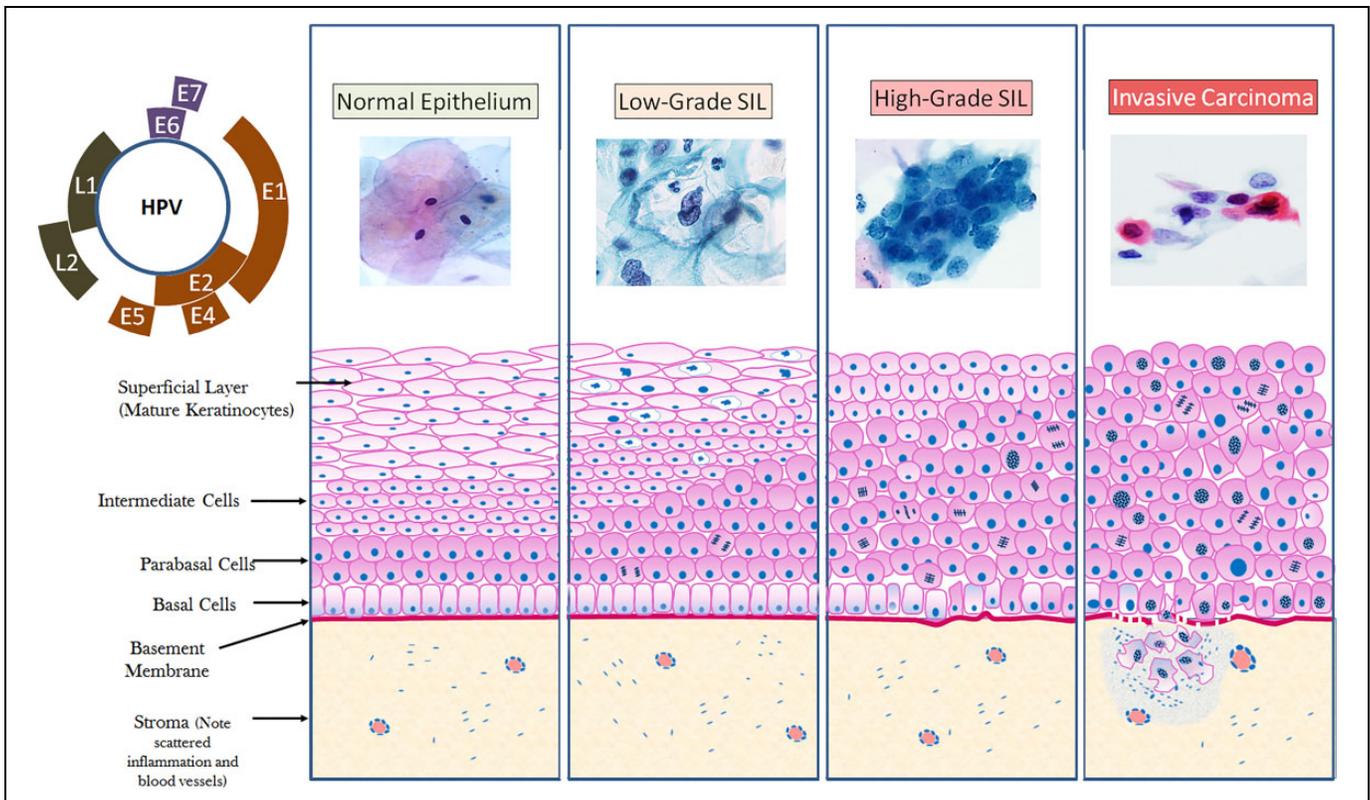
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**Figure 1.** Sketch demonstrating the progressive nature of the dysplastic changes leading to the development of cancer. To the left is a representation of the HPV genome with its associated early and late antigen proteins. HPV indicates human papillomavirus.

(HPV) co-testing results from that time demonstrated positivity for HPV type 16. As per American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines, the patient was recommended to undergo further testing with colposcopy but was lost to follow-up.

The patient's current cytologic evaluation is consistent with high-grade squamous intraepithelial lesion (HSIL), and HPV testing remains positive for HPV type 16. Gonorrhea/chlamydia testing is negative. The patient is now scheduled for an immediate colposcopy and loop electrosurgical excision (LEEP).

## Questions/Discussion Points

### What Are Your Preliminary Working Diagnosis and Its Differential?

This patient cytology results previously demonstrated an LSIL, which has since progressed to an HSIL with persistent HPV infection. The SILs are virally driven neoplastic proliferations of cervical epithelial cells and are precancerous. Epidemiological studies have demonstrated the time-dependent progressive nature of these lesions (Figure 1). The differential diagnosis may include other types of cervical lesions, such as squamous metaplasia, reactive changes, or glandular changes. Invasive squamous cell carcinoma of the cervix (when a precancerous lesion invades beyond the basement

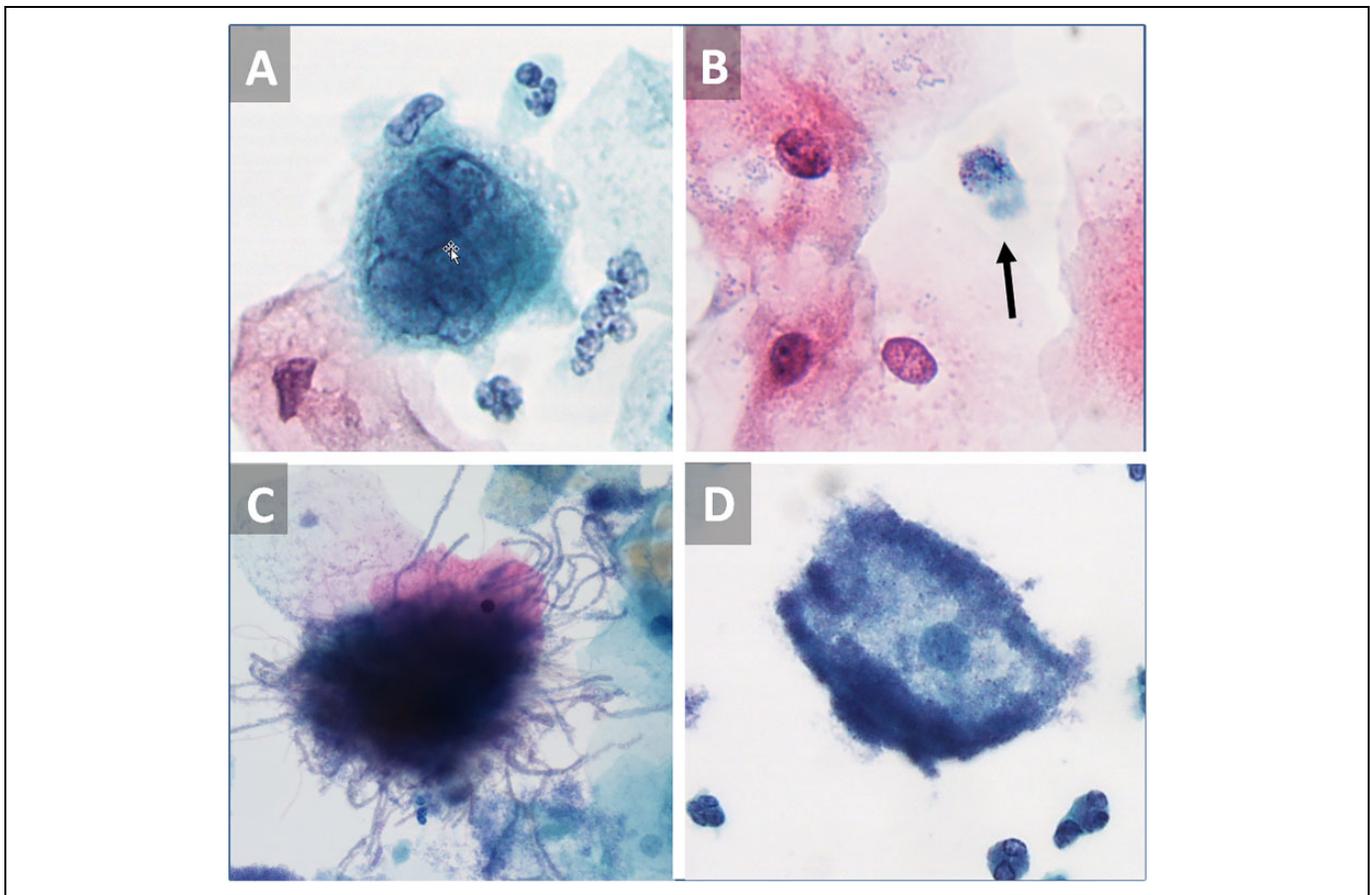
membrane) should not be ruled out and is the primary concern for this patient.

### What Screening Modalities Are Recommended to Detect Cervical Lesions and How Do They Impact Disease Progression?

Cervical cytologic screening was introduced decades ago and is still the most effective cancer prevention test today. For example, cytology screening that is only performed twice in a woman's life can reduce her risk for cervical cancer by 43%.<sup>1</sup> Cytologic testing and HPV co-testing for HPV infection decrease the prevalence and mortality of cervical cancer. Furthermore, regular screening allows for early intervention and reduces progression of disease.

Since cervical SIL is mostly a disease of reproductive-aged women, the American College of Obstetricians and Gynecologists (ACOG) recommends women aged 21 to 29 years have a Pap test every 3 years without HPV testing. Women aged 30 to 65 years should have a Pap test and co-testing for HPV every 5 years.<sup>2</sup>

HPV testing is not recommended for women younger than 30 years because the prevalence of HPV positivity is very high in these women, and the vast majority will completely clear their infections within 1 year.<sup>3</sup> However, for women older than 30 years, liquid-based Pap screening in conjunction with HPV co-testing has a lower false-negative rate and will detect a greater number of patients at risk compared to Pap testing alone.



**Figure 2.** Common infectious findings on Pap smear: (A) herpes simplex virus, (B) trichomonas vaginalis (arrow pointing to trichomonal parasite), (C) actinomyces, (D) Clue cell (indicating a shift in vaginal flora).

In 2014, the ATHENA (Addressing THE Need for Advanced HPV diagnostics) study examined the ability of HPV testing alone to identify women at risk of developing cervical squamous lesions. The study demonstrated that primary screening using only HPV testing can be an effective screening strategy in some patients. Based on data from this study, the Food and Drug Administration (FDA) approved the use of 1 HPV test (Roche Cobas HPV test) for primary cervical cancer screening (without cytology) in women aged 25 years and older.

Although HPV testing may be able to identify the presence of nucleic acids from high-risk HPV types and serial testing can demonstrate persistent infection, Pap testing still has several advantages over HPV testing alone. Since the Pap smear is not designed solely around HPV, it can provide tremendous additional information about the cervical and sexual health of a woman. Although not initially designed for this purpose, the Pap smear can identify numerous infections, including herpes, trichomonas, actinomyces, and bacterial vaginosis (Figure 2). It can even identify the presence of cancer cells from other gynecological sites, such as endometrial carcinoma. Additionally, Pap staining, which is designed to identify the lesions produced

by HPV infections, can provide information about how progressive the disease has become whereas HPV testing alone cannot.

#### *Why Is Cervical Cancer, in Particular, a Disease for Which It Is Appropriate to Create a Screening Program?*

In 1968, James Wilson published a World Health Organization pamphlet that addressed objective principles which any successful screening program must meet.<sup>4</sup> The principals were primarily not only related to the disease itself but also addressed case identification through testing. In brief, Wilson established that in order for a screening program to be successful, the associated disease must be a public health burden, there should be a pathophysiologic understanding of the disease, there must be a predisease state in which we can identify possible cases, there should be a cost-effective test for the disease and there should be an available treatment for the disease. Cervical cancer, along with our understanding of HPV-related neoplasia and progression to cancer, successfully meets all of these requirements and, therefore, was historically a prime candidate for a screening strategy.<sup>4</sup>

### ***Explain the Pathophysiologic Process That Occurs in the Development of Squamous Intraepithelial Lesion/Cancer: How Is Human Papillomavirus Involved?***

The HPV genotypes which have a documented association with squamous lesions and can progress to invasive cancer are termed “high risk.” Other “low-risk” genotypes may cause other lesions such as genital warts but do not progress to cancer. Of the high-risk HPV types, genotypes 16 and/or 18 have been identified in the majority of cervical cancers.

The pathogenesis of cervical cancer requires 2 biologically interrelated features: a productive HPV viral infection and an epithelial neoplastic process. According to this model, there is initially an infection with a high-risk type of HPV that persists and progresses to a pathologically defined precursor lesion and ultimately to invasion<sup>3</sup> (Figure 1).

Squamous lesions develop after HPV infected the basal or primitive cells of the immature squamous epithelium through defects or breaks in the skin or mucous membranes. The early region of the HPV genome includes transforming regions E6 and E7 whose corresponding proteins bind to and inhibit the host cell–regulatory proteins, p53 and Rb. This causes unrestricted cell proliferation and blocks cellular apoptosis. Most SIL starts at the squamocolumnar junction of the transformation zone of the cervix. Viral DNA replication occurs mostly in the superficial and intermediate cell layers of the squamous epithelium. As these infected cells move to the epithelial surface, differentiation-specific transcriptional factors from the host cells stimulate the production of viral capsid proteins and subsequently intact virions that produce characteristic cytologic and histologic changes<sup>3</sup> (see Figure 1).

### ***Describe the Natural History of Human Papillomavirus Infections and Its Relation to Neoplasia***

Most HPV infections are transient, becoming latent or undergoing immunologic clearance within 1 to 2 years of diagnosis.<sup>1</sup> However, infections with high-risk types of HPV clear more slowly and persistence of infection increases the likelihood of developing high-grade lesions. Prevalence of HPV infection peaks in the late teens to early 20s, while the incidence of cervical cancer in unscreened populations ranges from 35 to 55 years of age. Since HSIL is more common than invasive cervical cancer, this suggests that only a small portion of HSIL progresses to malignancy.<sup>1</sup> We, therefore, understand that although the development of invasive cervical cancer requires persistent infection with high-risk types of HPV, it is not always sufficient to cause cancer. Other associated risk factors include cigarette smoking, long-term use of combined oral contraceptive pills, and immunosuppression.<sup>3</sup>

### ***Describe the Cytopathological Features of Some Common Cervical Infections Which Can be Found on the Pap Stain***

Cervical cytology has relatively high specificity for most organisms and can help guide clinical management (see Figure 2).

- A. Herpes simplex virus (HSV): The classic features of HSV infections on Pap staining are described as the “three M’s of herpes”: multinucleation, margination of chromatin, whereby the rim of the individual nuclei appears darker staining than the center, and molding which is seen as each nucleus indenting the neighboring nuclei. With the accumulation of viral particles, the nuclei may develop a “ground glass” appearance.
- B. *Trichomonas vaginalis*: The parasitic organisms are visible with liquid-based preparations. They appear as pear-shaped organisms with an eccentrically located nucleus, eosinophilic cytoplasmic granules, and flagella.
- C. *Actinomyces*: Clumps of woolly filamentous organisms which are usually deeply staining and may demonstrate acute angle branching.
- D. Clue cells: Irregularly shaped squamous cells covered with darkly staining coccobacilli. These cells typically indicate a change in the cervical microbiological flora and may be an indication of bacterial vaginosis.

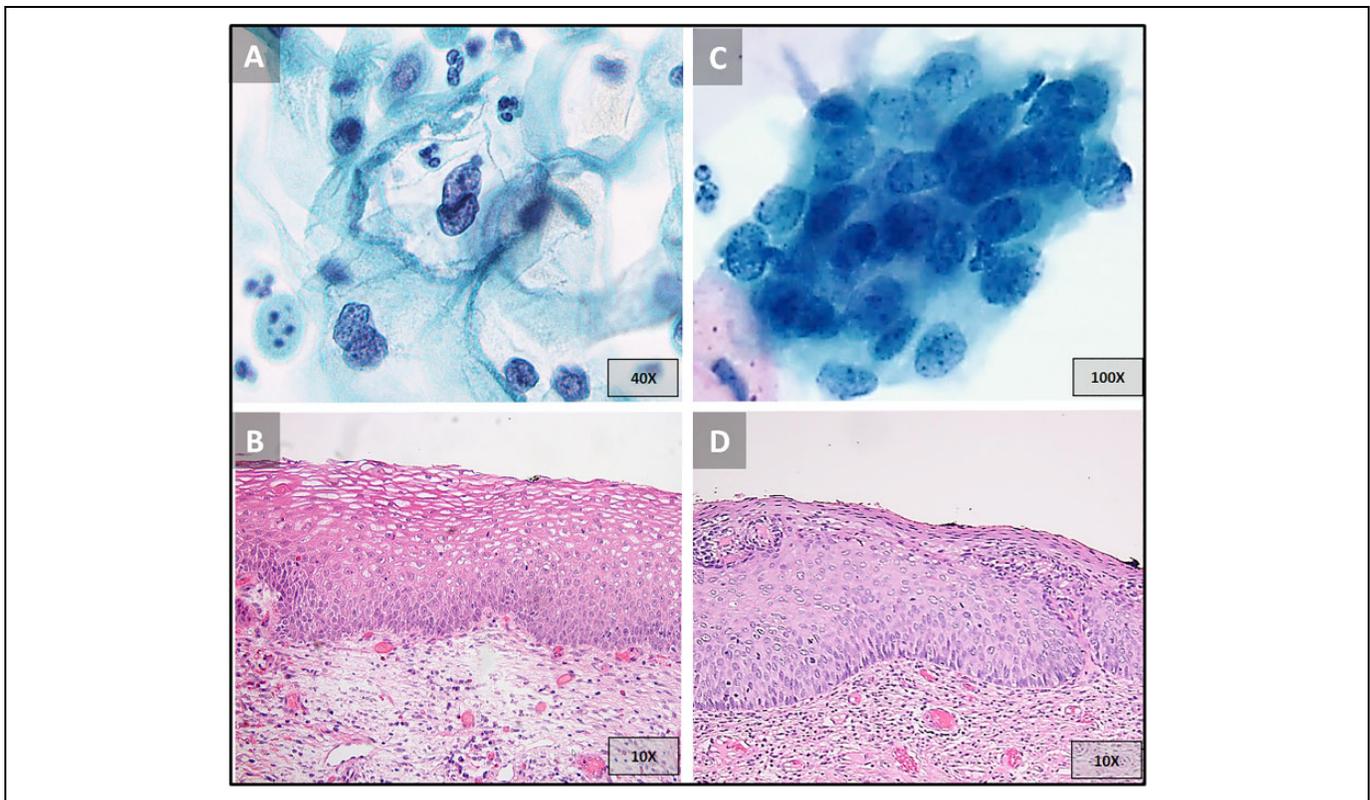
### ***What Systems Are Used to Classify Lesions Identified by Cervical Cytology? How About Classification of Cervical Histopathology?***

The Bethesda System (TBS) is used to categorize cytological diagnoses, while the Lower Anogenital Squamous Terminology (LAST) project describes histological findings associated with HPV throughout the anogenital tissues, including the cervix.<sup>5</sup> The Bethesda system utilizes a 2-tiered system of classification (LSIL or HSIL) and previously histopathologists utilized a 3-tiered system (cervical intraepithelial neoplasia [CIN]-I, CIN-II, and CIN-III). Large studies have demonstrated significant diagnostic variation between CIN-II and CIN-III; additionally, CIN-II lesions were found to demonstrate similar rates of progression of disease to CIN-III in some instances. For these reasons, the LAST project has adopted a 2-tiered diagnostic system and both LAST and TBS are in line with one another.<sup>6</sup>

### ***Compare and Contrast Cytopathology and Histopathology in the Images***

- A. Cytology LSIL:

Cells have enlarged nuclei, about 4 times the size of a normal intermediate cell nucleus. The nuclei are darker (hyperchromatic) with wrinkled irregular nuclear membrane contours. Cells may be multinucleated as seen in the image and the chromatin is finely granular. Areas of clearing around the nucleus (koilocytosis), a hallmark of LSIL, are also seen (Figure 3).



**Figure 3.** Cytologic and histologic images of low-grade SIL (A, B) and high-grade SIL (C, D). SIL indicates squamous intraepithelial lesion.

#### B. Histology LSIL (Previously CIN-I):

Nuclei are enlarged and irregularly shaped. There is also darkening of the chromatin (hyperchromasia). Koilocytes with their large vacuoles can be seen in the middle layers, while the superficial layers still resemble a fairly normal basket weave appearance of unaffected epithelium. Overall, there is increased thickness of the epithelium.

#### C. Cytology HSIL:

The degree of nuclear atypia is increased, such that the nuclei of these cells appear more hyperchromatic and irregular. The amount of cytoplasm decreases as nuclear to cytoplasmic (N:C) ratio increases. The classical features of a mature squamous cell with abundant cytoplasm are no longer seen, and these cells appear less differentiated.

#### D. Histology HSIL:

Undifferentiated neoplastic cells which no longer resemble the normal squamous cells are present in all layers of the epithelium. The lower layers of the epithelium (basal and parabasal) are thickened, and abnormal mitotic figures are visible. There is nuclear crowding, and cellular variation (pleomorphism) is seen. These areas may appear “bluer” under the microscope because there is less pink cytoplasm. The N:C ratio is high due to scant cytoplasm.

### *What Is the Recommended Management of Abnormal Cervical Cancer Screening Tests and Cancer Precursors? How Should You Proceed Now With More Advanced Disease Present?*

The ASCCP *Updated consensus guidelines for managing abnormal cervical cancer screening tests and cancer precursors* outlines the recommendations for managing patients with cervical cytological abnormalities. Although it takes many years for HSIL to progress to invasive cancer, untreated higher grade lesions are also more likely to persist and progress than regress.<sup>3</sup> Therefore, appropriate disease follow-up and management are crucial.

Since our patient now presents with evidence of HSIL on cytology, it is recommended that she undergoes an immediate LEEP or colposcopy with endocervical assessment. Management by ASCCP guidelines differs depending on the colposcopy results. If the biopsy confirms HSIL, either excision or ablation of the “transformation zone” is recommended, followed by co-testing at 12 and 24 months.<sup>1</sup>

### *What Other Factors Aside From Screening May Alter the Future Burden of This Disease on the Population?*

The HPV is an immunologically cleared infection. As such, it was a prime candidate for the development of a multivalent HPV vaccine. Currently, there are 3 FDA-approved HPV vaccines on the

market: Gardasil, Gardasil 9, and Cervarix. The Cervarix vaccine is designed for immunity against HPV 16 and HPV 18, the 2 most common high-risk HPV types that cause about 70% of cervical cancer. Gardasil adds coverage for 2 additional low-risk HPV genotypes (6 and 11) which are the major cause of genital warts. Gardasil 9 extends coverage further to 5 additional high-risk HPV genotypes associated with cervical cancer. These vaccines consist of nonreplicative viral-like particles which provoke an immune response and provide humoral immunologic memory.<sup>7</sup> Currently, several international health-based organizations recommend HPV vaccination be a part of all routine immunization programs. It is important to note that screening following immunization is still required and we have discussed several additional benefits to screening beyond identification of HPV infection.

### Describe Some Possible Therapies That Are Being Developed to Treat Human Papillomavirus–Associated Lesions and Cancers

While the initial therapy of squamous lesions is currently complete excision, other novel therapies are currently being evaluated. We have discussed vaccines to prevent infection that are widely available and utilize viral structural proteins. There are currently in development vaccines that help a person's immune system to clear the HPV after infection. Most of these vaccines target HPV oncoproteins E6 and E7.<sup>8</sup> This treatment is anticipated to become clinically available in the near future to decrease the HPV-associated disease burden. Furthermore, research on a pelvic sentinel lymph node biopsy procedure, immunotherapy, and targeted drug therapy is also being conducted.

### Teaching Points

- Characteristic cytologic and histologic features, such as koilocytosis, enlarged nuclei, and increased N:C ratio, allow pathologists to diagnose and classify different grades of squamous cervical pathology from low-grade to high-grade squamous lesions and ultimately to carcinoma.
- Classifications based on these features allow one to predict the biologic behavior and malignant potential of these precancerous lesions.
- HPV infection, particularly with high-risk types of HPV, is not only a major risk factor for developing cervical SIL/cancer but also a crucial factor in the pathogenesis of this disease.
- Adequate screening for cervical precancerous pathology may include both cytology Pap and ancillary HPV testing as each method has distinct advantages for the detection of pathologic conditions.
- Cervical disease and its associated diagnostic testing are a paradigmatic model of disease screening and intervention and remain one of the most successful screening programs ever devised.
- Several organizations, including the ACOG and the ASCCP, regularly publish guidelines related to screening and management of the cervical cancer.

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