Human Papillomavirus

EILEEN M. BURD1,2 and CHRISTINA L. DEAN1

1Emory University School of Medicine, Department of Pathology and Laboratory Medicine, Atlanta, GA 30322; 2Emory University School of Medicine, Department of Medicine, Division of Infectious Diseases, Atlanta, GA 30322

ABSTRACT Individuals with inherited immunodeficiencies, autoimmune disorders, organ or bone marrow transplantation, or infection with human immunodeficiency virus (HIV) are at increased risk of infection with both low-risk and high-risk human papillomavirus (HPV) types. Chronic immunosuppression provides an environment for persistent HPV infection which carries a higher risk of malignant transformation. Screening guidelines have been developed or advocated for processes that have detectable premalignant lesions, such as anal cancer or cervical cancer. For other anatomic locations, such as cutaneous, penile, and oropharyngeal, a biopsy of suspicious lesions is necessary for diagnosis. HPV cannot be cultured from clinical specimens in the laboratory, and diagnosis relies on cytologic, histologic, or molecular methods.

INTRODUCTION
Human papillomavirus (HPV), a member of the Papillomaviridae family, is a small (8kb), nonenveloped, double-stranded DNA virus. HPV has a predilection for infecting cutaneous and mucosal epithelial cells (1). Infection with HPV is associated with a wide range of pathology. HPV is the etiological factor in benign cutaneous warts and juvenile respiratory papillomatosis, as well as low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), a precursor to cancer, and invasive carcinoma (2). Harald zur Hausen, a German virologist, was the first to describe the association of HPV and cervical cancer in the 1970s (3, 4). It is now understood that HPV is necessary in the development of cervical cancer and is also associated with carcinoma of the vulva, vagina, penis, anus, and oropharynx (5, 6).

HPV does not grow well in tissue culture and is classified based on its DNA sequence. Currently, more than 200 types of HPV have been fully sequenced (1, 7). Human papillomaviruses are generally grouped into five genera (alpha, beta, gamma, mu, and nu) based upon tissue tropism and subsequent host pathology (2, 7). HPV types within the alpha group have been the focus of a considerable amount of research due to their ability to cause both cutaneous and mucosal pathology (2, 4). Individual types within this genus are further classified into high-risk HPV and low-risk HPV, depending on their oncogenic ability. HPV6 and HPV11 are low-risk types and are most commonly associated with benign anogenital warts (condylomata acuminata) (1, 8). Although HPV16 and HPV18 are the most prevalent types causing HSIL and cervical carcinoma, at least 12 high-risk types have been identified (9, 10). Of note, not all individuals infected with high-risk HPV will go on to develop cancer (2, 4). Elucidation of the life cycle and pathogenesis of HPV has largely come from studying HPV16 infections of the cervical epithelium (4).

Within the circular genome of HPV, eight genes are encoded, including genes expressed in both early and late stages in the life cycle. The infection produced by HPV can follow one of two pathways and is either productive or nonproductive (abortive or transforming). Regardless of the pathway, HPV requires access to the basal lamina, and such contact is likely achieved through small areas of damage to the epithelium (7). Capsid proteins, L1 (major coat protein) and L2 (minor coat protein), facilitate entry into the basal layer keratino-
cytes (7, 11). L2 is thought to also be involved in allowing the viral genome to enter the host cell nucleus where replication ensues via proteins E1 and E2 as well as the host cell’s replication machinery (7, 12). The E1 gene product is a viral DNA helicase, which is recruited by the binding of the viral transcription factor encoded by the E2 gene (2). E2 is also involved in anchoring the viral episome to the host cell genome (13).

Initial viral replication in the basal cells seems to be independent of the host cell cycle, and viral genomes are amplified to densities of approximately 50 to 200 copies per cell (2, 8). In the productive pathway, viral DNA copies are maintained at low copy number until the cell moves through the epithelium in the process of differentiation. Virus replication and assembly occurs in postmitotic differentiated squamous epithelial cells as they move toward the epithelial surface. E6 and E7 are regulated by E2, and while their precise role is somewhat uncertain, they are associated with driving cell cycle progression to allow HPV DNA replication in the mid-layers of the epithelium. Virus gene expression is tightly controlled, and viral DNA is amplified as extrachromosomal nuclear plasmids with viral copies increasing about two to four logs (2, 7, 8, 14). No viremia is produced and replication takes place entirely within the cell, thus producing little immune response from the host (15). At the epithelial surface, keratinocytes are considered terminally differentiated, and they die and are removed from the body by natural processes. Large amounts of virus are released from the epithelial surface for transmission to other individuals. At this point, HPV DNA may be found intracellularly as well as extracellularly (1). This has important implications for diagnostic testing. These steps that lead to productive virus synthesis in the superficial epithelial layers can be followed by either low- or high-risk HPV types, although productive infections never lead to cancer because the cell cycle is halted and infected cells leave the body. Most HPV infections become undetectable after several months (16). Current thinking is that productive infection is supported in the cellular environment at the ectocervix but is inefficiently supported by the cells at the endocervix, where nonproductive infection is favored (2).

In nonproductive infection, HPV gene expression becomes deregulated and the normal life cycle of the virus cannot be completed. The HPV types associated with nonproductive infections are designated as high-risk because of their association with progression to cancer. The high-risk carcinogenic types of HPV currently designated by the International Agency for Research on Cancer (IARC) are HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV59 (9). HPV68 is classified as probably carcinogenic, and HPV26, HPV30, HPV34, HPV53, HPV66, HPV67, HPV69, HPV70, HPV73, HPV82, HPV85, and HPV97 have been associated with rare cases of cervical cancer and are considered probable carcinogens (9). The E6 and E7 proteins in high-risk HPV types are functionally different from low-risk types and serve to stimulate proliferation of infected basal and suprabasal cells, allowing enlargement of the lesion. Long-term persistence of HPV is a necessary foundation for nonproductive infection. Persistence facilitates integration of the viral gene into the host cell chromosome. Although some cancers continue to harbor episomal HPV DNA, integration of viral DNA into the host cell genome is a common step associated with further deregulation and overexpression of the E6 and E7 oncogenes and carcinogenic progression (12). Integration occurs by chance, and E6/E7 overexpression typically results when HPV DNA integration occurs in the E1/E2 region and causes disruption or deletion of E2 gene sequences. This leads to loss of feedback control and overexpression of the E6 and E7 oncogenes as well as disruption or silencing of the downstream early genes that are required for regulated viral replication. The E6 protein prevents cellular apoptosis by degrading p53 and contributes to the accumulation of genetic mutations that lead to immortalization. The E7 protein stimulates cell proliferation when it binds with the retinoblastoma (Rb) protein and also stimulates host genome instability (17). The E5 protein appears to interfere with apoptosis and enhances the transforming ability of the E6 and E7 proteins (2, 12, 18). After the initial replication, viral DNA is stably maintained at an almost constant copy number during successive divisions of the basal cells (19). This is thought to occur because the viral genomes replicate once along with cellular DNA during the S-phase of the host cell cycle and are divided equally into the two daughter cells (19).

Even in this setting, most nonproductive infections are eventually cleared (as measured by current molecular tests), 50% within one year and 90% within two years, by way of a T-cell-mediated immune response (2, 8). High-grade lesions and cancers usually occur in individuals who do not resolve their infection and who maintain oncogene expression for years or decades.

There is growing evidence supporting the ability of some HPV types to persist in a long-term latent state in basal epithelial cells after apparent resolution of infection (20). Latent infection has been shown to be a risk factor for the development of disease at the same site at a
later point in time. Studies in animal models suggest that HPV genome replication can continue even though gene expression is suppressed by the activity of memory T cells (20). Since this type of latency is immune-mediated, subsequent immunosuppression can lead to reactivation of infection in these experimental systems. Although the duration of the latent state in humans is not known, it is a possible explanation for new HPV detection as a result of aging immune systems in older women (20). It appears that similar silent infections can result from low-titer infections or infections in epithelial cells that do not completely support the HPV life cycle (20). In this situation, changes in the local environment that occur with mechanical irritation, wounding, or exposure to ultraviolet light can initiate reactivation (20).

**Epidemiology and Risk Factors**

**Immune-Competent Patients**

HPV is the most common sexually transmitted infection (STI) worldwide. The majority of individuals who are sexually active will be infected with HPV at some point in their lifetime (6). Close to 80 million people in the United States are currently infected with HPV, and another 14 million become newly infected every year (21). Anogenital warts are a common clinical manifestation of HPV infection, and there are roughly 350,000 new cases in the United States every year (6, 22). Persistent infection with high-risk HPV is the most significant risk factor for developing carcinoma (2, 6). Cervical and oropharyngeal carcinoma comprise the majority of the newly identified HPV-associated carcinomas, and most of these cases are due to infection with high-risk HPV types, HPV16 and HPV18 (6, 23). Immune-competent persons infected with HPV generally have a slow progression to high-grade, precancerous lesions and subsequent carcinoma (24). However, most people with competent immune systems are able to clear HPV infections with no sequelae. Individuals who have compromised cell-mediated immunity, such as those with human immunodeficiency virus (HIV), AIDS, or solid-organ transplant (SOT) recipients, have shown accelerated progression to high-grade lesions and, thus, are at an increased risk to develop HPV-associated carcinomas (24–26).

**Immunocompromised Patients**

**External skin**

**Cutaneous warts**

HPV infection is very common among HIV-positive individuals, due to the sexually transmitted nature of both viruses (23). Hence, this patient population is at an increased risk for developing HPV cutaneous and mucosal disease. Cutaneous disease including common warts, epidermodysplasia verruciformis-like lesions, squamous cell carcinoma (SCC), and basal cell carcinoma is more prevalent in HIV-positive individuals (27, 28). These cutaneous lesions tend to be associated with HPV types usually found in the genital tract, and they are more likely to be aggressive and difficult to treat (28). Although the role of HPV in the oncogenesis of skin cancer has not been fully elucidated, there have been many reported cases of high-risk HPV types associated with SCC of the distal digits and periungual skin in HIV-positive individuals (28). These carcinomas require aggressive treatment and tend to recur.

Benign anogenital warts, or condylomata acuminata, are the most common clinical manifestation of HPV infection in both immune-competent and immunosuppressed individuals. Roughly 90% of anogenital warts are attributed to low-risk types, HPV6 and HPV11 (6). Similar to nongenital cutaneous lesions, HIV-positive patients often present with widespread lesions that can be more aggressive and difficult to treat (28, 29).

HPV-induced cutaneous warts and SCC also pose a major health risk for SOT recipients who are receiving immunosuppressive therapy. In fact, it has been reported that SOT recipients are at a higher risk for developing skin cancer than those with HIV or AIDS (30). This phenomenon has not been fully elucidated, but research suggests that certain immunosuppressive drugs, as well as an individual’s humoral immune response, may play a role (30, 31). The 10-year cumulative incidence of skin cancer in this population reaches 10 to 40% (32). Renal transplant recipients appear to be at a particularly high risk for developing HPV-related cutaneous warts and skin cancers (32, 33). Immunosuppression, ultraviolet light, and infection with high-risk HPV types are all important risk factors in patients with a history of SOT (33, 34).

Hematopoietic stem cell transplant (HSCT) recipients, especially those with chronic graft-versus-host disease (cGVHD), are at an overall increased risk for developing a secondary malignancy, with cutaneous SCC being the most frequent lesion identified (35). However, the association with cutaneous SCC and HPV in this patient population has not been well studied.

**Epidermodysplasia verruciformis**

Epidermodysplasia verruciformis (EV) is an autosomal recessive dermatologic condition characterized by persistent HPV infections of the skin that can transform into
carcinoma (25, 34). In fact, nearly 50% of patients with EV will develop skin cancer by the time they reach their fifth decade of life (34). There are specific HPV types associated with EV (EV-HPV), but HPV5, HPV8, and HPV14 are most commonly associated with malignancy in these patients (34). Whether EV-HPV is a causal factor in the development of cutaneous malignancy is still debated, and the oncogenesis is poorly understood (36). Recently, an acquired EV, or EV-like, syndrome has been described in immunosuppressed patients infected with EV-HPV, specifically those with HIV and SOT recipients (37). Case reports for this entity are rare and progression to malignancy has not yet been documented.

Oropharyngeal cancer
Individuals with HIV are at an increased risk of developing HPV-related cancers, including head and neck cancer (HNC). Over 400,000 new cases of HNC occur every year, making it the sixth most common cancer worldwide (38). HPV is detected in roughly 25% of cancers arising in the oropharynx, and in the general population, detection is directly correlated with the number of recent oral sex partners (39). More than 80% of HNCs are caused by oncogenic HPV16, and this is the most frequently detected HPV type in HIV-positive patients (40, 41). Up to a 3-fold increase in the incidence of oral HPV infection has been demonstrated in individuals infected with HIV (40). Contrary to HIV-negative individuals, oral HPV infection in those with HIV is associated with increased numbers of lifetime oral sex partners (40). It is still not clear whether CD4 count and/or AIDS diagnosis has an effect on the prevalence of oral HPV infection (39). Additionally, the effect of antiretroviral therapy (ART) on oral HPV prevalence has not been fully elucidated, but some studies suggest that there may be an increase in oral lesions and HPV persistence in individuals receiving ART (39).

SOT recipients are also at an increased risk for developing HPV-related cancers. Oropharyngeal carcinoma is the third most common HPV-related cancer identified in SOT recipients, after vulvar and anal carcinomas (42). Factors such as age, race, transplanted organ, and certain immunosuppressive drugs may affect the incidence of HPV-related oropharyngeal carcinoma (42). Oropharyngeal carcinoma is frequently found in long-term survivors of HSCT. As in cutaneous SCC, these lesions are strongly associated with cGVHD (35). Again, the association between HNC and HPV has not been fully elucidated in HSCT recipients; however, several case reports suggest that these lesions may be HPV-driven (43).

Anal cancer
Anal cancer is a rare malignancy, with an incidence rate of around 2 per 100,000 for both men and women (44). A significant risk factor for anal SCC is coinfection with high-risk HPV and HIV (45). More than 90% of anal SCCs are associated with persistent HPV infection, and the majority of these cases are attributed to HPV16 and HPV18 (45, 46). The prevalence of anal HPV detected when screening men and women with HIV surpasses 90%, though not all will have abnormal pathology. The incidence of anal SCC is increased 30-fold in HIV-positive individuals and 80-fold in HIV-positive men who have sex with men, compared to HIV-uninfected individuals (45, 47). A notable increase in the incidence of anal SCC was reported with the introduction of ART, which was attributed to the increased lifespan of HIV-positive individuals. However, a recent study suggests that these numbers may be stabilizing (47). Epidemiological studies have shown that 20 to 50% of SOT recipients have detectable anal HPV and a 10-fold increase in the relative risk of developing anal SCC (48, 49). Similar to HIV-positive patients on ART, the incidence of HPV-associated anal cancer has increased in SOT recipients who remain on immunosuppression for long periods of time, likely due to increased survival (48).

Penile cancer
Penile SCC and penile HSIL are rarely encountered diseases in the general population. In the United States, penile SCC accounts for less than 0.5% of all cancers in men (50). HPV is associated with roughly 50% of penile cancer cases, and oncogenic HPV16 is the most common type detected (51, 52). Individuals infected with HIV have a 2- to 3-fold increase in risk of developing penile SCC (28). Up to 4% of the HIV-infected population may have precursor lesions (HSIL), but the progression to cancer only occurs in about 5 to 30% of cases (28). Other risk factors include tobacco use, poor hygiene, phimosis, and lack of circumcision (28).

Cervical cancer
With over 500,000 cases every year, cervical carcinoma is the fourth leading cause of cancer in women worldwide (53). It is now known that HPV is necessary for cervical cancer to occur, and its pathogenesis has been investigated extensively. Risk factors for cervical carcinoma include tobacco use, young age of sexual debut, multiple sexual partners, high-risk sexual partners, history of other STIs, and a history of genital SIL or carcinoma (54). Women with HIV/AIDS and SOT recipients,
in particular renal transplant, have an increased incidence of persistent HPV infection and, thus, are at an increased risk for developing cervical carcinoma (25, 55). Of note, some studies suggest that SOT recipients on immunosuppressive therapy do not have an increased incidence of cervical cancer (42, 55). HIV-infected women have up to a 22-fold increased incidence of cervical cancer, and CD4 count is inversely proportional to abnormal cervical pathology (24, 25, 56). Because of the high prevalence, cervical carcinoma was classified as an AIDS-defining disease in 1993 (25). It is still unclear whether implementation of ART in HIV-positive women decreases the incidence of cervical cancer in this risk group (57).

Cervical carcinoma is the third most common secondary malignancy in HSCT recipients (35, 58). Women who receive long-term treatment for cGVHD and those with an unrelated HLA-matched donor are at greatest risk for developing SIL after transplantation (58, 59). Whether this is due to reactivation of HPV or to infection posttransplant needs to be further investigated.

LABORATORY TESTING

Skin Lesions

Diagnosis

Immunosuppressed patients should be vigilantly followed for early diagnosis of skin cancer. Lesions in immunosuppressed patients tend to grow more rapidly and can be more invasive (60, 61). In addition, there is a strong association for development of additional squamous cell carcinoma lesions in patients who have been previously diagnosed (62).

Cutaneous warts can generally be diagnosed by examining the affected area of skin, which may be extensive in immunocompromised patients. Warts do not generally need to be biopsied to make the diagnosis or to determine what treatment may be necessary, unless there is concern that the changes could be cancerous. There is some suggestion that persistent low-risk beta-HPV-induced warts may progress to actinic keratosis and skin cancer in immunocompromised patients since squamous cell carcinoma lesions and warts can be found jointly in this population (61, 63). For this reason, biopsy or surgical excision specimens for histopathologic assessment should be obtained from all suspicious lesions. The squamous cell carcinomas that develop in immunocompromised individuals have characteristic morphological features of HPV-induced lesions (63). Some phenotypic differences suggestive of a more aggressive process have been noted in skin cancers of transplant recipients compared to those found in immune-competent patients (64). These differences include higher levels of immunohistochemical staining for p53 and transforming growth factor-beta (TGFβ) proteins, along with low levels of phosphorylated mTOR and P70S6K, and a higher incidence of a spindle cell component, indicating epithelial-to-mesenchymal transition (64, 65).

Oropharyngeal Cancer

Screening

Because some parts of the oropharynx are difficult to visualize, oropharyngeal squamous cell cancers are often detected in later stages. A number of efforts have been made toward early detection but they have achieved only limited success. Current guidelines from the U.S. Preventive Services Task Force and the American Dental Association suggest that screening using conventional visual and tactile examination may facilitate early diagnosis of oropharyngeal and other oral cancers, but there is not sufficient evidence to support the use of additional screening tests (66, 67).

Diagnosis

The diagnosis of oropharyngeal cancer (OPSCC) is made by microscopic examination of biopsy tissues or exfoliated cells. Biopsy is often preferred since exfoliative cytology does not detect all cancers.

The histopathologic terminology used to describe HPV-related OPSCC has been inconsistent. Rather than describing specific histologic features, OPSCC tumors are often described by the presence of HPV and/or cellular changes due to HPV. The National Comprehensive Cancer Network and the College of American Pathologists have recommended routine testing for HPV in all OPSCC (68). It is essential for accurate diagnosis that only specimens obtained from the oropharynx are tested for HPV, since involvement of HPV with oral tumors outside of the oropharynx is negligible and may not be specific to the tumor. Since HPV-positive tumors tend to have better clinical outcomes, HPV testing can be helpful as a prognostic indicator (69). While metastatic disease is not common with OPSCC, and metastases in HPV-positive tumors are not preceded by bulky or advanced oropharyngeal disease, HPV testing may help indicate the site of the primary tumor (70). In the future, since prognosis is good, HPV testing may also allow for more directed and less aggressive therapy for OPSCC than therapy for smoking-related oral tumors. Other future uses of HPV testing could potentially include the assessment of response to treatment and monitoring for recurrence of disease following treatment.
While testing is recommended, the types of test(s) that should be used to detect HPV-associated OPSCC are not specified. The FDA-approved nucleic acid amplification HPV tests for cervical cytology are not approved for oropharyngeal specimens. Furthermore, tests that assess transcriptional activity or specifically localize HPV to the tumor may be more relevant since HPV can occasionally be detected in the absence of disease (71, 72). Numerous tests, such as RT-PCR for E6/E7 mRNA, HPV DNA-based in situ hybridization (ISH), and immunohistochemistry (IHC) for p16, p53, Ki67, proliferating cell nuclear antigen, and other markers have been investigated (73–76). HPV DNA ISH and p16 IHC have emerged as the most reliable of the methods. HPV DNA ISH allows for visualization of the virus in tumor cells and has been found to be highly specific, but not as highly sensitive. Modifications that include the use of nonfluorescent chromogens and changes in signal amplification steps have resulted in increased sensitivity (76). Because there will be some p16 staining in nearly all squamous cell carcinomas, the use of tumor-suppressor protein p16 IHC has been found to be highly sensitive as a surrogate marker of transcriptionally active HPV infection; unfortunately the marker is not entirely specific (76). When a positive p16 IHC stain is defined as strong nuclear staining plus cytoplasmic staining, which is present in at least 50% of tumor cells, then IHC is sufficiently sensitive and specific (76). Regardless of HPV status, p16 IHC staining alone is considered to be the most useful prognostic indicator for patients with known OPSCC (76, 77). Outcomes for p16 IHC-positive tumors are not significantly different whether they are HPV-positive or negative, but outcomes are significantly better compared to tumors that are negative for p16 (77). Along with HPV DNA ISH or HPV PCR, p16 IHC is recommended to help resolve focal or weak p16 staining, p16-negative tumors with typical HPV histologic morphology, and p16-positive tumors that do not have typical HPV histologic morphology (76) (Fig. 1). In patients with a known primary OPSCC, p16 IHC may be used instead of HPV testing of fine-needle aspiration biopsies to determine if the cancer has spread to the local lymph nodes (76).

**Penile Cancer**

**Diagnosis**

Early recognition of penile cancer is based on visual examination, and changes in penile skin color, thickening, ulcers, bumps, flat growths, or other lesions may warrant further investigation. Flat penile lesions, in particular, seem to be associated with HPV (78). A biopsy is needed to make an accurate diagnosis of cancer. Squamous cell carcinoma is the most common type of penile cancer, and most squamous cell lesions have distinctive features that allow their classification as HPV-related or non-HPV-related. Most penile cancers are not related to HPV and are usually classified as keratinizing squamous cell carcinomas (78, 79). Others are associated with high-risk HPV infection and are warty, basaloïd, or mixed (basaloïd/usual, warty/basaloïd, warty/usual) histologic types that are HPV ISH-positive and show strong diffuse p16 IHC staining and the presence of lymphovascular invasion (78, 79). Although PCR is often considered to be the gold standard for detection of HPV, none of the commercially available tests are FDA-approved for use in men. A recent study showed good correlation between PCR results and a combina-

![FIGURE 1](image.png) Algorithm for diagnosis of oropharyngeal squamous cell carcinoma.
tion of morphology, p16 IHC, and/or HPV DNA ISH (80). The authors recommend that in order to achieve the highest diagnostic specificity, morphology, p16 IHC, and HPV DNA ISH should be considered together and all should be positive (80).

The importance of determining the HPV status in penile lesions is often stressed because of the impact on preventing transmission to sexual partners or to identify partners who are already infected and at increased risk for HPV-associated malignancies (80). HPV status may also be helpful in the differential diagnosis of primary high-grade urothelial carcinoma versus HPV-related basaloid or warty/basaloid squamous cell cancer of the distal penile urethra (80). Both tumors are found with nearly equal frequency and can look histologically similar, especially when biopsies are small (80). The use of both HPV DNA ISH and p16 IHC is recommended since although typically associated with HPV, overexpression of p16 has been found in up to one-half of urothelial carcinomas (80).

Determining HPV status as a prognostic factor in penile cancer remains unclear. There are few reported studies that have examined HPV-positive precursor lesions and their potential to progress to cancer, and results have been inconsistent (78). Several studies from Europe have concluded that HPV status as determined by PCR and/or p16 IHC positivity in penile squamous cell carcinoma is favorably associated with cancer-specific survival (81–83). In a cohort of patients in North America, HPV status as determined by high-risk HPV ISH and/or p16 IHC was not predictive of outcome (recurrence, progression, overall mortality, or disease-specific survival) (84). A trend toward delayed progression was associated with high-risk HPV ISH positivity in this study, although the association did not reach statistical significance (84). In another North American study, p16 IHC positivity was not associated with stage, histologic grade, lymph node status, lymphovascular invasion, lymph node metastases, or overall survival in patients with penile SCC (85). Lack of p16, however, was significantly associated with recurrence, especially in patients who had lymph node involvement at diagnosis (85). Additional studies are needed to establish the role of HPV status as a potential prognostic indicator.

**Anal Cancer Screening**

Analogous to cervical cancer, the ability to detect HPV and identify precursor dysplastic lesions in the transition zone of the anal canal could allow for the development of screening and prevention programs. Systematic anal cancer screening is recommended by some groups, while others suggest that more information is needed before screening processes other than visual and digital examination can be recommended.

There are currently no formal recommendations for routine anal cancer screening in the United States; however, some programs, such as the New York State Department of Public Health AIDS Institute, have established screening algorithms (86). Screening is proposed only for high-risk groups, including SOT recipients, men who have sex with men, women with abnormal cervical or vulvar histology, all HIV-infected men and women, and any patient with a history of anogenital condylomas. It has become clear from multiple studies that testing for HPV is not useful for anal cancer screening because the prevalence of HPV and frequency of mixed infections are unusually high in at-risk populations, and the consequences of infection are not well understood (87). Cytology, however, may be useful; preliminary recommendations for screening men who have sex with men include obtaining anal cytology at baseline and annually for those who are HIV-positive and biennially for those who are HIV-negative (88) (Fig. 2). Either conventional Papanicolaou-stained smears or liquid-based cytology can be used. Anal cytology smears are evaluated using the same Bethesda nomenclature as for cervical cytology, with abnormalities designated as atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion (ASC-H); and LSIL and HSIL (Table 1). The koilocytic changes associated with HPV may not be as pronounced in anal cytology, although binucleation, multinucleation, and cytoplasmic keratinization may be more prominent than in cervical lesions (86). There is also a higher incidence and lower specificity of atypical squamous cells of undetermined significance in the anal canal (86).

**Diagnosis**

Similar to cervical cytology, there is substantial interobserver variation in interpretation of anal cytology, and histological confirmation is important. Histological grading is also subject to interobserver variability. Standardized terminology for epithelial lesions in all anatomic locations of the lower anogenital tract was developed in 2012 by the Lower Anogenital Squamous Terminology Standardization (LAST) Project to replace older nomenclature and eliminate some of the subjectivity in interpretation (89). According to older nomenclature, precancerous anal lesions were termed anal
intraepithelial neoplasia (AIN) and were categorized as grade 1, 2, or 3 (mild, moderate, or severe), based roughly on the distribution of the abnormalities within the epithelium. The LAST terminology is LSIL and HSIL and may include subgrading using the older –IN terminology (Table 1) (89). Clearly identifying a lesion as –IN 2 can be difficult, and p16 IHC is recommended to determine the appropriate classification (Fig. 2). Diffuse, strong p16 staining in the precancerous lesion supports classifying the lesion as HSIL (89). Absence of p16 staining, or presence of minimal patchy staining, supports reducing the classification to LSIL (89).

Opinions differ as to the optimal management of precursor lesions, and the success of treatment in the prevention of development of anal cancer has not been widely established (90, 91). Studies are needed to better characterize the natural history of HPV-associated anal lesions and to provide evidence for effective treatment.

**FIGURE 2** Algorithm for screening and diagnosis of anal cancer in high-risk groups. There are currently no formal recommendations for routine anal cancer screening in the United States.

**TABLE 1** Cytological and histological classification of anal dysplasia and cervical dysplasia

<table>
<thead>
<tr>
<th>Natural history</th>
<th>Bethesda 2001 cytology</th>
<th>LAST cervical intraepithelial neoplasia histology</th>
<th>LAST cervical and anal intraepithelial neoplasia histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal infection</td>
<td>NILM ASC-US ASC-H LSIL</td>
<td>Negative atypia</td>
<td>Negative atypia</td>
</tr>
<tr>
<td>Precancer</td>
<td>HSIL Carcinoma AGC</td>
<td>LSIL (formerly CIN 1) HSIL (formerly CIN 2, CIN 3, CIS) Correlates with a variety of histologic outcomes including (111): Negative or benign CIF 1 CIF 2/3 Microinvasive squamous cell carcinoma Carcinoma in situ Cervical adenocarcinoma Endometrial adenocarcinoma Endometrial hyperplasia</td>
<td>LSIL (formerly AIN1) HSIL (formerly AIN2, AIN3, CIS) Carcinoma N/A</td>
</tr>
<tr>
<td>Cancer</td>
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*NILM, negative for intraepithelial lesion and malignancy; ASC-US, atypical squamous cells unspeciﬁed signiﬁcance; ASC-H, atypical squamous cells cannot exclude HSIL; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AGC, atypical glandular cells; CIN, cervical intraepithelial neoplasia; CIS, carcinoma in situ; AIN, anal intraepithelial neoplasia; AIS, adenocarcinoma in situ.*
**Cervical Cancer Screening**

The current most widely recognized cervical cancer screening guidelines were updated and independently published in March 2012 by the American Congress of Obstetricians and Gynecologists (ACOG), the United States Preventive Services Task Force (USPSTF), and the American Cancer Society (ACS) in partnership with the American Society for Colposcopy and Cervical Pathology (ASCCP) and the American Society for Clinical Pathology (ASCP), with an interim update provided in January 2015 (92–95). These guidelines are focused on screening algorithms for immune-competent women with average risk for HPV infection and do not make recommendations for immunocompromised women.

Data are limited, but most studies seem to indicate that HPV infection is detected with greater frequency in immunosuppressed patients and that immune deficiency increases persistence of HPV and thereby increases the risk of cervical dysplasia and invasive cervical cancer (56, 96–99). There is some evidence to suggest that high-grade precursor lesions progress more rapidly to cervical cancer in immune-suppressed women (56). For HIV-infected women, no apparent reduction in HPV-associated cervical cancer is afforded by highly active antiretroviral therapy (100).

The Centers for Disease Control and Prevention (CDC), National Institutes of Health (NIH), and the Infectious Disease Society of America (IDSA) jointly published updated cervical cancer screening guidelines for HIV-infected women in 2009 (101, 102) (Table 2; Fig. 3). These guidelines recommend that HIV-infected women engaged in sexual intercourse should be screened using cytology (Papanicolaou-stained smear) at 6-month intervals in the first year following diagnosis of HIV infection and then annually after that as long as the cytology results remain normal (101, 102). Women with HIV should not begin screening until age 21 even if their HIV diagnosis was before age 21. There are no specific guidelines regarding screening of transplant recipients for cervical cancer. However, it is generally agreed that it is prudent to screen annually beginning at age 21 rather than every 3 to 5 years as is recommended for normal-risk women (99). Screening plans may be modified for individual patients, depending on the degree of immune suppression and other factors (99). Some experts would recommend continued cytology screening every 6 months in women with CD4 counts less than 200/mm³ or a history of HPV infection.

The initial biennial cytology screening is recommended given concerns about the relative insensitivity of cytology and that an abnormal lesion may be missed with a single cytology smear. The rate of abnormal cytology is high among HIV-infected women and has been reported as atypical squamous cell of undetermined significance or LSIL in 37% and as HSIL in 5%, with normal cytology in the remaining 58% (56). Prevalence of HPV is also high among HIV-infected women with normal cytology and has been reported as 57.4% in South/Central America, 56.6% in Africa, 32.4% in Europe, 31.4% in North America, and 31.1% in Asia (56). In contrast, HPV detection in the absence of apparent disease is found in about 11 to 12% of immune-competent women. Also of note is that the rate of

<table>
<thead>
<tr>
<th>TABLE 2 Cervical cancer screening guidelines</th>
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</thead>
<tbody>
<tr>
<td><strong>Normal-risk women (92–95)</strong></td>
</tr>
<tr>
<td>Age at initiation</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
</tr>
<tr>
<td>Age 21–29 years</td>
</tr>
<tr>
<td>Age 30–65 years</td>
</tr>
<tr>
<td>Discontinuation of screening</td>
</tr>
<tr>
<td>After hysterectomy</td>
</tr>
<tr>
<td>HPV vaccinated</td>
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</table>

*Providers should consider screening within 1 year of onset of sexual activity (101).
¹Some experts would recommend continued cytology screening every 6 months in women with CD4 counts less than 200/mm³ or a history of HPV infection.
²Annual cervical cytology screening is recommended for immunosuppressed women without HIV (99).
³Data insufficient in this population.
infection with multiple HPV types is higher for HIV-infected women than for the general population (56). This higher rate of multiple HPV infections is found in both the presence and absence of abnormal cytology (56).

The utility of detection of high-risk HPV or HPV genotyping in conjunction with cytology as is endorsed for the general population has not been determined for immunosuppressed women. The concern is that HPV DNA tests may be less specific in immunocompromised women and might not be as effective for screening because of the high prevalence and persistence of HPV among women with HIV (56). Specificity and positive predictive value may be improved by detecting E6/E7 mRNA, rather than HPV DNA as has been found in the general population, but this screening strategy has not been studied in immunosuppressed populations. Studies that compare detection of high-risk HPV with cytology and development of precancerous and cancerous lesions are needed. Results of pilot studies have raised concerns about normal cytology and unrecognized high-risk HPV infection, especially in HIV-infected women 21 to 30 years of age (98, 103, 104). Follow-up observations in one of the studies showed that HIV-infected women with normal cervical cytology and high-risk HPV were 4-fold more likely than women without high-risk HPV to have an abnormal finding on colposcopic examination (103). From these results, it appears that detection of high-risk HPV and early colposcopic examination could particularly be of benefit in screening of immunosuppressed women.

HPV16 is associated with the majority (48 to 72%) of high-grade cervical lesions in normal-risk women (105, 106). A large meta-analysis as well as subsequent studies have found that HPV16 is less prevalent (32 to 51%) among HIV-infected women with high-grade cervical lesions, and that other subtypes (11, 18, 33, 51, 52, 53, 58, and 61) and infection with multiple HPV types are significantly more common (56, 105, 107). These findings suggest that genotype testing may have less of a role in this population.

Diagnosis

Cytology

Cervical cytology using Papanicolaou-stained smears has been the standard screening test for detection of premalignant lesions and cervical cancer since 1941. Liquid-based, thin-layer systems are a more recent technology for preparation of smears that requires placing cervical specimens into a vial of liquid preservative. The specimens are processed to remove debris and reduce excessive white and red blood cells. The remaining specimen is then sedimented if the BD SurePath (BD Diagnostics–TriPath, Burlington, NC) system is used, or filtered if the ThinPrep (Hologic Inc., Marlborough, MA) system is used, to produce a monolayer of cells on a glass microscope slide, which is then stained. The liquid-based pro-
cess improves disease detection by making diagnostically relevant cells more clearly visible and results in fewer unsatisfactory slides when compared to conventionally prepared smears (108). Stained slides are examined manually using a standard light microscope or by an imaging system (e.g., ThinPrep Imaging System, Hologic Inc.) that uses a computer to automatically scan the slide and mark potentially abnormal cells with larger or darker nuclei for review by a cytotechnologist and interpretation by a pathologist for final diagnosis. The consistent and objective evaluation of cytology smears afforded by imaging systems is thought to improve diagnostic accuracy (109).

The Bethesda System has been the standard terminology used for reporting cervical cytology since 1988. The system has been modified several times, and the nomenclature currently in use was developed in 2001 (110) (Table 1). Precancerous cytologic abnormalities in squamous epithelial cells are stratified in several categories. Atypical squamous cells (ASC) are the most common abnormal finding and are divided into ASC-US and ASC-H. LSIL is mild dysplasia caused by HPV infection and characterized by squamous cells with nuclear atypia, perinuclear halo, and dense cytoplasm. HSIL is a more severe abnormality that includes moderate to severe dysplasia and carcinoma in situ. If LAST terminology is accepted, squamous histology and cytology descriptions will be consistent (Table 1). The Bethesda system also allows for description of glandular cell abnormalities which are caused by HPV but are far less common and are categorized as atypical glandular cells (AGC) with identification of whether they are endometrial or endocervical if possible (111). “Adenocarcinoma in situ” and “AGC favor neoplastic” are included as subcategories of AGC and can be difficult to detect because the ability to completely sample the endocervical canal for cytologic analysis is somewhat limited and glandular lesions may be inaccessible.

Cervical cytology has been very effective in cervical cancer screening programs but carries a false-negative rate of about 5% to 20% (112). Inadequate sampling of the transformation zone and small lesion size may account for much of the reduced sensitivity, but there is also some interobserver variability as well as screening errors by cytotechnologists and interpretive errors by pathologists that contribute. Steps to reduce laboratory reading errors were mandated under the Clinical Laboratory Improvement Act of 1988 (CLIA), which requires that a random sample of at least 10% of cytology smears are rescreened by a pathologist or senior cytotechnologist for quality control (113). In addition, all slides assessed as positive after a single examination should be reviewed a second time for verification.

### Molecular HPV tests

HPV DNA testing in HIV-infected women or adolescents is not recommended at this time, although it has now been incorporated into cervical cancer screening for normal-risk women because of the limitations of cytology (114) (Table 3). The most common commercially available molecular tests are designed to detect HPV DNA from high-risk types and generate a pooled result without identification of the specific type present. There are important differences among the various tests. The tests include the Digene Hybrid Capture 2 High-Risk HPV DNA Test (Qiagen, Redwood City, CA) using hybrid capture technology, the Cervista HPV HR test (Hologic Gen-Probe Inc., San Diego, CA) using fluorometric invader technology, and the cobas HPV real-time PCR test (Roche Molecular Systems Inc., Pleasanton, CA). The cobas HPV test generates an aggregate result and indicates the presence of HPV16 and/or HPV18. Other tests are designed specifically to detect HPV16 and HPV18 (or HPV18/45). Commercially available genotyping assays include the Cervista HPV16/18 test and the Aptima HPV16 18/45. Many studies have determined that detection of HPV E6/E7 mRNA is more specific than DNA-based assays when measured against clinical disease with a histological diagnosis of moderate cervical intraepithelial neoplasia (CIN 2) or worse (115, 116). Currently, the only available test that detects HPV mRNA is the Aptima HPV Assay (Hologic Gen-Probe Inc.). The HPV molecular tests are approved by the FDA for cervical cancer screening in combination with a Pap test among women over age 30 and for follow-up testing of women with abnormal cytology results. The cobas HPV test was recently approved as a primary screening test.

It is important to note that HPV tests, as approved by the FDA, are performed on a fixed volume of sample.

**TABLE 3 Role for HPV testing in HIV-infected women**<sup>a</sup> (119)

<table>
<thead>
<tr>
<th>Normal-risk women</th>
<th>HIV-infected women</th>
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<tbody>
<tr>
<td>Triage ASC-US cytology result</td>
<td>Triage ASC-US cytology result?*</td>
</tr>
<tr>
<td>Cotest with cytology for women ≥ age 30 years</td>
<td>Follow-up after colposcopy or treatment (loop electrosurgical excision procedure/cone biopsy)</td>
</tr>
<tr>
<td>Postmenopausal women with LSIL</td>
<td>Follow-up after colposcopy or treatment (loop electrosurgical excision procedure/cone biopsy)</td>
</tr>
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<sup>a</sup>Data insufficient in this population.
randomly obtained from the collection vial used for liquid-based cytology or a cervical brush in preservative fluid. Because cytology analyzes only cells and HPV tests analyze cells plus extracellular material, they are not exactly comparable (117). The extracellular material examined in HPV tests may contain a large amount of nucleic acids from free virions as are found in productive transient infections. When HPV tests are performed using an enriched cellular fraction obtained after centrifugation rather than a random whole sample from the collection vial, the specificity of the HPV test for detection of corresponding abnormal cytology is improved (117). Another aspect is that even molecular HPV tests with a high analytical sensitivity of about 20 to 400 HPV DNA copies per reaction are not sensitive enough to detect all cancers (118). The lower limit can easily be reached when few clonal (abnormal) cells are present in the sample.

Management
There is controversy about management of abnormal cytology in HIV-infected women. ASCCP guidelines in 2001 presented separate recommendations for the management of ASC-US in women with HIV infection and other immunosuppressive conditions. These separate guidelines were eliminated in 2006 in favor of managing immunosuppressed women in the same manner as the general population. ASC-US is managed in the general population either by repeat cytology (at 1 year is acceptable) or by high-risk HPV testing, which is preferred (119). Other guidelines suggest that there are not enough data to recommend HPV testing alone in the management of ASC-US and suggest that either immediate referral to colposcopy or repeat cytology in 6 to 12 months should be used (120). Some authorities recommend that all HIV-infected women with atypical squamous cell of undetermined significance be referred for only immediate colposcopy and directed biopsy, with further treatment based on those results (102).

For any lesion greater than ASC-US (i.e., ASC-H, LSIL, HSIL, or AGC), referral for colposcopy is recommended (102, 119). It is also recommended that HIV-infected women with cervical HSIL should be referred for anal cytologic screening as they are at increased risk for anal dysplasia and cancer (102).

Treatment monitoring
Although HPV is more difficult to treat and more likely to recur with increased immunosuppression, cervical cancer precursors in HIV-infected and other immunosuppressed women should generally be managed according to ASCCP guidelines (119). Treatment may include ablative (e.g., cryotherapy, laser vaporization, electrocautery, diathermy, cold coagulation) or excisional (e.g., loop electrosurgical excision procedure, laser conization, cold knife conization) to remove or destroy the abnormal cells. An excisional procedure is indicated in patients with recurrent or persistent high-grade CIN or if colposcopy is unsatisfactory (119). For HIV-infected adolescents, standard treatment guidelines for adolescents and young adults are considered to be safe and effective (119). Recurrence is more common in these patients, and close observation as defined in the guidelines for management of CIN 1 and CIN 2 is important. For patients with CIN 1 who may not be compliant, following the more stringent management guidelines for CIN 2 may be preferable (119).

The success of treatment has been monitored using a wide variety of follow-up approaches including repeat cytology, HPV testing, cytology and HPV cotesting, or colposcopy alone or in combination with cytology or HPV tests. There are no established guidelines for testing, and follow-up intervals vary among reports from 3 to 12 months (119, 121). Although posttreatment data from randomized trials are not available, HPV testing is generally considered to be more sensitive than cytology and may allow for earlier diagnosis of persistent or recurrent disease, while a negative HPV test may allow for less intense management (119, 121). Further work to refine follow-up strategies is needed for the general population as well as specifically for immunocompromised patients.

Prevention
Prophylactic vaccination
The FDA has approved two LI (major capsid protein) virus-like particle vaccines that also contain adjuvants to enhance both major histocompatibility complex class I (to activate cytotoxic [CD8+] T lymphocytes) and class II (to activate T-helper [CD4+] lymphocytes) presentation pathways. Gardasil (Merck & Co., Whitehouse Station, NJ) is a quadrivalent vaccine that protects against disease caused by HPV types 6, 11, 16, and 18, and Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) is a bivalent vaccine that protects against HPV types 16 and 18. The quadrivalent vaccine is approved for both females and males 9 to 26 years of age while the bivalent vaccine is approved only for females 9 to 26 years of age. The vaccine is administered in three doses, with the first dose of the quadrivalent or bivalent vaccine recommended for girls 11 to 12 years old and the quadrivalent vaccine for boys 11 or 12 years old, but either vaccine can be administered as early as age 9 years. The second
dose should be administered 1 to 2 months after the first dose, and the third dose 6 months after the first dose, with a minimum interval of 12 weeks between the second and third dose (122). Catch-up vaccination using the standard dosing intervals is also recommended for females and males aged 13 through 26 years old if they were not previously vaccinated or if they have not completed the three-dose series (122). Vaccination in the general population induces a cell-mediated immune response and a strong humoral immune response with antibody titers several times higher than typically seen after natural infections (123, 124). Vaccination is recommended regardless of the presence of abnormal cervical cytology, as it may protect against one or more HPV types to which the individual has not been exposed. In addition, the vaccine has been shown to be effective in older individuals, and vaccination up to age 45 has been recommended by some groups (125).

Several published studies have shown that HPV vaccines are well tolerated in immunocompromised individuals and that a specific humoral immune response was produced with high rates of seroconversion in HIV-infected individuals after three doses and 100% seroconversion after four doses in one study (25, 90). Seroconversion rates, however, have been less than optimal in vaccinated organ transplant recipients (126). Although immunosuppression does not prevent successful immunization, most studies have found that antibody titers are lower in immunocompromised individuals than in immunocompetent individuals (25, 90, 126). Among HIV-infected individuals, antibody titers are higher in those who are on treatment with antiretroviral agents. While safety has been established, optimal dosing schedules and the efficacy of vaccination in immunocompromised individuals will require additional study.

The Advisory Committee on Immunization Practices (ACIP) and IDSA recommend HPV vaccination for immunocompromised men and women, including those with HIV infection, as well as for men who have sex with men. Vaccination is recommended regardless of the severity of immunosuppression and regardless of history of abnormal cervical cytology. The dosing schedule for immunocompromised individuals is the same as for immunocompetent individuals, with the three-dose series given at age 11 or 12 years and at age 13 through 26 years if not previously vaccinated (102, 122). Immunocompromised individuals should continue to have routine cervical and anal cancer screening tests and visual and digital pelvic and anorectal examinations even if they have been vaccinated.

Therapeutic vaccination

There is limited literature regarding therapeutic HPV vaccination. Clearance of a naturally acquired HPV infection is mediated by a specific cell-mediated immune response in which dendritic cells stimulate T-helper type 1 lymphocytes, which then elicit the production of cytotoxic T lymphocytes that attack infected cells. The ability of an HPV vaccine to stimulate a specific cellular immune response could potentially have a therapeutic effect (124). The current FDA-approved L1 VLP vaccines were designed to prevent HPV infection primarily by stimulating a neutralizing antibody response and were not intended for therapeutic purposes. Several short-term studies suggest that the current vaccines may be somewhat effective in patients with existing HPV infection, while other studies have not found a significant effect (125, 127–129).

Vaccines that are designed to boost specific T-cell responses could potentially be therapeutically effective. Since the L1 and L2 structural capsid late proteins appear only in fully differentiated epithelial cells later in the course of infection, therapeutic vaccines have been directed toward stimulating T-cell responses to the HPV E6 and E7 viral oncoproteins, which are continuously expressed throughout the course of infection (130). Approaches have included administration of peptide antigens or recombinant proteins, live viral vector vaccines, whole-cell vaccines, and plasmid DNA vaccines as well as administration of E7-pulsed dendritic cells (130). DNA vaccines have been favored since they are stable, safe for multiple administrations, can provide sustained release of antigenic proteins, and can be delivered by a variety of methods (130). Modifications that lead to enhanced immunogenicity are being investigated (130).

Effective therapeutic vaccination in the face of CD4+ T-cell compromise or depletion is of concern in some primary immune deficiencies and in HIV-infected individuals. In a murine immunodeficiency model, a DNA vaccine against HPV E6 and E7 was shown to be capable of generating a specific CD8+ T-cell-mediated immune response in the absence of CD4+ T cells and was protective when challenged with subcutaneous administration of E6- and E7-expressing tumor cells, although no antibody response was detected (131). Further investigations are needed to determine the long-term effect of therapeutic vaccination in management strategies for both immune-competent and immunocompromised individuals. A chimeric vaccine that contains preventive and therapeutic components may ultimately be ideal and could be used for both treatment and prophylaxis (123).
REFERENCES


Human Papillomavirus