The development of cervical cancer and its precursors: what is the role of human papillomavirus infection?

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Human papillomavirus (HPV) is a significant health care burden in the United States. The majority of sexually active men and women will be infected with HPV at some point in their lives and are subject to developing human papillomavirus-associated disease. Current estimates suggest that 20 million Americans are currently infected, and more than 6 million new infections occur each year. The prevalence of human papillomavirus is highest in populations in their late teens and early twenties, with nearly half of all new human papillomavirus infections occurring within 3 years of first intercourse. HPV is the necessary cause of genital warts, cervical intraepithelial neoplasia, and invasive cervical cancer. As such, human papillomavirus is responsible for significant medical morbidity and health care costs. Screening with cervical cytology has significantly reduced mortality rates; however, approximately 3900 women will die in 2005 from cervical cancer in the United States. Human papillomavirus DNA testing has shown promise in identifying high-grade abnormalities as an adjunct to traditional cytology, and should be used according to guidelines established by the American Cancer Society and the American College of Obstetricians and Gynecologists. The epidemiology of HPV infection and a brief introduction to the natural history of HPV infection will be presented here.

Keyword
Human papillomavirus (HPV), cervical cancer, genital warts

Epidemiology
The prevalence of human papillomavirus (HPV) infection is underestimated because of the subclinical nature of most infections [1], the lack of broad population screening for HPV by molecular testing [2,3], and limited reporting, as clinicians are not required to report cases of HPV infection to the Centers for Disease Control and Prevention (CDC) [1]. Nevertheless, the development of more sensitive and reliable assays for detecting HPV DNA within the past two decades has provided a greater understanding of the magnitude of HPV infection [1,4]. HPV is the most common sexually transmitted infection in the United States [4]. An estimated 15% of the population is currently infected with HPV [1], but because HPV infections are usually transient, this number is far below the lifetime risk of getting one or more HPV infections, which is likely to be at least 75% (Fig. 1) [1]. Moreover, the estimated 1-year incidence of HPV infection is at least 5.5 million [4,5].

HPV infection is most prevalent in young women and adolescents, most likely as a result of increased transmission during the early years of sexual activity, or possibly a lack of previous exposure that might generate a protective immune response [6–10]. Alternatively, young women may be more susceptible to infection during adolescence for biological reasons. The transformation zone of the cervical epithelium undergoes a process of squamous metaplasia during puberty that exposes normally protected basal cells to infection [11]. In one study using a polymerase chain reaction (PCR)-based DNA amplification system to detect HPV DNA, 32% of women 16–24 years old tested positive for HPV DNA compared with 4% of women aged 45 years and older [9]. In a more recent study, the prevalence of HPV infection ranged from 36% in women younger than 25 years of age to 2.8% in women aged 45 years and older [8]. The prevalence of HPV infection is extremely high in sexually active adolescent females, with up to 64% of young women testing positive for HPV DNA [12].

The rate of acquisition of HPV is extremely high compared with that of other sexually transmitted infections. For example, among women between the ages of 18 and 35 years, the rate of new HPV infection reported from the Young Women’s Health Study was 2.9% per month [13], with a 32% cumulative incidence of new HPV infection during a 2-year period [14], rising to 43% over 3 years [15].
The rate of new infections with high-risk, oncogenic HPV types is higher than with low-risk types. High-risk HPV infections are more common than infections caused by low-risk, nononcogenic types, as evidenced by the higher cumulative probability that a woman would become newly infected with a high-risk HPV type during a 12-month follow-up period (0.32) compared with that for nononcogenic types (0.18) [13,16].

Because HPV infections are typically asymptomatic in men, prevalence rates of HPV in men have been difficult to assess. Testing is complicated by the fact that sample collection from the external skin, whether male or female, has generally been inadequate for molecular HPV testing [17]. Most published studies have been conducted outside the USA in men attending sexually transmitted disease (STD) or university clinics, or among the male partners of women with HPV infection [17]. The reported prevalence rates of HPV in men range from 16–45% [17]. Additional information will need to be collected to determine the actual prevalence rates in men, but since the virus is sexually transmitted, it is likely that prevalence is similar in both sexes. Nonetheless, it is well appreciated that both men and women develop genital warts. At least 1% of sexually active adults are currently carrying genital warts in the USA [1]. Therefore, strategies centered on preventing HPV infection in both men and women will probably produce the greatest public health benefit.

Transmission of HPV infection

HPV is transmitted by genital contact with an infected partner. For infection to occur, the virus must have access to the basal epithelial cells, either in epithelium that is naturally thin and immature, such as the transformation zone of the cervix or the anal verge, or through microscopic tears or abrasions in the external genital skin or the introital or vaginal mucosa [18]. Studies among initially virginal women strongly confirm the sexually transmitted nature of HPV infection. In a 2-year study of 205 women, all virgins were HPV DNA negative for genital HPV types and seronegative for HPV 16, whereas 35% of women with two or more partners were HPV DNA positive and 23% seropositive for HPV 16 [19].

Intercourse is not absolutely necessary for transmission, because the transmission of HPV infections may manifest on external anogenital sites, and subsequently spread by self-inoculation to other areas [14,20]. However, the age of onset of intercourse is designated to be the age of onset of the risk of cervical neoplasia in the American Cancer Society (ACS) and the American College of Obstetricians and Gynecologists (ACOG) guidelines. Whereas genital–oral transmission may be a possible route of infection, the literature has not reached a consensus regarding whether or not HPV can be transmitted orally [14]. Although rare, recurrent respiratory papillomatosis in young children can occur from the transmission of HPV 6 or 11 from a mother to a newborn baby, other HPV types have been detected on neonates but have not been proven to cause disease [21,22]. Transmission by inanimate objects such as environmental surfaces and clothing has been hypothesized but not conclusively documented [23–25].

The relationship of HPV infection with genital neoplasia

Cervical cancer is the third most common gynecological malignancy and a serious public health issue in the USA (Fig. 2) [26]. One recent study estimates that the lifetime risk of cervical cancer would be 3.67% in the absence of cervical cancer screening, with a lifetime cervical cancer mortality risk of 1.26%, and a peak incidence of cervical cancer of 81/100 000 at age 50 [27]. However, because of cervical cancer screening with the Papanicolaou (Pap) test, the estimated number of new cases will be down to 10 370 (approximately 1.5% of all new cancer cases in
women) and mortality will be down to 3710 (approximately 1.3% of cancer-related deaths in women) in 2005 [26]. This is a reduction in risk of greater than 75% from the estimated 40 000–50 000 cervical cancers that would probably have occurred in 2005 in the absence of screening [28].

A causal relationship exists between HPV infection and cervical cancer, as well as a significant fraction of vaginal, vulvar, penile, and anal cancers [29]. Virtually all cervical cancers contain HPV DNA – an estimated 99.7% prevalence in cases worldwide – consequently, HPV holds the highest worldwide attributable fraction ever identified as the cause of a major human cancer [30]. Oncogenic HPV types 16 and 18 cumulatively account for approximately 70% of all cervical cancers, and are associated with a more than 200-fold increased risk of developing invasive cancer [31]. Infection with high-risk HPV types most commonly results in subclinical infections [32]; therefore, most infected women harbor HPV DNA without showing cytological or histological changes, or have changes that are so transient that they are not detected by routine cytological screening [33]. One study reported that 36% of women with normal cervical cytology tested positive for HPV DNA [34], but most evaluations of women over the age of 30 years report high-risk HPV detection to occur in 3–10% of women with normal concurrent cervical cytology [35–37]. Persistent HPV infections can cause changes in the cervical cytology of squamous epithelia that may progress to noninvasive cervical intraepithelial neoplasia (CIN) 2/3, and less frequently and many years later, to invasive cervical cancer.

Clinical manifestations: low- and high-risk HPV infection

A common manifestation of low-risk HPV infection is condylomata acuminata, or genital warts. Genital warts are polypoid, often cauliflower-like growths that generate infectious virus and have a low-to-negligible risk of malignant progression. Over 90% of condyloma acuminata are caused by infection with low-risk HPV types 6 or 11. One study detected HPV 6 in more than 90%, HPV 11 in 32%, and HPV 6 or 11 in 97% of genital warts tested for HPV DNA [38]. Exophytic genital warts are composed of fronds of connective tissue covered by an anacanthotic squamous epithelium. Characteristic cellular changes in the superficial layers of the epithelium include keratinization, multinucleation, and atypical karyocytes, characterized by perinuclear cytoplasmic vacuolation and nuclear enlargement, hyperchromasia, and irregularity. Histologically, genital warts can be distinguished from CIN by the absence of nuclear atypia in the basal layers of the epithelium [39]. Although genital warts are medically benign, they represent a significant economic burden and source of morbidity. The CDC reports that in 2003 there were an estimated 264 000 visits to physicians’ offices for genital warts (Fig. 3) [40]. Some warts may spontaneously resolve, but treatments, when necessary, are typically painful and generally aim to remove the wart, either through excision, desiccation or immune modulation, and often must be repeated. Costs to treat genital warts vary considerably, from as low as US$200 (for simple surgical excision or desiccation) to as high as US$6000 (for IFN-α2b therapy) [41]. An effective immune response to HPV is most responsible for clearance and ultimately determines the cost [42].

Cervical infection with low-risk HPV types may manifest as noninvasive, low-grade squamous intraepithelial lesions (LSILs), also referred to as CIN grade 1 (CIN 1),
and occasionally as high-grade CIN 2, but rarely as CIN 3 [39]. However, most CIN of any grade is due to high-risk types of HPV [43]. High-risk HPV types are called ‘high-risk’ because of their association with cervical cancer, yet these HPV types are also the most common types in women with no detectable HPV manifestation [44].

The risk of developing LSILs decreases over time after incident HPV infection: at 4 months, the hazard ratios are 6.14 for low-risk HPV types and 13.03 for high-risk HPV types; by 60 months, the hazard ratios are 0.73 for low-risk HPV types and 3.37 for high-risk HPV types [45]. Most untreated cases of LSIL regress within 2 years, as assessed by one or two normal Pap smears (Fig. 4) [46]. One evaluation of all relevant studies on the natural history of cervical neoplasia between 1952 and 1992 estimated that 60% of CIN 1 cases regress, 30% persist, 10% progress to CIN 3, and 1% progress to invasive cancer [47]. A more recent meta-analysis estimated that 47% of LSILs will regress to normal, 21% will progress to high-grade squamous intraepithelial lesions (HSILs), and 0.15% will progress to cancer [48].

HSILs include CIN grades 2 and 3 and is associated with persistent infection in high-risk HPV types [49,50]. High-risk types, mostly 16 and 18, are found in 50–80% of high-grade lesions [39], and the detection of HPV type 16 DNA is highly predictive of CIN 3 [51]. Additionally, women infected with high-risk types 16 and 18 have a greater chance of progressing to CIN 3 or cancer than do women infected with other oncogenic strains [52,53]. Compared with low-grade lesions, high-grade CIN has lower rates of spontaneous clearance (30–40%), are much higher rates of progression to cancer without treatment (> 12%) [47,50]. By 2 years, it is expected that 35–40% of CIN 2 will regress to normal, and 1.44% of CIN 3 will progress to invasive cervical cancer [48].

Persistent HPV infection with high-risk HPV types is the most important risk factor for developing cervical cancer precursor lesions and invasive cervical cancer [54–57]. The risk of developing CIN 3 is 14 times higher for women who have had at least three positive tests for high-risk HPV compared with women who have had negative tests [58]. Infections with high-risk HPV types are more persistent than those with low-risk types. For women aged 18–35 years, the median time to clearance for high-risk types is 9.8 months, significantly longer than for low-risk HPV types (4.3 months) [13]. HPV 16, the highest risk HPV type [16], is more likely to persist than any other HPV type [15,59–61]. Persistence is also associated with older age [15,62], infection with multiple HPV types [15], and with compromised immunity [63,64].

**Risk factors for HPV infection**

The foremost risk factor for acquiring HPV infection is sexual activity. Among men [65] and women [15,34, 66–68], the risk of acquiring HPV dramatically increases with the number of lifetime sex partners. Another variable that is just as important in determining a woman’s risk of HPV infection is the number of current and previous partners of her partner [14,69]. In men, circumcision reduces the risk of the acquisition and transmission of HPV infection [70]. A recent report from the National Institutes of Health (NIH) and a detailed review of the published literature both conclude that there is no consistent epidemiological evidence that the use of latex condoms reduces the risk of HPV infection [71,72].
Although latex condoms provide an impermeable barrier to particles the size of HPV [73,74], they do not offer protection from HPV infections on anatomical sites that are not physically covered [72,75]. However, failure to use a condom is associated with higher rates of genital warts and cervical cancer [71,72]. Additionally, several studies have determined that the use of condoms reduces the risk of genital herpes and chlamydia, both of which may indirectly contribute to HPV infection and the development of cervical cancer [76,77].

Immunocompetency also has a significant impact on the ability to clear HPV infections. A greater rate and incidence of infection has been observed in immunosuppressed renal transplant patients [78] and patients infected with HIV [79,80], compared with patients who are not immunocompromised. Additionally, immunity can be normal but immunocompetency for HPV may be genetically determined to some extent. Hence, certain human leukocyte antigen (HLA) markers are associated with a higher risk of cervical cancer, and some individuals with otherwise normal immunity have a very difficult time resolving their genital warts [39].

Other risk factors associated with cervical cancer include high parity (five or more pregnancies) [81] and smoking [76,82–85]. A recent study reported an association between active and passive cigarette smoking and cervical neoplasia, providing evidence that even passive smoking is a risk factor for cervical cancer [85]. Long-term use of oral contraceptives (OCs) has also been associated with an increased risk for cervical cancer. One study showed that more than 5 years of OC use is associated with a 2-fold increased risk of cervical cancer, with risks increasing to more than 4-fold by 10 years of OC use risk among women with active HPV infections [86,87].

Cytological screening and DNA testing procedures

Regular cervical screening has had a significant impact on the incidence and mortality associated with cervical cancer [26]. The goal of cervical screening in the USA is to identify precancerous lesions so they can be removed prior to progression of invasive cancer [49]. The survival of women with preinvasive lesions (CIN 2/3) is nearly 100% [26]. Approximately 90% of women with cervical cancer survive 1 year, and 5-year survival rates are nearly 75% [26]. Approximately 92% of women diagnosed with, and treated for, early-stage invasive cervical cancer survive 5 years [26]. It is estimated that 50% of women diagnosed with cervical cancer have never received a Pap test, and an additional 10% have not been screened within 5 years of diagnosis [88].

Although cervical screening has significantly decreased the mortality associated with HPV infection, the sensitivity of the Pap smear is generally less than many believe it to be. The Agency for Health Care Policy (AHCPR) and Research determined that the conventional Pap smear is only approximately 50% effective at detecting cervical lesions of all grades, and that the conventional Pap smear is more accurate when detecting high-grade (CIN 2/3) than low-grade lesions. Liquid-based cytology has been reported to detect between 26 and 103% more cases of CIN 2/3 than the conventional Pap smear; however, the degree to which sensitivity is increased is unknown [33]. Poor sensitivity has driven the expectation that cytology should be repeated annually. Novel approaches to cervical screening, such as HPV DNA testing, are now being developed to increase the sensitivity of cervical cancer screens. In March 2003, the US Food and Drug Administration approved the Hybrid Capture® 2 HPV DNA test (HC2) for use in the primary cervical screening of women aged 30 years and above when used in combination with cervical cytology. The high-risk panel of HC2 detects the presence of one or more of 13 high-risk HPV types in exfoliated cervical cells and was previously approved in March 2000 for the management of women with a Pap result indicating atypical squamous cells of undetermined significance (ASCUS) [89]. HPV DNA testing is very useful for detecting clinically relevant lesions – in the majority of studies HC2 has a sensitivity of 90–98% for detecting CIN 2/3 [90]. In addition, minor HC2 crossreactivity with low-risk HPV types has little effect on its clinical performance as a general screening test, but some decrease in specificity when used as a triage test [91]. Most studies indicate that women with a concurrent normal cytology result and a negative HC2 test have a substantially decreased risk of developing CIN 2/3 or cervical cancer relative to those for whom the only screening information is a normal conventional cytology result [33]. However, a positive HC2 result is not an absolute indicator that a high-grade lesion exists or will develop.

The combined use of cytology and HPV DNA testing increases sensitivity but decreases specificity [92]. The cumulative incidence of CIN 3 or cancer in a 45-month study of women who tested negative for HPV infection using both cytology and HC2 was approximately 1.6/1000; and remained low at 0.8% at 10 years [93]. Hence, the combination of HPV testing and cervical cytology provide not only greater reassurance that cervical precancer and cancer have not been missed, but also predict the level of risk for the future, which is not obtainable through screening with cytology alone.

‘Reflex’ HPV DNA testing is the procedure of performing an HC2 high-risk HPV test directly from the remaining cells in a liquid-based Pap test vial when the cytology interpretation is ASCUS. ‘Reflex’ HPV DNA testing has been recommended as a convenient and cost-effective
Reducing the burden of cervical cancer and HPV-related diseases through vaccination

approach for the management of women with ASCUS [94]. This may also be done by co-collecting a separate HPV DNA test at the same time as cervical cytology and holding the HPV test vial until the Pap result returns. If the Pap result returns as ASCUS, the co-collected vial is sent to the laboratory to be tested for HPV, whereas all vials from women having normal, atypical squamous cells but cannot exclude HSIL, atypical glandular cell (AGC), LSIL, or HSIL Pap tests are discarded [33].

Guidelines for cervical cytology

The ACS and the ACOG endorse either the conventional Pap test or liquid-based preparations (LBP) for cytological screening [33,49]. The ACOG recommends the following practices to optimize cervical cytology [33]: cells should be collected before bimanual examination, and care should be taken to avoid contaminating the sample with lubricant. If cervical samples are to be collected to test for STDs, cell collection for cervical cytology should be undertaken first. Ideally, the entire portion of the cervix should be visible when the sample is obtained. Routine swabbing of the discharge from the cervix may result in cytological samples of scant cellularity. In an effort to reduce air-drying artifact, the specimen should be transferred and fixed as quickly as possible.

A ‘satisfactory’ specimen for cytological analysis has been defined by the Bethesda 2001 Workshop, a consensus workshop convened by the National Cancer Institute and cosponsored by 44 professional societies [50]. There should be at least 8000–12 000 well-visualized squamous cells for conventional smears and 5000 squamous cells for liquid-based preparations. There should be at least 10 well-preserved endocervical or squamous metaplastic cells. A specimen is considered ‘partly obscured’ when 50–75% of epithelial cells cannot be visualized; specimens with more than 75% of epithelial cells obscured are ‘unsatisfactory’.

The Bethesda 2001 Workshop established the following terminology for reporting cytology results [50]: samples may be categorized as ‘negative for intraepithelial lesion or malignancy’ if considered to be within normal limits or benign cellular changes are detected. ‘Other’ applies to cases in which there are no morphological abnormalities in the cells that would be suspicious for CIN but abnormal findings are present, such as cells suspect for ovarian or other cancer. ‘Other’ also applies to findings that may indicate some increased risk, such as benign-appearing endometrial cells in a woman 40 years of age or older. Atypical squamous cells (ASC) may be qualified as being of ‘undetermined significance’ (ASCUS) or ‘cannot exclude high-grade squamous intraepithelial lesion (ASC-H’). All cases of ASC are considered to be suggestive but not definitive for squamous intraepithelial lesion (SIL). Noninvasive squamous intraepithelial lesions may be classified as LSIL, which includes CIN 1 (mild dysplasia) and cytological findings consistent with HPV infection (koilocytic atypia). Alternatively, they may be classified as HSIL, which combines CIN 2 and CIN 3 (moderate dysplasia, severe dysplasia, and carcinoma in situ).

Glandular cell abnormalities less severe than adenocarcinoma may be classified into the following categories: AGC, either endocervical, endometrial, or glandular cells not otherwise specified; atypical glandular cells, either endocervical or glandular cells favor neoplasia; and endocervical adenocarcinoma in situ. The term ‘atypical epithelial cells’ may be used for cases where a squamous vs a glandular origin cannot be determined. An intermediate category ‘atypical endocervical cells, favor neoplastic’ and ‘atypical glandular cells of undetermined significance, probably neoplastic’ apply to cases showing some features suggestive of, but not sufficient to reach an interpretation of, adenocarcinoma in situ [50].

Guidelines for programs of cytological screening and HPV DNA testing

According to the ACS, ACOG and the US Preventive Services Task Force (USPSTF), cervical cytological screening should begin within 3 years of first sexual intercourse or by 21 years of age, whichever comes first [33,49]. Nonetheless, adolescents who may not need a cervical cytology test should obtain appropriate preventive health care, including an assessment of health risks, contraception, prevention counseling, screening, and treatment of STDs.

It is generally agreed that women with an intact cervix should receive cervical cytological screening for cancer every 1–3 years, depending on age, prior test results and risk factors associated with cancer. For women younger than 30 years, ACS recommends cervical screening annually with the conventional Pap smear or every 2 years using liquid-based cytology [49]. ACOG recommends annual cytology screening regardless of the type of Pap test utilized [33]. For women aged 30 years and older, it is generally recommended that screening intervals be lengthened if the patient is not currently in an accelerated follow-up due to abnormal cervical cytology or previous cervical treatment. ACS and ACOG recommend that women aged 30 years and older who have had three consecutive negative cytology results may be screened every 2–3 years, women with a history of in utero diethylstilboestrol (DES) exposure, HIV infection, or who are immunocompromised may require more frequent screening, and women with a history of HSIL or cancer should be screened annually [33,49]. For women aged 30 years and older, as an alternative to cytological testing alone, both the ACS and ACOG provide as an
option cervical screening with the Pap test combined with HPV DNA testing. Both organizations suggest that women who test negative using both cytology and molecular testing for HPV DNA should be screened no more frequently than every 3 years. The ACS, ACOG, and USPSTF all recommend discontinuing cervical screening in women who have undergone hysterectomy for benign reasons. ACS and ACOG recommend that women with a history of CIN 2/3 at the time of the hysterectomy continue screening after hysterectomy until three consecutive negative cytology results are achieved [33,49].

No consensus has been established concerning the upper age limit for cervical cancer screening among low-risk women. The ACS suggests that screening may be ceased in women who are aged 70 years and older with an intact cervix, after three consecutive negative cervical cytology tests, and no positive cytology tests within the past 10 years [49]. Women positive for HPV DNA should continue screening at the discretion of their health care provider. The USPSTF recommends ceasing screening in women older than 65 years if they have had consistent negative test results and are not otherwise at high risk of cervical cancer [95]. The ACOG does not define an upper age limit for discontinuing screening [33].

Consensus guidelines are available for the management of women with cervical cytological abnormalities and cervical cancer precursors [96,97]. These evidence-based guidelines were developed in 2001 by an expert consensus conference sponsored by the American Society for Colposcopy and Cervical Pathology [96,97].

Prophylactic HPV on the horizon

Vaccines are currently being developed to reduce susceptibility to HPV infection and persistent infection. A recent study projected that an effective vaccine targeting high-risk HPV types could prevent 1500 deaths annually from cervical cancer if all 12-year-old girls currently living in the USA were vaccinated [98]. HPV vaccines have shown encouraging success in clinical trials [99]. A vaccine for HPV 16 given to adolescent girls demonstrated 91% efficacy in preventing HPV 16 infection and 100% efficacy in preventing persistent HPV 16 infection [99].

Although the majority of low-grade cervical lesions spontaneously regress, and genital warts pose little threat of malignancy, diagnosis with either of these clinical manifestations can evoke great levels of anxiety in women. Many women initially equate diagnosis with an abnormal Pap smear as indicative of cervical cancer. Moreover, women diagnosed with low-grade lesions may have lower self-esteem, decreased sex drives, and suffer from extremely high anxiety levels [100]. Therefore, vaccines that protect against the greatest number of cervicovaginal disease-causing HPV types will be most efficacious.

Two multivalent vaccines have been developed to prevent HPV infection. A quadrivalent vaccine that protects against HPV types 6, 11, 16, and 18, and a bivalent vaccine protecting against HPV 16 and 18 were both over 90% effective in reducing vaccine-type persistent infections and CIN [101,102]. Widespread acceptance of these vaccines should significantly reduce the incidence of HPV-associated disease, thereby alleviating a significant fraction of morbidity associated with HPV infection.

References

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