Human Papillomavirus

Epidemiology and Public Health

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• Approximately 15 types of human papillomavirus (HPV) infection cause virtually all cases of cervical cancer. Human papillomavirus 16 is the major type, accounting for approximately 50% of cases. The major steps of cervical carcinogenesis include HPV infection, viral persistence and progression to precancer (as opposed to viral clearance), and invasion. Human papillomavirus is the most common sexually transmitted infection. However, most HPV infections become undetectable by even sensitive HPV DNA testing within 1 to 2 years. The prevalence of infection peaks at young ages and declines thereafter, perhaps as the result of HPV type-specific acquired immunity. Most HPV infections are neither microscopically evident nor visible, making HPV DNA detection the diagnostic reference standard. Poorly defined immunologic factors are the major determinants of viral outcome. Smoking, multiparity, and long-term oral contraceptive use increase the risk of persistence and progression. Other sexually transmitted infections (eg, *Chlamydia trachomatis*), chronic inflammation, and nutritional factors might also play a role. Overt, long-term viral persistence in the absence of precancer is uncommon. New prevention strategies can be derived from the evolving knowledge of HPV carcinogenesis. Human papillomavirus vaccination is the ultimate prevention strategy, and large-scale trials are already underway. In the meantime, HPV DNA diagnostics are more sensitive although less specific than cytology, permitting a consideration of lengthened screening intervals. In terms of public health education, clinicians and patients will need to shift discussions of the mildly abnormal Papanicolaou test to consideration of HPV infection as a common sexually transmitted infection that rarely causes cervical cancer.

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Infections with oncogenic types of human papillomavirus (HPV) cause virtually all cases of cervical cancer and precancerous intraepithelial lesions.[1] The cervical transformation zone is a circumscribed ring of tissue with unique susceptibility to HPV carcinogenicity. The process of cervical carcinogenesis can, therefore, be assessed visually (colposcopy and related techniques), microscopically (cytology and histology), and molecularly (HPV DNA tests and serology). This unique access to surveillance and measurement has led to a relatively advanced understanding of the critical steps that lead from the normal cervix to cancer, compared to what is known about cancer development in other tissues. Such understanding has led, in turn, to several excellent preventive strategies that can now be considered in addition to traditional cytology. This brief article will summarize some evolving concepts regarding HPV molecular epidemiology and prevention of cervical cancer.

Before reading this article, the practicing pathologist might reasonably question the relationship of epidemiology to his or her everyday work. A natural tension exists between the real-life variability of the patient and the artificial clarity of research.[2] An inescapable requirement of epidemiology is reproducible classification of the risk factor (HPV) and disease (cervical intraepithelial neoplasia [CIN] and invasive cancer). Of course, biologic variability does not lend itself to the simple categories required for research. Clear classifications of disease are particularly difficult when subtle steps in carcinogenesis are being addressed rather than frank malignancy. Specifically, the evident morphologic spectrum from CIN 1 to CIN 2 to CIN 3 is a histopathologic reality, but it has proven difficult thus far to create fine pathologic categories that are reproducible enough for epidemiologic study and prevention algorithms.[3]

The definition of HPV infection is also problematic. There are more than 100 types of HPV, with more than 40 anogenital types, of which approximately 15 are oncogenic.[1] Human papillomavirus 16 is uniquely oncogenic, accounting for approximately one half of cervical cancers.[1] Multiple HPV type infections constitute more than a quarter of infections. Detection and typing are dependent on choice of viral assay.

Despite the complexity of pathology and HPV measurements, some strong epidemiologic conclusions are possible and are of great relevance to gynecologic pathologists. A consensus is emerging over a simple view of cervical carcinogenesis that favors ''lumping'' rather than ''splitting.'' If accepted, this altered view of cervical carcinogenesis will reshape clinical practice in the next few years.

As shown in the Figure, there are 3 major, necessary

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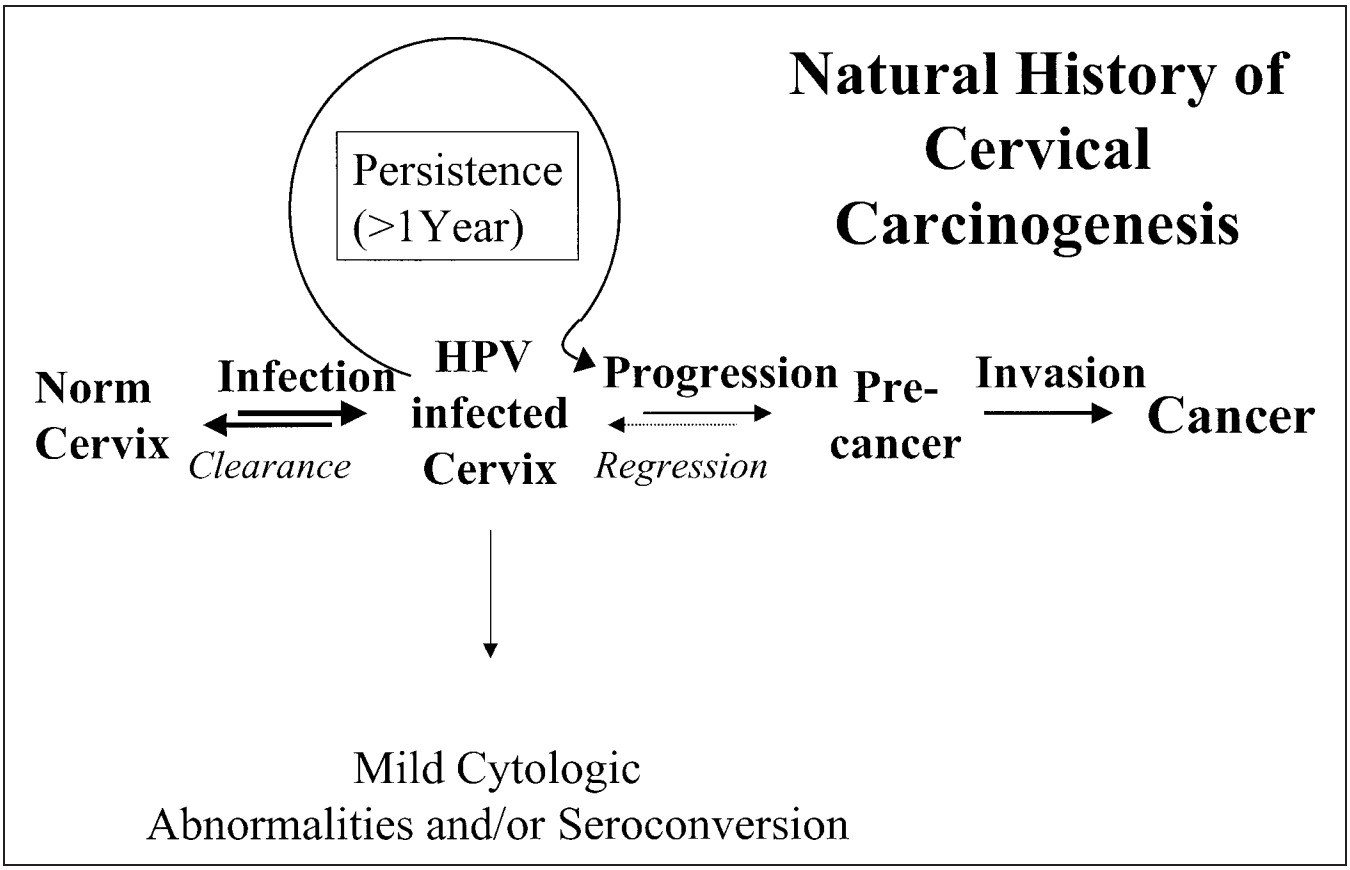

For natural history studies, cervical carcinogenesis can be viewed simply as 3 steps, including human papillomavirus infection, progression to cervical precancer, and invasion.

steps in cervical carcinogenesis that can be reproducibly distinguished, studied, and used in prevention programs. These steps include HPV infection, progression of infection to precancer, and invasion. Each of these broad categories is discussed in more detail in the following sections. ''Backward'' steps occur also, namely clearance of HPV infection and the less frequent regression of precancer to normalcy. The remainder of the discussion will be organized with reference to the steps shown in the Figure.

TRANSMISSION AND ACQUISITION

Human papillomavirus infection is studied best at the molecular level because most infections are not microscopically evident or visible to the clinician.[4] There is a strong link of HPV transmission as measured by DNA assays (the reference standard of detection) to numbers of sexual partners.[5] Detection of HPV DNA is especially linked to number of recent sexual partners, concordant with an acute and typically resolving sexually transmitted infection. Human papillomavirus serology is not sensitive, in that many infected women are not seropositive. But serology is specific, in that noninfected women are negative. Therefore, it is an important confirmation of HPV sexual transmission that virgins are seronegative.[6]

Considered as a group, anogenital HPVs are the most common sexually transmitted infections.[7] Tens of millions of women in the United States have been infected, and all should realize there is no stigma to such an exposure. Human papillomavirus infections are easily transmitted, presumably through microscopic tears in the surface of epithelium that commonly occur during sexual intercourse. Infections of the introitus and vagina are as common as cervical infections (an important clue regarding the importance of the transformation zone to carcinogenesis), but rarely result in cancer.[8] Human papillomavirus can be transmitted from one woman to another woman, as has been shown for women who have sex with women.[9] Anal intercourse can result in anal HPV infections and anal neoplastic lesions in men and women.[10,11] There have been only a few epidemiologic studies on HPV transmission by nonsexual routes, such as environmental fomite or vertical transmission, but clearly both are far less important than sexual transmission.

Each HPV type is a separate genetic species and should be considered a separate sexually transmitted infection. Because all oncogenic types are transmitted by the same sexual route, concurrent multiple (type) infections are very common. The currently available data, which are limited, seem to indicate that HPV types influence each other minimally[12]; however, more studies of multiple infections are important to guiding vaccine strategies (ie, to confirm the impression that preventing one type, HPV-16 for example, will not increase the acquisition or persistence of other oncogenic HPV infections).

Assessing HPV transmission in studies of sexual partners is difficult because comprehensive measurements of HPV infection of the male and female are prone to error, especially given multiple types and even variants of types, making the distinction between persistence, recurrence, and acquisition very difficult. It is also difficult to get re-

liable data on sexual behavior and sexual networks. It seems clear, however, that condoms are not completely protective, probably because infection is pan-genital.

THE MALE CARRIER

Like the vagina and vulva, the penile skin hosts HPV frequently, while cancer develops very rarely. Circumcision reduces the likelihood of HPV infection, probably due to the reduction of infection-prone noncornified epithelium. Thus, male circumcision slightly decreases the risk of cervical cancer among female sexual partners.[13] Like the vulva, only a fraction of penile cancers are related to HPV with the remainder related to chronic inflammation.[14] Human papillomavirus–related lesions can be easily and commonly observed by acetowhitening (highlighting of the lesion with 5% acetic acid) and the HPV can then be typed. Sometimes they appear to be precancerous microscopically, but the risk of invasion is quite low. There is currently no reliable way of measuring HPV infection of the entire cornified epithelium of the penis. Examination of male partners of women with identified HPV infections is of unclear value.

PREVALENCE OF HPV INFECTION

The typical age of cervical HPV infection is similar to other sexually transmitted infections, with a large peak following sexual initiation.[15] In the United States and Western Europe, infection prevalences decline sharply and reach very low levels by age 50 years, consistent with viral transience as well as low incidence at older ages.[16] However, in other populations there is not a big decline in HPV prevalence with age; rather, the curve rises again or never substantially falls.[17] Such variation in age patterns is important to screening strategies in other countries and is, therefore, a source of much conjecture and active study. Some, but not all, studies of highly exposed women, such as prostitutes, have shown a significant decrease in the HPV prevalence with age despite continuously high sexual activity, suggesting that loss of viral detection and the development of HPV type-specific immunity to reinfection occur.[18] The most common oncogenic type, HPV-16, is also the most common type in the general population. However, several nononcogenic types, like HPV-53, HPV-61, and HPV-62, are also very common.

PERSISTENCE VERSUS CLEARANCE

Anogenital HPV infections tend to clear, as do warts anywhere on the body. Cervical HPV infections remain detectable by polymerase chain reaction for a median of approximately a year, with HPV-16 tending to persist longer than other types.[19–21] As a result, the processes of HPV acquisition and clearance dynamically oppose each other in each cohort of women, to produce the characteristic age distributions as infections are transmitted sexually when women have new partners and then cleared. The major unresolved question of HPV natural history relates to viral latency. In follow-up studies lasting up to 10 years, it is evident that virtually all HPV infections become nondetectable by sensitive HPV DNA tests, usually within 2 years, except for those that lead to precancer. Little else is known about latency, what might cause reemergence like that seen in renal transplant patients and HIV-immunosuppressed women, and what fraction of cancers arises following a period of latency. Answers to these questions will greatly affect prevention strategies reliant on HPV DNA detection.

MICROSCOPIC ABNORMALITIES

Only a small minority of women with HPV detectable by DNA assays have microscopic abnormalities diagnosed.[4] Thus, screening for precancer and cancer using HPV DNA tests will be more sensitive than cytology, but less specific. The fraction of HPV infections that are cytologically evident depends on the choices of molecular test and cytologic method. Microscopic and visual diagnoses are prone to subjectivity, particularly when mild or equivocal changes are involved.[22] Therefore, misclassification is always a concern when epidemiologists consider how best to relate HPV infection to microscopic diagnoses (including histology that depends on colposcopic recognition of abnormalities). Human papillomavirus type is very important, because nononcogenic HPV infections can cause cytologic abnormalities without implying risk of cancer. High HPV viral loads are linked to cytologic abnormalities.[23] Ultra-low viral loads are associated with microscopic normalcy and with low risk of subsequent precancer or cancer, but in the clinical setting the prognostic value of increasingly high viral loads is not at all established.[17,24]

As a result of these issues, it is still not clear whether microscopically evident mild abnormalities represent a separate natural history stage from HPV detected by DNA testing alone. The authors personally favor considering all HPV infection, whether microscopically evident or not, as a single stage in potential carcinogenesis, with shadings of risk defined primarily by HPV type. Even among the oncogenic types, HPV-16 is uniquely risky, and even for HPV-16 (and other oncogenic types), variants are relevant to natural history. Human papillomavirus persistence is a necessary state for the emergence of precancer, but a large fraction of precancers arise from HPV infections in the absence of lower-grade, microscopically evident abnormalities. In histologic terms, it seems that a sizable percentage of CIN 3 cases arise from oncogenic HPV infections without transiting through diagnosed CIN 1 or even equivocal microscopic lesions. However, the certainty of this statement is limited by practical considerations of screening frequency in prospective studies. Because the detection of cytologic abnormalities rises with screening intensity, it is probable that rapidly progressing CIN 1 or equivocal cytologic abnormalities would be missed (while longer-duration CIN 3 would be found) with typical screening intervals.

PROGRESSION TO PRECANCER

Human papillomavirus infections (even with oncogenic types) are so common that getting infected might no longer be the usual limiting factor in cervical carcinogenesis. The critical step for most women might be whether precancer develops as an outcome of persistent oncogenic infection. Of note, it is the persistence of an oncogenic type (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68, and possibly a few more types) that is strongly linked to precancer.

It is a nontrivial task to define *precancer.* This admittedly vague term avoids the use of CIN 2, CIN 3, carcinoma in situ, or other terms that are important for histopathology, but which can confuse epidemiologic study due to variability in meaning. There is substantial heterogeneity in

the microscopic diagnosis and biological meaning of CIN 2 lesions in particular.[3] Some certainly represent acute HPV infections of particularly bad microscopic appearance that, however, are destined to regress, while others are incipient precancer destined to persist with high risk of invasion. Nononcogenic HPV infections are capable of producing lesions diagnosed as CIN 2, showing that this level of abnormality is not a sufficient surrogate for cancer risk. Of course, following the emphasis in the United States on safety and concern over loss to follow-up, treating CIN 2 is a valid clinical strategy to provide a margin of safety, given that it is not yet possible to know which lesions pose a threat.

Human papillomavirus infections are necessary but not sufficient causes of cervical cancer. Certainly, individual differences in immunologic responses to HPV play a critical role in determining the fate of the infections. Studies of HPV and immunity are underway, but optimal biomarkers of the successful immune response are not yet available. Epidemiologists need to confirm prospectively the other etiologic cofactors that promote HPV progression, which have been established by multiple case-control studies of cervical cancer. These include smoking, multiparity, and long-term oral contraceptive use.[25–27] Less defined cofactors include other sexually transmitted infections, such as *Chlamydia trachomatis*, chronic inflammation, and diet.[28–30] So far, only smoking has been confirmed as a risk factor for precancer or cancer in cohort studies of women infected with HPV.[31,32] With the exception of immunodeficiency and age, no cofactor identified to date is important enough to merit separate screening or clinical management protocols.

RISK OF INVASION

Cervical intraepithelial neoplasia grade 3 lesions tend not to regress over short-term follow-up; however, even among CIN 3 lesions, risk and timing of invasion versus eventual regression are matters of probability. The longer CIN 3 persists, the higher the risk of invasion. Thus, age is a critical epidemiologic factor that does merit consideration in clinical management. The median age of women with CIN 3 is approximately 30 years.[15,17] Cervical intraepithelial neoplasia grade 3 might be diagnosed earlier than age 25 years, but invasive cancer in women younger than 25 years is extremely rare. To catch the tiny number of such cases requires screening efforts among millions of younger women. In other words, in a poor-resource area or a culture wishing to balance safety against excessive intervention, screening for cervical cancer could start later than it currently does. Even screen-detected cases of invasive cancer tend to be approximately a decade or more older than women with CIN 3, suggesting a long average sojourn time in the precancerous state. The median age moves toward even older ages as the quality of screening decreases, but the average stage of cancer at diagnosis also worsens.

PUBLIC AWARENESS

Pathologists and clinicians should realize that the public perception of cervical cancer screening and prevention will soon change profoundly. Although the concept of CIN 2 and 3 as cancer precursors will not change, discussions of a mildly abnormal Papanicolaou test or CIN 1 will inevitably be replaced with those regarding HPV infection. This is a scientific advance but will not be comfortable for everyone. Because HPV infections are mainly sexually transmitted and long-term latency is possible, proper communications must inform honestly without unduly alarming women. The clarification of the natural history of HPV infection makes women safer. There is no new epidemic in the last few years. Nonetheless, the medical community needs to understand the challenge of switching communications. It is not clear who will take the lead in such communications. To date, it has not been possible to speak with one voice regarding issues as fundamental as proper screening intervals.

HPV VACCINES

Prevention of HPV infection would prevent cervical cancer, and animal models support the promise of virus-like particle (VLP) vaccines, which represent the outer protein shell of HPV without infective DNA.[33] Early trials in humans have demonstrated that intramuscular injection of VLP vaccines produce high titers of type-specific antibody with minimal side effects.[34] There are a few large-scale vaccination trials now underway and preliminary results are promising.[35] These early vaccines are focusing on HPV-16 and a few other important types, but a vaccination strategy would eventually require polyvalent vaccines. One type of VLP vaccine is purely prophylactic, containing only the VLP shell and aiming at eliciting neutralizing antibody. Ideally, it would be targeted at adolescent girls before the initiation of sexual intercourse. Another (''chimeric'') type of vaccine also contains some of the early proteins of HPV in a noninfective state (no DNA and mutated to ensure nononcogenicity) in order to elicit therapeutic cell-mediated immunity to enhance viral clearance in addition to producing neutralizing antibody for prophylaxis. Success with these early vaccines will lead to next-generation trials, and it is likely that many modifications will be required before a version applicable and affordable for worldwide use is available.

CONCLUSION

Trials of preventive strategies like prophylactic vaccination are already proceeding with justifiable scientific optimism. Human papillomavirus DNA diagnostic assays are being introduced into routine clinical practice to triage equivocal cytology and will eventually be introduced into general screening. However, epidemiologists working on etiology, molecular pathogenesis, and diagnostics are still very interested in understanding what exists between the causal risk factor and the disease endpoint, namely, the natural history of HPV leading to anogenital neoplasia. Such efforts may provide new or improved strategies for cervical cancer prevention. The benefits of better understanding of cervical carcinogenesis will also be applicable to understanding carcinogenic processes at other organ sites.

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