Should Family Physicians Test for Human Papillomavirus Infection?

An Affirmative View

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From the global perspective, cervical cancer is the second most common human malignancy, with an incidence approximating 450,000 cases per year. In second-world and third-world countries, the deaths from cervical cancer (mostly women under the age of 40 years) exceed the mortality rate for any cancer affecting both sexes. In South America the lifetime risk of a woman developing cervical cancer is about 5%. Since exfoliative cytology has proven too expensive and too difficult for developing nations, our best prospect of breaking this tragic cycle lies with the intervention of an automated technological test (rather than with attempts to further refine labor-intensive ones). In the West, four decades of cytologic screening have reduced incidence rates to sixth among female malignancies. Nonetheless, cervical cancer remains a disease of prime importance. Enormous sums are spent in running mass screening programs and in the diagnosis or treatment of patients with abnormal cells found on cytologic testing. Despite hefty public health expenditure, this entirely preventable cancer has not yet been eradicated in any community.

The overwhelming majority of cervical cancers arise within a field of squamous metaplasia, affecting the everted columnar epithelium of the transformation zone.[1] Exposure to coital carcinogens deviates this otherwise physiologic process into a spectrum of abnormal epithelial proliferations, some of which can evolve into invasive cancers. Specific human papillomaviruses (HPVs) are now firmly incriminated as the agent responsible for initiating cervical intraepithelial neoplasia (CIN). Whether these HPVs also play a role in promoting progression from precursor to invasive disease, however, is not yet known (Figure 1).[2] Although there is a steadily mounting body of evidence implicating the cancer-associated types as promoters, it is equally clear that oncogenic HPV infection alone is not sufficient to produce malignancy in an immunocompetent host.

Clinicopathologic Grouping of the Mucosotrophic HPVs

More than 60 HPV types are currently recognized, most of which were isolated from nongenital skin (cutaneotropic viruses). About a third of the known HPVs (the mucosotropic types), however, are most often detected in the anogenital or acrodigestive tracts. Based on nucleotide homology between the various viral genomes and on distinctive type-specific disease associations, anogenital HPVs can be subdivided into four main categories.

HPV Types 6, 11, 42, 43, and 44

HPV types 6 and 11 are two highly related viruses responsible for two main forms of disease: papillomas of the upper airways, and benign exophytic condylomas affecting the external genitalia, the lower third of the vagina, and the anal canal. Detection of HPV type 6 or HPV type 11 in minor lesions of the transformation zone has fostered a mistaken belief that these types account for the majority of minor cervical atypia. In fact, HPV type 6 or HPV type 11 probably causes only about 15% of flat condylomas or mild dysplasias.[3] Apart from an association with verrucous cancer, there is no convincing evidence to link HPV types 6 or 11 with genital malignancy. Earlier data associating HPV types 6 or 11 with cervical cancer probably represent laboratory errors as a

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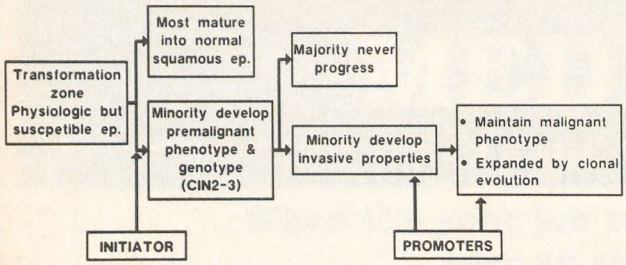

Figure 1. A diagrammatic representation of the standard model for the pathogenesis of solid tumors. Initiators transform physiologic but susceptible squamous metaplasia into permanently altered precursor epithelium. In contrast, promotors act at various other points in this cascade, such as developing invasive properties, maintaining a malignant phenotype, or favoring clonal expansions.

result of cross-reactivity of the HPV probes with other cancer-associated types. Included within this group are three recently cloned viruses, HPV types 42, 43, and 44, which are all closely related to HPV types 6 and 11 at the nucletoide level. HPV types 42, 43, and 44 are found in a small proportion of low-grade cervical, vulvar, and penile lesions, but have not yet been detected in an invasive cancer.

HPV Type 16

Worldwide, HPV type 16 is the viral type detected in about 50% of high-grade intraepithelial neoplasia and invasive cancer (mainly squamous, but HPV type 16 is also present in about one third of adenocarcinomas).[4] Moreover, elsewhere within the anogenital tract, HPV is found in at least 85% of high-grade lesions.[5] Thus, from the clinical perspective, detection of HPV type 16 apparently identified a patient at risk for the whole gamut of HPV-associated malignancies, namely, squamous cancer of the cervical transformation zone, adenocarcinoma of the cervical canal, and squamous carcinomas of the vagina, vulva, or anus. Moreover, earlier reports of detecting HPV type 16 in up to 84% of normal tissues have now been retracted on the basis of specimen contamination. Subsequent studies have placed the true latency rate for HPV type 16 within the range of 1.5% to 5%, depending on population characteristics.

HPV Types 18, 45, and 56

HPV type 18 shows a different distribution pattern from HPV type 16, being the second most prevalent type (20%) in invasive cervical cancers (especially aggressive adenocarcinomas of young women) but relatively uncommon (5%) in minor-grade lesions. On the basis of this skewed distribution, it has been hypothesized that HPV type 18 infection may set the stage for rapid progression from precursor to malignancy—perhaps too rapidly for reliable cytologic detection.[6] HPV types 45 and 56 are two relatively rare types, closely related to HPV type 18 at the nucleotide level, that share broadly similar patterns of disease association.[7]

HPV Types 31, 33, 35, 39, 51, and 52

These recently cloned types are often classified together as the intermediate-risk group. From the clinical perspective, this intermediate group presents certain similarities. Research undertaken by ourselves and colleagues has found that this group of HPV types tend to be overrepresented (25%) in CIN 2-3 but underrepresented (10%) in invasive cancer. Second, these more recently isolated viruses are usually found in squamous carcinomas of the cervix rather than in cervical adenocarcinomas or in other lower tract squamous cancers. From the molecular perspective, most of these intermediate-risk viruses resemble HPV type 16.

The Scientific Basis for HPV Testing

In contrast to the weaker, probably spurious associations between cervical cancer and other sexually transmitted pathogens, the relationship of cervical cancer with HPV infection is strong, consistent, plausible, and specific (Table 1). Koch's postulates have been fulfilled for the relationship between HPV type 11 and CIN: (1) Viral DNA is readily demonstrable in most lesions. (2) Skin warts have been experimentally transmitted to human volunteers by vaccination with cell-free filtrates. (3) Typical histologic changes of condylomas and cervical dysplasia have been induced by HPV type 11 infection of human cervical cells grafted beneath the renal capsule of nude mice. (4) Virion production has been documented within these HPV-infected transplants.[7]

Although not yet successfully cultured in Kreider's nude mouse model, HPV types 16, 18, 31, 33, 35, and 56 can immortalize (the first step in malignant transformation) human keratinocytes in cell culture. Histologic patterns essentially identical to CIN 3 can be produced by infecting human keratinocytes both in vitro and in vivo.[8,9] Moreover, progressive potential within precursor lesions is largely defined by HPV type.[10]

The role (if any) of HPV in promoting progression from CIN to cancer has not yet been defined. Nonetheless, there is a lot of provocative evidence suggesting that these viruses may play pivotal roles at many points along this long road. At least in tissue culture, the early ge-

Table 1. Evidence Implicating Oncogenic Human Papillomaviruses (HPVs) as Either Initiators or Promotors

Evidence HPV Infection Linked to the Pathogenesis of Cervical Epithelial Neoplasia (CIN) 2–3
1. Cancer-associated HPVs are found in 90% of CIN 2–3 vs 10% of normal women, yielding a relative risk estimate of 80 :1
2. CIN 1 is indistinguishable from condyloma, but viral cytopathic effect decreases with increasing levels of premalignant transformation
3. Noninfected cervical epithelium becomes senescent after 10 passages in cell culture; however, cervical keratinocytes are immortalized by oncogenic HPV infection
4. Histologic features of CIN 2–3 can be reproduced in vitro and in vivo by oncogenic HPV infection of previously normal human keratinocytes
5. Progressive potential of minor cervical atypia is influenced by HPV type
HPV Infection Linked to Progression From CIN 2–3 to Invasive Cancer
1. Cross-sectional data show strong, consistent relationship between specific HPV types and both precursor and invasive disease
2. HPV-immortalized human cells can eventually develop tumorigenic (invasive) properties with long-term culture
3. Animal papillomaviruses of analogous genetic organization produce invasive cancers in several species
4. Viral genome (especially E6 and E7) is continuously transcribed within cancer cells and cervix-cancer-derived cell lines
5. E6 and E7 viral proteins bind two cellular "antioncogenes" (p 53 and p RB) that control cell growth rates
6. HPV DNA is episomal in benign lesions, but integrated into the cellular genome of most cancer cells
7. Integration destroys the viral negative control gene (E2), but preserves the transforming genes (E6 and E7)

nomic region of oncogenic (but not nononcogenic) HPVs provides all the means for inducing cellular aneuploidy. Moreover, there are now reports of HP°V-immortalized cells acquiring invasive properties, simply by continued growth in vitro for 2 to 4 years.[11] In contrast to the "balanced" transcription of viral genomes seen in productive infections, malignant tissues are characterized by diminution of the negative regulatory effects of the viral E2 gene, and "unbalanced" expression of the viral transforming regions, E6 and E7.[12] Significantly, proteins transcribed from the viral E6 and E7 genes appear to inactivate two important human "anti-oncogenes" (p53 and pRB), involved in regulating cell division.[13,14] Finally, HPV genomic sequences tend to integrate into the host chromosomes at about the time that malignant cells acquire invasive properties.[15] Somewhat suggestively, linearization of the circular viral episomes prior to integration invariably preserves the E6 and E7 genes, but disrupts the regulatory viral E2 gene.

Viral testing cannot be justified simply on the grounds that HPV infection appears causally related to cervical neoplasia. If both condylomas and cancer contained HPV type 16, there would be no advantage to viral testing. Alternatively, even a surrogate marker could have great diagnostic value if it provided reliable insight into differing natural histories of morphologically similar lesions. Clearly, because of distinctive type-specific disease associations, knowing the HPV status can help discriminate genuine precursors from benign mimics.

The biggest problem facing currently available testing methods is that about 10% of apparently healthy women carry chronic, latent infections by clinically significant HPV types.[12,16] It is likely that future techniques will solve this problem by testing for evidence of viral expression rather than simple DNA colonization. For example, exon-specific mRNA production by the E4 and L1 genes would indicate potential infectivity, while unbalanced E6 and E7 mRNA in basal cells might prove a reliable guide to the risk of neoplastic change. Likewise, assays for virus-specified protein production would separate latent from clinically expressed infection.[2] As discussed below, however, present tactics for distinguishing between significant and nonsignificant results need to take a different approach.

In examining the role that viral testing can play in the 1990s, applications can be subdivided into research uses, quality control assurance, diagnostic workup, and well-woman screening. The value in the first application is not in question. Likewise, while few would argue over the use of viral testing for quality assurance, such problems are basically the preserve of the pathologist. The subject of this debate is whether first-generation viral tests (those based on the detection of HPV DNA) are of value to the clinician. In framing the answers to this question, it is necessary to differentiate screening from diagnostic strategies.[17] Screening tests are relatively simple procedures, designed to separate well persons from those with a high probability of having the disease under study. Screening tests are not intended to be diagnostic, but simply to identify people who warrant a formal workup. In contrast, diagnostic tests are more complex but more reliable procedures that aim at the precise identification and quantification of any underlying disease. Diagnostic tests are usually administered by a physician in response to suggestive symptoms and signs or in the further evaluation of a positive screening test. Diagnostic tests are performed on patients, whereas screening tests are performed on apparently well persons.

Diagnostic Uses of HPV Tests

Once a health problem has been identified, the use of HPV testing is noncontentious, since it conforms to the basic philosophy of clarifying the nature and prognosis of any underlying disease. Of course, many problems still

remain. Optimal methods of cell sampling, intermittent patterns of exfoliation, and potential interaction with other clinical events still need clarification. Such difficulties, however, are simply obstacles to be overcome, not reasons to abandon HPV testing. Of course, it would be desirable if any decision to apply routine testing was first evaluated in clinical trials, rather than by sporadic introduction of the prototype test.

In contrast to routine use, selective diagnostic testing in difficult clinical situations is perfectly reasonable. In particular, HPV testing would probably be of immediate value in the triage of minor-grade lesions. Historically, women with CIN 1–flat condyloma have presented a therapeutic dilemma. Either option—long-term surveillance or empiric treatment— represents a compromise. Nevertheless, HPV testing offers an accurate tool for individualizing between minor-grade dysplasias that seem to warrant treatment and other lesions with no apparent precursor potential.

Screening Uses of HPV Tests

The most controversial but perhaps the most important question is whether we should apply HPV testing to the screening of well women. On the one hand, Papanicolaou smears have been instrumental in securing a two-thirds reduction in cancer deaths in Western society. On the other hand, modern screening programs appear to be facing increasing difficulties. In the United States average age-adjusted mortality of cervical cancer for all age groups has decreased over the last decade by about 18%.[18]

Mortality rates among women younger than 45 years, however, have remained stable during this period, while incidence has actually increased in this subset.[19] Moreover, maintaining or improving upon screening benefits promises to become increasingly difficult, as the birth cohorts who were teenagers and young adults during the "sexual revolution" approach menopause (the modal age for cervical cancer). For example, a careful analysis of 81 cervical cancers occurring in Rhode Island in 1987 showed that failure to attend for screening accounted for only 15% of cancers in women aged 20 to 39 years, compared with 65% of cancers in the 40 to 69-year group.[20] In contrast, 67% of the younger women had had a negative smear within 3 years of diagnosis, compared with 35% of the older group (Table 2).

Forty years of screening appear to have changed the natural history of cervical cancer. Slow-growing, less aggressive cases tend to be detected, while rapid-transit, more aggressive cases are apt to fall through the screening net. Hence, physicians will soon be forced to reappraise critically traditional cervical screening programs. The key to the problem is understanding that no screening test is ever perfect, as the distributions of healthy and diseased individuals will always overlap (Figure 2). Hence, in practice, actual cutoff points are chosen to give the best compromise for the disease in question.

Table 2. Circumstances Associated with the Occurrence of Invasive Cancer in a Population-Based Survey of Rhode Island Women

Papanicolaou Smear History	Percent Aged 20 to 39 Years (n = 33)	Percent Aged 40 to 69 Years (n = 48)
Never had smear, or interval between smears > 3 years	15	65
False-negative or rapid transit	67	35
Neglected positives	18	0
Total	100	100

Cutoff criteria for the Papanicolaou smear have traditionally maximized specificity for two principal reasons. First, in the days when diagnostic standards for the Papanicolaou smear were being framed, an abnormal report generally led to a somewhat injurious intervention (namely, cone biopsy). Second, since false-positive results incur substantial but unnecessary fees, the cost-benefit ratio is more closely tied to specificity than to sensitivity. Historically, imperfect sensitivity was compensated by the assumption that false-negatives would be detected by repeat screening, before progression to invasive cancer could occur. In the 1990s, however, these assumptions are becoming increasingly hazardous, risk-

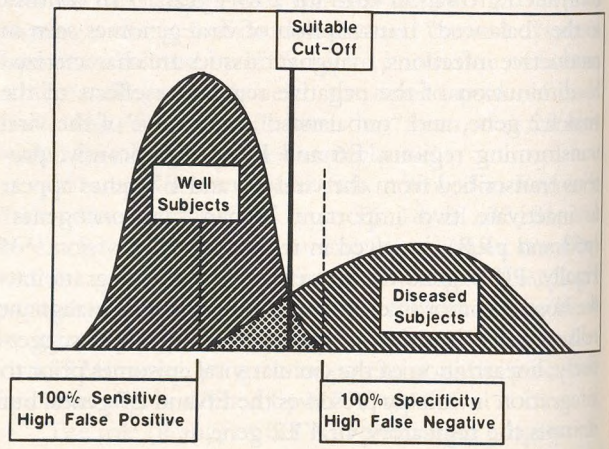

Figure 2. Because the distribution of well and diseased individuals always overlaps, no single screening test can ever be perfect. Attempts to maximize sensitivity lead to loss of specificity, and vice versa. Hence, actual cutoff points are a compromise.

ing a calamitous illness for the patient and litigation for the physician.

After four decades of experience with exfoliative cytology, it seems doubtful that new advances can be secured by adaptations of the Papanicolaou smear alone. Rather, the best prospect of further reducing cervical cancer among screened women will probably depend on the incorporation of such adjunctive measures as cervicography or HPV testing. To test this hypothesis, we screened 1012 Michigan women using cytologic studies, cervicography, and DNA hybridization.[21] Within this database, cytologic studies detected only 12 (52%) of the 23 high-grade lesions, despite the expense of recalling 8.7% of the women. Any attempt to reduce triage expenses by recalling just patients with major cytologic lesions would have caused an unacceptable reduction in sensitivity. Similarly, attempting to improve sensitivity by recalling all of the women who had dyskaryotic smears would result in a major erosion of specificity, too great to justify the marginal increase in sensitivity so attained.

Cervicography and hybridization were both effective screening tests, each being comparable to cytologic testing. Since each method measures a different aspect of the disease, however, it is likely that combinations could produce added benefits. Obviously, any simple combination of cytologic testing plus hybridization will undoubtedly increase sensitivity. As there are now two sources (rather than one) from which false-positives will accumulate, however, two test regimens are at risk of unduly eroding specificity. Such an error will lead to excessive patient recall, further swamping of colposcopic facilities, and the destruction of any cost-benefit ratio.

The use of three tests was sufficiently effective to allow the choice of more restricted endpoints (just high-grade lesions), while still delivering an 83% sensitivity for a 7% recall rate. This performance was encouraging, but had two weaknesses: it was discomforting to miss 17% of high-grade lesions, and the reduction in recall percentage from 8.7 % to 7% was too small to provide cost savings sufficient to offset the extra expenditure. Optimal performance within this model system was obtained using more selective endpoints. Namely, recall was restricted to (1) those women who had a high-grade morphologic abnormality on either cytologic studies or cervicography, and (2) patients with minor-grade aberrations in whom an oncogenic HPV type was detected. This strategy detected 96% of the high-grade lesions at the cost of recalling only 4% of the population (Figure 3). The reduction in recall for colposcopic triage and empiric therapy (88 vs 40 women) produced savings sufficient to essentially offset the costs of the expanded screening profile. Moreover, since the three-test regimen detected 22 cases (vs 12 for cytologic studies alone), the actual detection costs per case was 40% cheaper in the three-test model.[21]

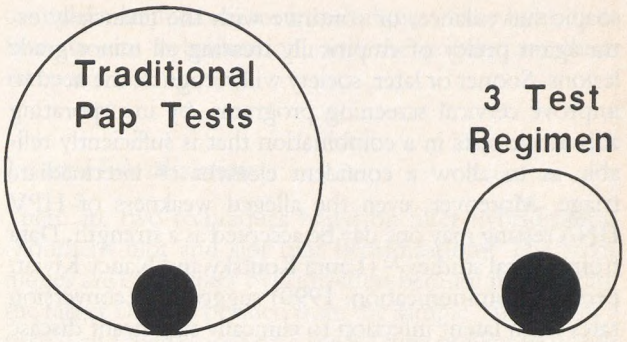

Figure 3. Comparing the relative efficiencies of suggested three-test regimen with traditional Papanicolaou smear programs. The proportion of CIN 2–3 is shown within the shaded circle, while the overall number of patients recalled for colposcopy is represented by the open circles.

Conclusions

Naysayers maintain that test reliability has not been fully investigated, that predictive value needs further clarification, and that we need a better understanding of the natural history of HPV infection. These arguments are true, but the emphasis is wrong. Of course we must continue to evaluate viral testing in formal clinical trials, and of course we must be sensitive to the emotional impact of finding sexually transmitted HPV DNA in asymptomatic women. But the heart of the issue is whether viral testing is scientifically rational. Would viral testing help to discriminate trivial from significant disease in the context of a diagnostic workup? Can viral testing help improve the sensitivity and specificity (and it must be both) of cervical screening programs? If the answer to any of these questions is "yes," then the premise that viral testing can be clinically useful is proven. All that remains is to decide on the best time frame and format for the introduction of these methods.

We are witnessing the birth of a new technology. Existing tests are prototypes that will soon be swept away by improved methods. Even without additional technical advances, the discriminating use of first-generation HPV DNA tests could improve patient care and lower health costs. The regimens outlined above represent provocative models rather than standards of care; however, we are confident of the wisdom of these arguments. Sooner or later society will find it cheaper to individualize the management of minor-grade lesions rather than to continue with the logistically impossible task of trying to keep 5% to 10% of the female population under colpo-

scopic surveillance, or continue with the financially extravagant policy of empirically treating all minor-grade lesions. Sooner or later, society will recognize the need to improve cervical screening programs, by incorporating adjunctive tests in a combination that is sufficiently reliable as to allow a confident element of intermediate triage. Moreover, even the alleged weakness of HPV DNA testing may one day be accepted as a strength. Data from several studies [22] (Laura Koutsky and Nancy Kiviat, personal communication, 1990) suggest that conversion rates from latent infection to clinically significant disease approach 20% per year!

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An Opposing View

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Human papillomavirus (HPV) infection of the lower genital tract has increased in incidence and now may be the most common sexually transmitted viral disease.[1,2] It is well documented that HPV is highly associated with condylomata and intraepithelial neoplasias.[3–5] There is increasing interest among family physicians to develop technical skills of colposcopy and androscopy so they can identify the clinical manifestations of HPV.[6] It is important for family physicians to become aware of this virus, particularly the methods to detect it.

Several years ago it became possible to isolate HPV from genital tract lesions using molecular hybridization techniques. Initially this process was quite laborious. Today procedures based on molecular hybridization have been modified so that they are easy to do and simple to

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Table 1. Summary of Available Human Papillomavirus Detection Tests.

Product	Method	Number of Tests per Kit	Cost/Test ($)
Digene	In situ	20	15
Enzo	In situ	40	10
ONCOR	Southern blot	60	7
Virapap	Slot blot	50	7
ViraType	In situ	20	15

learn. Several kits are now commercially available for hospital laboratories (Table 1). As the clinical manifestations of HPV become publicized, more patients will request the test. In some instances, information about the HPV status of a patient will affect management. Because HPV-related lesions are sexually transmitted, the emotional impact of whether a patient has such an infection may be profound. It is imperative that the physician be as accurate as possible. This discussion will center on the data now available on the clinical utility of testing for HPV. After a brief review of the basics of HPV testing, we will focus on clinical settings where such tests are useful.

HPV Testing: Molecular Hybridization Techniques

Molecular hybridization is the basis for all the important tests available for HPV detection. In brief, the technique involves attaching single-stranded nucleic acid (either DNA or RNA) from a sample, called the target, to a labeled probe. The probe is usually labeled with either radioactive or biotinylated nucleotides that can be detected by autoradiography or standard immunohistochemical methods.[7,8] The target and probe will attach (hybridize) because of the hydrogen bonds formed between complementary nucleotides (for DNA this would be G-C and A-T). Homology is a reflection of the degree of base-pair matching between the target and probe. If the degree of homology is high (ie, there is substantial G-C and A-T matching), then the target-probe complex will tend to remain hybridized and resist efforts to separate (denature) it. On the other hand, target-probe complexes with poor homology will disassociate easily. The number and strength of the hydrogen bonds in hybridized DNA with good homology is much greater than for antibody and antigen associations. This translates into a much higher sensitivity and specificity for molecular hybridization when compared with prior methods. The ability of this technique to reliably detect the presence of HPV is exceptional. As few as 10 viruses per 1 million cells of tissue can be detected. There are three tests based on molecular hybridization techniques: filter hybridization, in situ hybridization, and the polymerase chain reaction.

Filter Hybridization

There are two techniques based on filter hybridization (Southern blot and slot blot hybridization). The techniques are called filter hybridization because in each case the target DNA is purified from the sample (the patient's tissue) and then placed on a special type of filter to which it can bind. In slot blot hybridization, the DNA is added directly to the filter. In Southern blot hybridization, electrophoresis of the target DNA precedes transfer of the DNA to the filter. This step is done to remove possible impurities. The filter can then be treated with the labeled probe to determine whether the sample contains the DNA of interest.[9,10]

The major advantage of filter hybridization is its sensitivity. As few as one virus for every 100 cells can be detected.[11] When HPV infects tissue, it is common to find as many as 10,000 virus particles per cell. Filter hybridization should, therefore, easily identify such an infection. HPV-detection kits that use slot blot and Southern blot hybridization are now commercially available (Table 1).

There are two major disadvantages with filter hybridization. First, the tissue must be fresh or frozen. This criterion can present a problem for clinicians performing colposcopy in out-of-hospital settings. Second, in most cases radioactive probes are needed (ONCOR has recently introduced a nonradioactive filter hybridization kit) (Table 1). A wait of up to 1 week for results is involved along with the attendant problems in disposing of radioactive wastes. Each of these problems can be avoided using in situ hybridization.

In Situ Hybridization

With in situ hybridization the probe is applied directly to a tissue section. The probe is a "viral cocktail" containing specific segments of HPV DNA. Attached to each HPV-specific segment is a protein "label" (biotin). In the presence of HPV, the biotinylated DNA will concentrate in the nucleus of an infected cell. The tissue is then treated with a colorizing agent. The HPV-infected nuclei will turn a brilliant blue. Infected cells can be easily recognized under a standard light microscope.

The major advantage of in situ hybridization is that fixed, paraffin-embedded tissue, available from any patient who has had a biopsy, can be used. Testing, therefore, may be considered after the biopsy report is com-

pleted. Another important advantage is that one can use nonradioactive (biotin-labeled) probes.[11,12] Detection of a biotin-labeled target-probe complex is a simple technique available in most hospital laboratories. The test can be completed in a matter of a few hours. In short, in situ hybridization is an easy, rapid test for HPV that can also be used to tell which specific HPV type has infected the tissue. Why, then, even bother to use filter hybridization? The answer is contained in the detection threshold for in situ hybridization analysis. About 10 to 20 viruses must be present in the cell for the in situ test to be positive.[11,12] Although there is no problem in low-grade intraepithelial lesions, occult infection by HPV has far fewer particles. In situ hybridization is usually negative in such infections, whereas filter hybridization usually detects the virus.[11]

The Polymerase Chain Reaction

The purpose of the polymerase chain reaction is to amplify the amount of HPV DNA present in a patient's tissue sample by making copies of the HPV DNA. The procedure is done in the following steps: short, HPV-specific fragments are "manufactured" through a process called DNA sequencing. The resulting fragments are called primers. When a cocktail of the primers is added to a tissue sample, the primers will bind to homologous areas of the viral DNA. The result will be a strand of patient HPV DNA with multiple short HPV-manufactured segments attached. Gaps will exist between each of the fragments. An HPV-specific enzyme called Taq polymerase is then added. This enzyme fills in the gaps by synthesizing the missing segment of DNA. Next, the primer-target complex is denatured and the process restarted. With each cycle the amount of HPV DNA doubles. After 30 such cycles there are generally over a million HPV particles, which can then be detected by a characteristic electrophoretic pattern.[13,14]

The major advantage and disadvantage of this test are related. Because viral DNA is amplified, it is possible to detect very small amounts of HPV; as few as 10 viruses per 1 million cells. Even a small amount of contaminating HPV DNA, however, can result in a false-positive result. The technician must take great care that contamination does not occur. Another advantage of polymerase chain reaction is that paraffin-embedded tissue can be used. Further, there is no need for radioactive probes. The main advantage of polymerase chain reaction is that if the test is negative, one can be certain that the morphological changes in the tissue in question are not related to HPV infection. Currently the polymerase chain reaction test should be reserved for research laboratories.

When Clinical Testing for HPV is Not Indicated

Determination of HPV Type

A great deal of attention has focused on the different HPV types. It is often stated that HPV types 6 and 11 are "good" (not associated with cervical carcinoma) and that HPV types 16 and 18 are "bad" (associated with an increased cancer risk).[4,15–17] So should lesions associated with HPV 6 and 11 be left alone? We say no. As anyone who regularly treats vulvar or penile low-grade lesions (condylomata) knows, these lesions have a high rate of recurrence. As many as 70% will recur.[18] They can become large and are often distressful to the patient. The only possible use of specific HPV typing might be to determine which women carry HPV 18. This type, which occurs in less than 1% of the population, is detected in the majority of adenocarcinomas of the cervix. Adenocarcinomas are responsible for 5% to 7% of cervical cancers. As many as 50% of women with adenocarcinomas are missed on routine Papanicolaou smear testing.[19] Currently there is no information to suggest that a major change in the screening for adenocarcinomas of the cervix is warranted.

Detection of Occult Infection

Several investigators have shown that about 10% of women and men will have HPV DNA detected from cervical or penile swabs, respectively, but will have either normal Papanicolaou smears or no visible lesions.[20–22] Should these people undergo more extensive testing? At this stage there is no evidence to suggest that in the absence of a visible lesion (an acetowhite area found during colposcopy), these women are more likely to have cervical intraepithelial lesions (CIL). Indeed, some evidence suggests that these women may be less likely to develop CIL.[22] The emotional impact of telling a woman that she has a potentially cancer-causing virus in the cervix even though there is no evidence of disease is of course profound. Follow-up data do not exist at this time to justify alarming 10% of the population. The Papanicolaou smear continues to be the most effective screening tool in the detection of cervical intraepithelial disease. It has yet to be demonstrated whether HPV testing will increase the detection of cervical intraepithelial lesions relative to Papanicolaou smears in women who have normal Papanicolaou smears over time.

When Clinical Testing for HPV is Indicated

Detection of HPV DNA in Women with an Abnormal Colposcopy

In certain settings testing for HPV DNA can provide very useful information to determine who is at high risk for CIL or who does not have an HPV-related lesion. A common clinical problem for the colposcopist involves a woman who has an abnormal Papanicolaou smear (often squamous atypia) and an acetowhite lesion on colposcopy which on biopsy is negative for condyloma or CIL. What follow-up is indicated for such a patient? Repeat the colposcopy? Treat the cervix anyway? And what should the patient be told? It has recently been shown that HPV detection in this setting is an excellent way to determine who really is at high risk for CIL and who is not.[23] We propose HPV testing for all women with atypical Papanicolaou smears who have acetowhite lesions with biopsies nondiagnostic for CIL. Patients who are virus positive should undergo colposcopy again in 4 to 6 months, whereas those who are virus negative may be best followed by repeat Papinicolaou smears.

As an Aid to the Histological Examination of HPV-Suspected Lesions

Although in its classic form the histologic changes of HPV infection are easily diagnosed, it is not infrequent that the histological changes, though suggestive of a lesion, may be equivocal. Pathologists may try to be helpful by signing out such cases as "borderline, early, or equivocal for condyloma," but this may be confusing to the physician and especially the patient, who wonders, "Do I or don't I have this sexually transmitted disease?" Here, HPV testing by in situ hybridization can be very helpful. Over 95% of low-grade vulvar, penile, and cervical intraepithelial lesions will be positive by this methodology, whereas in normal tissue the in situ test is invariably negative.[17] It has been shown that in lesions clinically suggestive of low-grade infections (condylomata) where the histological changes are equivocal, HPV DNA can be detected by this test at a rate of 2% for the cervix and about 10% for the vulva and penis. HPV-positive cases are best considered low-grade lesions. HPV-negative cases are most likely mimics for which another condition, such as candida infection or chronic irritation, should be explored. These conclusions can be supported by testing with the highly sensitive polymerase chain reaction technique.

Summary

Recent advances in the field of molecular biology have expanded the knowledge of HPV manifestations. We have attempted to separate those circumstances when HPV testing may be useful in patient management and when it may not. Routine screening for HPV has not yet been shown to be useful. One must weigh informing 10% of patients that they have a potentially oncogenic virus against the ability of a repeat Papanicolaou smear to detect the lesion. On the other hand, HPV detection in the setting of an abnormal Papanicolaou smear and an acetowhite lesion that on biopsy lacks the histologic features of CIL does predict who is at high risk for CIL. HPV typing should not affect clinical management, and we do not recommend it. HPV detection by in situ hybridization is a very useful adjunct to histological analysis in genital tract lesions that are clinically suggestive of an HPV lesion but where the histological analysis is equivocal. This ability to distinguish true HPV lesions from its mimics is often crucial to the patient, given the implications of having a sexually transmitted disease.

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