Testing for Human Papillomavirus: Basic Pathobiology of Infection, Methodologies, and Implications for Clinical Use*

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New molecular biological evidence has recently emerged, strongly implicating the human papillomavirus (HPV) as playing an etiologic role in the development of neoplasias of the genital tract. As technologies advance, the ability to test for the presence of HPV has become simpler, more reliable, and less expensive. A great deal of controversy has arisen regarding the effective and proper utilization of these new tests in the management of HPV infections. This review will detail the new evidence implicating the putative role of HPV in neoplasia and the current methodologies available for assessing the presence of HPV in clinical samples and will describe the current controversy surrounding their utilization.

INTRODUCTION

It is estimated that there will be 13,000 incident cases of invasive cervical cancer and 50,000 incident cases of cervical carcinoma in situ in the United States in 1991 [1]. Since the advent of mass cytologic screening programs in the 1950s, the overall mortality of this disease has been reduced by 70 percent, but deaths continue in the unscreened or poorly screened patient populations. Recently, a resurgence in the number of cases of precursor lesions to cervical cancer has been seen, particularly in young, sexually active populations. Improved clinical methods for screening, diagnosis, and prediction are continually being sought and tested, the better to control this preventable disease.

A growing body of epidemiologic, cytologic, and molecular biologic evidence has linked the human papillomavirus (HPV) to cancers of the female genital tract. Data show that cervical cancers have the epidemiologic characteristics of venereally transmitted infections. HPV deoxyribonucleic acid (DNA) has been identified in the vast majority of specimens from cervical and vulvar neoplastic lesions. Furthermore, association of particular histologic patterns of neoplasia with specific HPV types has

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been demonstrated. In addition, newly emerging molecular genetic data show a potential biologic role of papillomaviral genes in the carcinogenesis sequence.

With this growing understanding of the association, and potential role of the virus in carcinogenesis, particularly cervical, the ability to identify evidence of HPV infection has been explored as a method to identify those patients at increased risk for the subsequent development of invasive cancers. The ability to define further the additional risk of disease progression by viral typing has also been postulated.

The purpose of this review will be to detail the evidence linking HPV to genital neoplasia, the methodologies currently available to detect HPV in clinical samples, and the clinical utility of the various detection strategies.

THE ASSOCIATION OF HUMAN PAPILLOMAVIRUS WITH GENITAL NEOPLASIA

The epidemiologic data supporting a venereal transmission pattern for condyloma and cervical neoplasia have been well reviewed by other authors [2,3]. A variety of infectious agents have been investigated with regard to their carcinogenic potential in genital neoplasia. It was not until the middle 1970s, when Meisels and Fortin [4] first recognized that the cytologically defined, pre-cancerous condition of koilocytosis [5,6] actually represented HPV cytopathic effect, that the association of HPV in cervical neoplasia was postulated. Classic cytologic studies had previously shown a progression of koilocytotic lesions to dysplasias and invasive carcinoma [7]. Hence, HPV became the prime infectious agent postulated to be etiologic in the development of lower genital tract neoplasia, thus spurring a tremendous research effort into the understanding of this association and its mechanisms.

Prior to the ability to detect HPV via immunopathologic and molecular biologic methods, the only way to confirm that a koilocyte was an HPV-related cell was by electron microscopic demonstration of virions. Numerous investigators have shown that, although koilocytes were identified in a high percentage of low-grade lesions, such as condylomata and slight dysplasias (CIN I), as the epithelial maturation abnormality increased (i.e., higher grades of dysplasia), koilocytes were found in fewer and fewer cases [8,9]. Our present understanding of the life cycle of the HPV explains this phenomenon, although the actual mechanism of development of koilocytosis is presently unknown. Completion of the viral life cycle with production of whole virions is most closely correlated with the cytopathic effect that we recognize as koilocytosis. Full viral replication can only occur in the presence of an entire intact viral genome and requires keratinocyte maturation for full expression of the viral life cycle. Therefore, in low-grade lesions, the host cellular environment is permissive for production of virions, and, hence, koilocytes are more frequent. In the higher-grade lesions, the epidermal cells lack the necessary maturation for full viral replication, and, in addition, the phenomenon of partial viral genomic integration into host genome may also occur. In this situation, the virus is not competent to replicate, and viral gene expression is altered such that koilocytosis is much less frequent.

Immunohistochemical approaches to the identification of viral antigen present in tissues were somewhat more productive than virion identification but still failed to identify HPV in the majority of high-grade lesions, because the procedure detects viral capsid antigen. Antigen production requires late gene expression, which is often lacking in high-grade lesions [9,10].

With the advent of molecular biologic techniques for the detection of viral nucleic acid molecules, it became clear how prevalent HPV DNA was, not only in low-grade lesions, but also in the high-grade dysplasias and invasive carcinomas. When sensitive techniques were employed, more than 90 percent of invasive cervical carcinomas of all histologic types were found to contain portions of the HPV genome [11]. Over 60 HPV types have been identified to date, with about two dozen of these having been found to be associated with genital tract pathology. In addition, it appears that there are certain virus types which are associated with lesions showing a low risk of progression, most commonly viral types 6 and 11. These are the types most likely to be found in exophytic genital condylomata [12]. Virus types 16 and 18 are the predominant types associated with cervical intraepithelial lesions and invasive carcinomas and hence have been labeled high-risk virus types [13,14]. It is important to note, however, that mere infection with a high-risk virus does not imply that a high-grade lesion is present, or that progression will occur. Most woman who are infected with these viral types show either no pathologic or low-grade changes [15,16], and classic morphologic studies show that, in a high proportion (approximately 90 percent) of patients, any disease which is present will fail to progress or will ultimately regress [7]. Other viral types, such as the 31, 33, and 35 group, seem to have an intermediate risk, because, while more frequently seen in high-grade genital dysplasias and carcinomas than types 6 and 11, they are much rarer in the human population, and less well characterized epidemiologically [11,13,17]. Specific viral types have also been more highly associated with specific histology and/or clinical behavior. For instance, HPV type 18 has been implicated in a more rapidly progressive form of cervical squamous cancer [18], as well as in the small-cell and adenocarcinoma variants of cervical cancer [19,20,21,22,23]. Infection with the "high-risk" viruses has been postulated to confer a higher risk of progression from low- to high-grade lesions. Complete prospective studies to confirm this hypothesis have not yet been completed, although preliminary data from Syrjanen and co-workers have suggested that cervical intraepithelial neoplasia (CIN) lesions associated with HPV type 16 do tend to run a more aggressive course [24]. This point is particularly important, because data have recently suggested that the most prevalent virus type found in samples from the cervix may be type 16 [15,25,26].

MOLECULAR EVIDENCE LINKING HPV TO CARCINOGENESIS

The genetic structure of the papillomaviruses has been reviewed extensively elsewhere [27]. Several key points regarding viral structure and genetic makeup are important to this discussion. The family of papillomaviruses are small, double-stranded DNA viruses, consisting of a central core of DNA, surrounded by an outer capsid of viral protein. Viral DNA consists of a covalently closed, circular, double-stranded molecule of about 7,900 base pairs. The viral genome is organized into three major areas. The first, representing about 15 percent of the total genome, is a non-coding segment known as the long control region (LCR), or upstream regulatory region. This region is responsible for much of the DNA replication origination function and includes several enhancers and promoters of messenger ribonucleic acid (mRNA) transcription. The second major portion of the viral genome, representing about 45 percent of the total, is the early, or E region. This segment contains at least seven open reading frames (ORFs), each of which codes for one or more proteins responsible for the maintenance of cellular transformation or viral DNA

replication [27]. The third portion of the viral genome, comprising 40 percent of the total, is the late, or L, region, which contains two ORFs coding for viral capsid proteins.

The body of experimental evidence implicating HPV as a potential agent in the development of cancer is increasing in size. Portions of the genome from high-risk viruses have been found in a variety of cervical cancer cell lines. HPV genes from the high-risk types have been shown to transform a variety of animal and human cell lines. In addition, extensive studies have been performed to understand the pattern of gene expression in these transformed cells and the cells of clinical carcinomas.

It has been shown that the HPV early coding genes, E6 and E7, are virtually always present in HPV-related transformed and cancer cells [28]. Both genes appear to be required together to immortalize human foreskin keratinocytes. E6 alone fails to elicit a response in the transfected cells, while E7 alone causes hyperproliferation without immortalization [29]. Transfection of the E6-E7 region of high-risk viruses alone is sufficient to transform several cell lines [30,31]. In human cancers and high-grade intraepithelial lesions, HPV DNA is often incorporated into the host genome [32]. This condition is in distinction to low-grade lesions, where the viral genome most commonly exists as an episome apart from the host's DNA. This incorporation of HPV genes has several uniform features. Integration preferentially disrupts the region of the E1 and E2 genes. These genes are associated with DNA replication control and the control of transcription, and, in the process of linearization and integration, these genes are damaged. These same genes are critical to the regulation of E6 and E7 through the adjacent LCR, the three of which are always retained. Hence the stage is set for poorly controlled replication and transcription of these particular genes [33]. Homologies of the E7 gene have been identified with the genes E1A of adenovirus and the large T antigen of the SV40 virus, both known transforming genes, and suggest the possibility that the E7 gene may play a similar role in human cervical cancers [34]. In addition, like the protein products of the E1A and large T antigens, the protein product of the E7 gene can bind to the protein product (p105-RB) of the retinoblastoma gene, a known tumor suppressor gene, and this p105-RB-E7 protein complex has been identified in cell culture [35]. The E7 gene products of HPV types 16 and 18 bind p105-RB with a higher affinity than do the E7 proteins of HPV types 6 and 11, hence suggesting a greater tumor disposition for the "high-risk" viral types [35]. Analogously, E6 protein has been found to bind to cellular protein p53, a protein which also has tumor suppressor activity [36]. Furthermore, there is some evidence to suggest that E7 gene products can interact with cellular oncogenes, such as ras, to promote cellular transformation [34,37].

Although the molecular evidence, as presented above, is gradually moving toward implicating the HPV, and specifically the high-risk types, as an effector in the carcinogenesis process, it must be noted that HPV infection alone is insufficient to initiate cancer development. Other, as yet undetermined co-factors, potentially tobacco smoking or other possible viral infections, appear to be necessary for ultimate neoplastic transformation in humans [3].

METHODS OF TESTING FOR HPV PRESENCE IN CLINICAL SAMPLES

Standard Cytologic and Histologic Examinations

The Papanicolaou smear and surgical biopsy specimens are effective methods of identifying viral cytopathic effect and secondary features which suggest HPV infec-

tion, when these changes are present. Molecular biologic evidence supports the concept that a finding of unequivocal cervical intraepithelial neoplasia (dysplasia, carcinoma-in-situ), arrived at by standard diagnostic criteria, implies that HPV DNA is present in the lesion. Unfortunately, in a large percentage of cases of cytologic atypia (pure mild nuclear enlargement, and elevated nucleus-to-cytoplasmic ratio of squamous cells), or the presence of only secondary (non-diagnostic) features of HPV infection [hyperkeratosis (HK) and parakeratosis (PK)], the correlation of the presence of HPV DNA with morphologic features is poor, approaching that seen in cytologically or histologically normal individuals [12,38,39]. In addition, any assessment of HPV type is not possible on routine light microscopy.

Immunopathologic Assessment of HPV Presence

Antibodies raised to bovine papillomavirus capsid antigen have been used extensively to identify the presence of HPV capsid antigen. Unfortunately, immunohistochemistry for HPV shares many of the same limitations as does routine light microscopy. Because late protein (capsid protein) formation is necessary to provide antigen for detection in this procedure, generally only low-grade CIN lesions can be marked with regularity. In several studies of condyloma and low-grade CIN lesions, only 50–60 percent of cases were positive with immunohistochemistry [10,12,40]. In our own series of cases, a small percentage of lesions (13 percent) with only secondary features suggesting HPV presence (HK and PK) were positive for antigen, and no biopsies of histologically normal tissue were positive [12]. As lesions become higher-grade, fewer and fewer are positive, in keeping with the previously noted biology of HPV expression in high-grade lesions [10]. In addition, no ability to assess HPV type is presently possible with standard immunohistochemistry, although reagents are currently under investigation which may be able effectively to label specific protein products of high-risk virus early genes [41].

Nucleic Acid Hybridization Technology for HPV Detection and Typing

The ability to detect, via nucleic acid hybridization, specific strands of DNA which code for unique sequences in HPV has been an important force in the advancement of our current description of HPV biology and forms the basis for the most commonly utilized tests for HPV detection and typing.

There are several methods of hybridization which can be performed, including dot blot, Southern blot, and in situ hybridizations. In addition, amplification procedures, such as the polymerase chain reaction (PCR), have been developed to increase the sensitivity of detection.

All nucleic acid hybridization procedures are based on the principle that two anti-parallel strands of DNA or ribonucleic acid (RNA) will bind specifically to one another via hydrogen bonding between complementary base pairs. Probes that bind to type-specific papillomavirus genetic sequences have been developed, which will allow not only recognition that HPV DNA (or parts of it) is present, but which can also allow distinction between the various types of HPV, based on nucleotide dissimilarities. The concept of the stringency of a reaction refers to the factors which favor duplex association; i.e., high-stringency reactions require that base pairs in the duplex match very closely, while low-stringency reactions require fewer matches to maintain detectable binding. For instance, a probe specific for sequences unique to HPV type 6 will bind only to HPV 6 DNA under conditions of high stringency but will

also bind to HPV type 16 under less stringent reaction conditions. Therefore, by altering the stringency conditions of a particular hybridization study, assays can be made more or less sensitive and specific. The details of the hybridization and stringency conditions are extensively reviewed elsewhere and are beyond the scope of this discussion [42,43].

Following the initial specific hybridization reaction between probe and target DNA or RNA, detection of this interaction must then take place. Probes may be labeled with a variety of markers, such as radioactive nucleotides (^{3}H, ^{35}S, ^{32}P), for autoradiographic detection; or with non-radioactive markers, such as biotin, which allow detection via an enzyme-linked colorimetric assay completely analogous to those utilized in standard immunohistochemical procedures.

TYPES OF HYBRIDIZATION ASSAYS

Dot Blot Hybridizations

These assays consist of a sample of DNA being placed in a specific area of a filter paper and then incubated with a solution containing the labeled probe, followed by stringency washes to remove non-hybridized, or incompletely hybridized probe. Detection of duplex formation is by exposure of the filter paper to X-ray film, in the case of a radiolabeled probe, or with a colorimetric reaction, in the case of a non-radioactive probe.

The advantages of this assay are ease of performance, rapid turnaround time, and relatively low cost. By utilizing probes directed against specific HPV types, under stringent reaction conditions, not only detection, but some typing of the virus is possible. The major disadvantages of this assay are that it appears to be less sensitive than is Southern blotting and some types of in situ hybridization, and that it lacks the ability to identify the location of HPV within the tissue, as the method is destructive of the histology of the sample. The most prominent commercial effort in HPV testing utilizes this type of technology. The test, as commercially available, has shown moderate sensitivity and excellent specificity [15,26]. One limitation common to all of the hybridization assays at present is the lack of readily available probes for all of the viral types associated with genital tract neoplasia. Although probes are available for the most prevalent viral types, a significant percentage of cases infected with other viral types may produce false-negative assays [15].

Southern Blot Hybridizations

This type of assay for the detection of DNA is considered the "gold standard" of the non-amplified hybridization procedures in terms of sensitivity and specificity. Studies in patients with documented CIN lesions have shown HPV DNA detection rates of greater than 90 percent with Southern blot [11,17,44]. In this assay, the nucleic acid is extracted from the sample and cleaved into smaller fragments with restriction enzymes. These fragments are then electrophoresed on gels for separation of the fragments based on size. The nucleic acids are then transferred on to filter papers and hybridized with probes directed against specific HPV nucleic acid sequences. Identification of duplex formation is the same as with dot blots. Southern blots have the advantage of very high sensitivity and specificity and the ability to distinguish easily between HPV types by identification of specific restriction patterns; their major disadvantages are that special equipment and expertise are

required to perform the assay, it is expensive, requires a fairly large sample, and, like dot blots, is destructive of the tissue, not allowing HPV localization and correlation with histology.

In Situ Hybridization (ISH)

In this procedure, the hybridization reaction is performed directly on the tissue section or cytologic specimen. The procedure is therefore an "in situ dot blot," and visualization of duplex formation is by either autoradiography (when radiolabeled probes are used) or colorimetric visualization, as in immunohistochemical analyses. The major advantage to this procedure is the ability to visualize the exact location of the HPV nucleic acid and therefore to correlate the exact histology with which it is associated, as well as allowing individual positive cells to be identified against a background of negative cells. In addition, the sensitivity and specificity are comparable to Southern blot when the more sensitive radiolabeled assays are utilized [12]. For such assays, the limit of sensitivity has been theoretically determined to be approximately 25 whole genomic equivalents per cell [42]. Hence, it is apparent that these procedures will fail to identify very low-level infected states. Generally, commercially available, non-isotopic ISH kits have been shown to be less sensitive than isotopic assays; however, several recently published reports have shown improved sensitivity with commercially available, non-isotopic probe kits [45,46,47]. In situ hybridization can also be performed on archival, fixed-processed tissue, eliminating the need for the fresh tissue necessary in the above-mentioned hybridization assays. The major disadvantage of the procedure is that, in its most sensitive form, it is time- and labor-intensive, although commercially available kits have more recently offered increased simplicity.

Amplification Technology

Newly developed methods for amplification of low-level DNA signals have recently been applied to the detection of HPV DNA. The most common procedure is the polymerase chain reaction (PCR) [48]. This procedure most often utilizes a thermostable DNA polymerase known as Taq, which, through the use of nucleic acid primers directed against specific target DNA sequences, allows exponential production of copies of target DNA, so that identification by the labeled probe can be achieved. Theoretically, this procedure is exquisitely sensitive, being able to identify single copy genes. The price for this high sensitivity is the high risk of contamination, leading to false-positive results. Hence, extreme caution must be exercised in specimen handling and in the use of control material. This procedure can be run on both fresh and recently fixed-archival tissues [15,49,50,51]. The sensitivity for detection of HPV DNA by PCR appears to be the highest of all the above-mentioned techniques. Bauer et al. [15] showed that, when compared with dot blot hybridization, PCR identified four times as many infected patients. The major advantage of PCR in this study was the procedure's ability to identify viral types for which probes were not available in the dot blot assay.

CLINICAL USE OF TESTING FOR HUMAN PAPILLOMAVIRUS

The simplistic approach to HPV testing would dictate that, if all patients who were infected with HPV, and in particular those infected with one of the intermediate or high-risk viruses, could be identified, these patients could be closely followed, and/or

treated, with the HPV infection being ablated prior to the sequential development of dysplasias and invasive cancers. Under this scenario, mass screening for HPV would be a very important tool in the prevention of cervical cancer. Unfortunately, at the present time there are no effective means of treating or eradicating HPV infections. Effective treatments are available only to control CIN lesions when they are detected by cytology or colposcopy. In addition, for a screening test to be effective, it needs to be very sensitive and preferably inexpensive [52]. In situations of relatively low disease prevalence (e.g., cervical cancer and its precursors), the test needs to be very specific to have a high predictive value for the disease in question. There is early evidence to suggest that, in fact, the most widely applicable test (least expensive and technically simplest), the dot blot hybridization, may not be as sensitive as is necessary. Several studies performing either dot blot [26,53] or Southern blot [39] screens on patient populations have found detection rates ranging from as low as 40 percent to as high as 80 percent of patients who have some evidence, either current or past cytologic abnormality, indicative of HPV infection. Many of these patients undoubtedly had eradicative procedures and were no longer infected, but presumably many had levels of HPV DNA below detection limits for the test, or HPV types which were not being probed for in the assay utilized. By comparison, studies utilizing PCR amplification technology have shown detection rates consistently greater than 80 percent in similar patient populations who have cytologic evidence of HPV infection [15,54]. In addition, it appears that the prevalence of HPV infection in the community among some epidemiologically defined high-risk populations may be as high as 80 percent [55], although it appears that reasonable estimates range from less than 5 percent in private patient populations to as high as 46 percent in sexually transmitted disease clinics and student health service populations [15,16,26,53]. Exact prevalence figures are unknown at present because broad-based population studies, using gold-standard techniques, have not yet been reported, and, clearly, prevalence data will vary considerably, dependent on the population studied and on the methods utilized in screening. On the other hand, evidence obtained over many years utilizing the Pap smear, colposcopy, and biopsy has shown that the prevalence of cytologic, colposcopic, and histologic abnormalities of CIN in the population, at 2–3 percent [7,24,56], is significantly lower than what has been estimated for the prevalence of HPV infection. Therefore, it appears that a large pool of "infected" individuals exists, who have, at the present time, no identifiable changes in the cervix. Syrjanen [24] has termed these types of infections as latent (only molecular biologic evidence of HPV DNA, without cytologic or colposcopic evidence of disease), or subclinical (molecular biologic evidence, plus minimal or inconclusive cytologic or colposcopic evidence of disease). Under the scenario of molecular biologic screening, these groups of patients would create a significant dilemma for the gynecologist, who must then decide how to utilize effectively this molecular biologic evidence of infection without clinical disease. The natural history of the HPV DNA-positive, disease manifestation-negative state is poorly known at the present time. No estimate can be made of the number of these individuals who will ever proceed to clinically significant illness (i.e., HPV DNA testing lacks specificity for the actual neoplastic disease process). In addition, classic morphologic studies have clearly shown a high regression rate at all levels of CIN, suggesting that, even when a lesion becomes manifest, there still exists a significant number of patients who will never progress to invasive cancer [7]. Other, as yet unknown,

factors must be involved in this progression. Therefore, mass screening for HPV DNA would create the potential for overzealous treatment, overwhelmed gynecologists, unnecessary patient anxiety, and tremendous cost, all for undocumented benefit [57]. In addition, because the test may lack sufficient sensitivity, and the prevalence of infection (not clinical disease) appears to be high in the population, there is the potential for significant numbers of infected individuals to be missed by any but the most sensitive tests.

An augmented screening approach, which has been suggested, is to perform HPV typing on all HPV DNA-positive individuals. This procedure would presumably serve to identify the patients who are postulated to represent a group at higher risk for progression and hence would need closer follow-up and/or treatment. In this scenario, a substantial number of patients would be placed in the HPV high-risk type-positive, disease manifestation-negative group, of which the natural history is again poorly known.

Therefore, it appears that, at the present time, the data would suggest an approach of screening, following, and treating patients using well-established criteria based on cytologic, colposcopic, or histologic abnormalities, about which the natural history is better understood. In a prospective study of a cohort of untreated patients in Finland found to have clinical infection (molecular biologic evidence of HPV infection, plus conclusive cytologic and colposcopic evidence of CIN), the most significant factors affecting the rate of progression of disease were the histologic grade of the abnormality at the time of the first biopsy, the degree of cellular atypia on the initial Pap smear, and the presence of HPV type 16 [24]. The overall progression and regression rates for CIN lesions in this study closely paralleled those rates well known from classic cytologic studies, suggesting again that the presence of a positive HPV DNA assay may not be adding significant information beyond routine cytology. The latter finding of the association in this group, between the presence of HPV type 16 and progression of disease, may add weight to the hypothesis that HPV type 16 latent and subclinical infections may also be at higher risk for progression to clinical disease, but no studies have yet been completed to confirm this conjecture. Also, the natural history of HPV type 16 histologically confirmed CIN lesions, by inference from classic data, may include a significant number of regressors [24]. In addition, cancers and high-grade dysplastic lesions have been known to occur, albeit infrequently, with "low-risk" viral types [58,59]. Finally, because HPV type 16 may be the most prevalent virus in the cervix, it is therefore the most commonly expected result of typing on samples from this site which test positive for HPV [14,15,25,26].

The problem of rapidly progressive cervical cancer [18] and its association with HPV type 18 may eventually warrant an approach which selectively screens for this viral type in the population, but further characterization of this disease entity must first take place.

At present, the only well-founded indication for performance of an identification and/or typing test for HPV in the clinical setting is under circumstances in which the result will guide follow-up and further patient management. If one believes that lesions of CIN I or higher require eradication, then HPV testing has no significant role when such lesions are identified by cytologic or colposcopic examination. In the case of the atypias, or minimally abnormal results on Pap smear, there has been some interest and rationale for HPV testing. In many cases of atypia, the cause is not related to infection by HPV but may be due to other inflammatory/infectious

conditions which are not pre-cancerous. Therefore, the absence of HPV, or the presence of a low-risk HPV type might warrant continued cytologic follow-up, whereas the presence of HPV, or of a high-risk HPV type, might warrant more aggressive investigation/follow-up or treatment. In this scenario, the test could be useful for choosing between alternatives in patient management. Even on this point there is significant disagreement among pathologists and among clinicians. Some would follow minimal Pap smear changes by cytology regardless of HPV status, while others would perform colposcopy on all patients with these changes for fear of missing a more serious lesion. As the detection of HPV DNA is poorly correlated with equivocal morphologic changes, the decision to follow versus colposcope the patient is best made on the basis of clinical judgment.

SUMMARY

At the present time, the only plausible clinical indication for the performance of an HPV DNA identification and/or viral typing relates to minimal or low-grade cytologic abnormalities which may be managed in differing ways, dependent on the outcome of the test. Further screening indications, particularly in high-risk epidemiologic populations, may be forthcoming with prospective, longitudinal studies, detailing the natural history of the HPV-positive, disease manifestation-negative patient (latent or subclinical infections), and a more thorough understanding of the epidemiology of "high-risk" virus-infected patients.

In the future, additional HPV-related screening tests, which have better predictive value for, or correlation with, cervical pathology, will need to be developed. These might include assays for HPV messages or gene products which would indicate the presence of active viral genetic activity. Such signals might better correlate with the presence of clinical lesions or high likelihood of progression, rather than with the simple presence of viral DNA.

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