The human papillomavirus is a double-stranded DNA virus enclosed within a protein capsomere. It causes a spectrum of disease. The best known manifestation of HPV infection is the venereally-transmitted genital wart, or condyloma accuminatum. In 1984, over 1 million people sought treatment for genital warts, compared to 400,000 for the better publicized Herpes Simplex 2. The peak incidence of the disease occurs within the age group 20-24 years. Both males and females are affected. The contagion rate is high, with 60-65% of patients developing infection after exposure, with an incubation period of 1-3 months (ref. 10).

Genital warts represent only the most visible end of the spectrum of HPV disease. In 1977, an association of HPV with cervical dysplasia and invasive carcinoma of the cervix was first suggested. Carcinoma of the cervix had been associated with sexual activity since 1842, when it was first noted that the disease was rare among nuns. Since then, documented risk factors for cervical carcinoma have included age at first intercourse and number of sexual partners. Because of this, sexually transmitted diseases have been investigated as possible causative agents of the carcinoma. In the early 1970's, the increased incidence of antibodies to herpes simplex 2 in women with cervical dysplasia and carcinoma made this sexually transmitted virus a prime suspect as the etiological agent (ref. 6). In 1977, Meisels et al. and Purola et al. simultaneously noted that certain flat abnormalities of the cervix, hitherto thought of as dysplasia, contained cells which were cytologically similar to those found in genital warts. The two sets of investigators suggested that cervical dysplasia might actually be the flat equivalent of the more exophytic condyloma accuminatum (ref. 5). The cells which showed cytological similarities were called koilocytes, derived from the Greek word meaning "hollow". These cells were characterized by enlarged, irregular nuclei and prominent peri-nuclear haloes. These cells were subsequently examined by electron microscopy. Numerous viral particles were demonstrated within the cell nucleus, and the cytoplasm contained a peri-nuclear rim of necrosis accounting for the "haloe" effect. Following this confirmation that koilocytes were actually virally infected cells, Jensen developed an antibody to the common papillomavirus antigen. This was used to stain tissue sections of condylomata and dysplasia, demonstrating viral antigen in both (ref.5).

Since then, viral DNA has been isolated from HPV-infected cells and cloned into bacterial plasmids. These clones have been used to construct labelled DNA probes for HPV. Probes have been used extensively in DNA hybridization
studies to document the presence of HPV in tissues and cells. DNA probes represent a far more sensitive method of detecting HPV than the morphological observation of koilocytosis, which depends on the presence of a productive infection with numerous replicating viral particles. Probes allow detection of low level and latent infection, in which there may be only one copy of the HPV genome per cell. Hybridization studies have led to several important discoveries. First, 90-95% of tissues showing cervical dysplasia and squamous cell carcinoma have been found to contain HPV DNA sequences. Second, HPV DNA has been found in every tissue culture cell line which has been derived from a cervical carcinoma. Finally, HPV from condylomata and low-grade dysplasia has been found in episomal form within cells, while HPV from high-grade dysplasia and carcinoma has been found integrated into the host DNA (ref 6). The significance of this is unclear, but it is thought that integration into the host DNA may be an essential step in the progression toward malignancy.

While molecular studies have shown a very close association between HPV, cervical dysplasia, and carcinoma, epidemiological studies have been less compelling. These studies have been hampered by the difficulty of testing for HPV infection in large populations, which has required sampling of cervical cells and the use of DNA hybridization technology. In 1987, Reeves published a case-controlled study comparing 46 women with invasive squamous cell carcinoma of the cervix with 51 matched controls from Latin America. Using filter in-situ hybridization, he found HPV in 91% of patients with carcinoma, but also in 63% of the control population (ref.7). The largest epidemiological study to date was published in 1986 by de Villers. He studied 9295 women who received cytological evaluation at 3 German hospitals. He found that 94.2% had normal cytology, 2.1% had evidence of koilocytosis, 3.0% had evidence of dysplasia, and 0.7% had invasive carcinoma. Of those with normal cytology (94.2%), 9% had HPV DNA using filter in-situ hybridization. Of those with dysplasia or carcinoma, only 35% had demonstrable HPV DNA (ref 7). A relatively small number of positive samples within the dysplasia/carcinoma group may have been detected for two reasons. First, filter in-situ hybridization is a relatively insensitive hybridization technique. Second, at the time the study was done, many of the recently discovered DNA types were not known about.

These epidemiological studies illustrate several points. First, there is a high background prevalence of HPV infection in the general population, ranging from 9% in one study to 63% in the other. Second, if HPV is a causative agent in the development of cervical carcinoma, it must be only one of many different factors which together eventuate in malignancy. Other possible co-factors which may play a role in the development of cervical carcinoma have been identified, and these include young age at first exposure to
HPV, altered immune status, cigarette smoking, and hormonal status (ref 5).

Once the association between HPV, condylomata, dysplasia, and carcinoma was made, the question arose of how to regard findings of HPV infection on Pap smear or physical examination. Should koilocytosis on a Pap smear be regarded as an incidental finding and not treated unless accompanied by evidence of dysplasia? Or should simple HPV infection be considered part of the spectrum of cervical neoplasia? A couple of studies have addressed this issue. Mitchell in 1986 published a study of 846 women who had evidence of HPV infection on cervicovaginal smear. Over 6 years, carcinoma-in-situ developed in 30 patients, showing a relative risk of 15.6 times the general population. For women less than 25 years, the relative risk was even higher—38.7x (ref 10). Syrjanen in 1986 followed 343 patients prospectively for a mean of 18 months. She divided patients into 2 groups: one with evidence of HPV infection and dysplasia, the other with evidence of HPV infection alone. Over sequential Pap smears performed every 6 months, the rate of progression in the group initially showing only HPV infection was significantly higher (15.4%) than the group which initially showed HPV and dysplasia (11.6%). The number of women (7) who progressed to carcinoma-in-situ over 18 months was the same in both groups (ref 9).

As a result of studies such as these, it has been widely accepted that condylomata should be regarded as "preprecursor" lesions and incorporated into the presumed continuum of epithelial changes preceding invasive carcinoma.

Patients may present for evaluation of HPV infection in several different ways. Most obviously, they may perceive genital warts and present for treatment. It has been shown that 70% of women with obvious external condylomata acuminate also have evidence of HPV infection of the cervix on colposcopy (ref 10). Patients may also present because they are partners of women with some form of HPV infection. Barrasso published a study in 1987 of 480 male partners of women with either condylomata or dysplasia. He found that 64% of the male partners also had evidence of HPV infection. Only 1/3 of the infected men had grossly evident condylomata, however. In 2/3 of the cases, HPV infection presented as flat lesions which were only visible with careful colposcopic examination following the application of acetic acid (ref 1).

Finally, women may present following the finding of koilocytosis or dysplasia on Pap smear.

The Pap smear was developed by Papanicolou in 1947. By allowing detection of pre-cancerous or dysplastic changes of the cervix, it has become the most effective screening tool for the prevention of cancer in use today. At present, the Pap smear also serves as a screen for the presence of HPV infection, as manifested by the presence of koilocytes among cervical cells. Although it has contributed significantly to a decrease in the rate of cervical carcinoma, the Pap smear is an imperfect screening device, and there are numerous
opportunities for error in its performance. Detection of
dysplastic or virally-infected cells depends first on
shedding of these cells from the cervix. Next, it depends on
adequate sampling of the cervical cells by the clinician.
The clinician must then smear the cells on a glass slide and
immediately fix the slide in alcohol. It must then be
adequately screened by cytotechnologists who are faced with
the assessment of an average of 30,000-50,000 cells per slide
and 90 slides per day. Cytotechnologists highlight abnormal
cells for review and final interpretation by pathologists.
Given this chain of events, the overall efficacy of the
screening procedure is rather remarkable. For women 35-64
years old, the level of protection from developing invasive
cervical cancer is estimated to be 93.5% if annual screening
is undertaken, 83.6% if screening is done every 5 years, and
64% if it is done every 10 years (ref 11). No research has
been done on the sensitivity of the Pap smear for detecting
productive HPV infection without evidence of dysplasia. For
detection of latent HPV infection of the cervix, the
sensitivity of the Pap smear is 0%, since diagnosis depends
on the presence of koilocytes which signify a productive
infection.

There has been an evolution of descriptive terms
characterizing the Pap screen from its inception until the
present. Papanicolou originally devised a 5 class system to
describe smears(Class I-normal, Class II-benign atypia, Class
III-dysplasia, Class IV-carcinoma in situ, and Class V-
cancer). The distinction between dysplasia and carcinoma-in-
situ was confusing, and seemed to obscure the idea of a
biological continuum in this disease. Thus, in 1973, the
term cervical intraepithelial neoplasia(CIN) was introduced
to replace the terms dysplasia and carcinoma-in-situ (ref
11). CIN I replaced mild dysplasia, CIN II moderate
dysplasia, and CIN III replaced severe dysplasia and
carcinoma-in-situ. While it clarified a sense of the
continuity between these conditions, this classification
system did not address such important issues as adequacy of
specimen, diagnosis of infectious agents, or hormonal
evaluation. In 1988, a consensus conference at the National
Cancer Institute developed a new method of Pap smear
reporting called the Bethesda System. This system attempts
to correct previous deficiencies of reporting by calling for
explicit evaluation of the adequacy of the sample, providing
an extensive outline of what should be included in a
descriptive diagnosis, and advocating that a specific
recommendation of action accompany each Pap report. Another
change within the Bethesda system is that the previous 3-
tiered classification system of dysplasia/CIN is collapsed
into 2 tiers called low-grade squamous intraepithelial
neoplasia and high-grade squamous intraepithelial neoplasia.
Koilocytosis as well as mild dysplasia/CIN is included in the
category of low-grade neoplasia, reflecting the evidence
which implicates simple HPV infection as a "preprecursor"
lesion to overt cervical malignancy.
were aneuploid and 14% were polyploid. In this study, the ploidy level of a lesion had more predictive value for its future course than did its traditional histological classification (ref 2).

DNA hybridization is the technology which has been used to document the presence of the HPV genome in all studies thus far. Several different methods of DNA hybridization have been used, and all have different advantages, requirements, and sensitivities. Southern blot is considered the standard in the field. It requires the extraction of a relatively large amount of DNA from a cell swab or biopsy. DNA is cut into fragments by restriction enzymes, and fragments are separated by gel electrophoresis and transferred to nitrocellulose filter paper. The filter is then hybridized to labelled HPV DNA probes and visualized appropriately. Hybridization can be conducted under varying conditions which allow for a greater or lesser percentage of base pair mismatches as complementary DNA strands anneal. Under high-stringency conditions, only 13% base pair mismatches are tolerated, while under low-stringency conditions 33% mismatches are tolerated. Low-stringency conditions have been used to search for new types of HPV. By allowing some mismatch of DNA, known HPV DNA probes will bind to unknown HPV types which contain some homologous sequences. High-stringency conditions are used with type-specific DNA probes to identify a specific HPV type. Southern blot is the only hybridization technology which can specifically type an HPV DNA sequence, based on its characteristic electrophoretic pattern. It has been the gold standard for detection and typing, and thus has been 100% sensitive when compared with other hybridization methodologies. Controls are run to rule out nonspecific hybridization (ref 7).

Dot blot hybridization requires extraction of a small amount of cellular DNA which is then denatured. The DNA is applied to a filter using a dot blot manifold which contains 96 wells. HPV DNA probes are applied to the multiple DNA dots. Multiple controls are needed to rule out false positivity. This method allows diagnosis of a type-related HPV rather than a definitive typing, because the DNA is presented in a dot rather than in a characteristic electrophoretic pattern. The sensitivity is 1 HPV genome copy per cell(ref 7).

In the tissue in-situ hybridization technique, labelled DNA probes can by applied directly to tissue sections. This technique is much more convenient; however, there is considerable sacrifice of sensitivity. Depending on the type of labelling used for the DNA probe, this technique can detect between 5-800 copies of the HPV genome per cell. Thus, it shows strongly positive labelling in more mature, superficial epithelial cells where the virus is actively replicating and numerous copies of the genome are present in each cell. These more mature cells are present in condylomata and low-grade intraepithelial lesions. However, in higher grade lesions, cells do not show maturation, and
the virus cannot actively replicate. Tissue in-situ hybridization rarely detects HPV in high-grade lesions which contain the viral genome in low copy number. New modifications which may increase the sensitivity of the technique are the use of DNA probes to detect RNA and the construction of RNA probes. The great advantage of this technique is that it may be used on archival material (ref 7).

Filter in-situ hybridization involves the application of DNA probes directly to cells obtained from cervical or other tissue scrapings. Cells are placed on a filter. DNA is denatured, and probes are then applied. Since the DNA is concentrated in one location, the specific type of HPV present cannot be ascertained and there is potential for nonspecific hybridization. Multiple controls are needed to deal with the latter problem. This technique has been estimated to be 1/3 to 1/2 as sensitive as the Southern blot or the dot blot, but its great advantage again is its relative convenience compared to those two techniques (ref 7).

Polymerase chain reaction (PCR) is a method of amplifying specific DNA sequences within a tissue sample. Use of this technology in conjunction with Southern Blot would increase the sensitivity of HPV detection to 1 copy of the viral genome per 10 cells (ref 7). Large scale studies using PCR have not yet been applied to HPV. When done, they may radically alter our understanding of the occurrence of the virus.

The use of DNA hybridization as the technique for detection of human papillomavirus has led to the discovery of multiple viral types, distinguished by heterogeneity of DNA sequences. To date, 57 types have been documented. Different types show specific predilections for certain types of tissue. Types 1 and 4 cause plantar warts while type 2 causes common warts of the skin. Types known to affect the genitalia are 6, 11, 16, 18, 31, 33, 35, 42, 45, and 51. Of these, types 6, 11, 16, and 18 have been most extensively studied. In aggregate, these studies have suggested that types 6 and 11 are most commonly associated with overt condyloma, condylomatous cervical abnormalities, and low grade dysplasia. These types are most likely to cause polyploid lesions which either persist or regress, but uncommonly progress to a higher grade. Types 16 and 18 are most commonly associated with high-grade lesions and carcinomas, and are likely to be aneuploid. These lesions tend to persist or progress, but are unlikely to regress. An illustrative study was published by Syrjanen in 1986. Using tissue in-situ hybridization, she typed 64 of 505 patients with HPV infection who were being followed prospectively for a mean of 18 months. She found HPV type 6 in 20%, 11 in 17%, 16 in 8%, and 18 in 5%. Of patients with type 6 infection, 60% had koilocytosis or CIN I, and 40% had CIN II or III. Of patients with type 16, 20% had CIN I and 80% had CIN II or III. 80% of patients with type 16 progressed to a higher
grade lesion over time, while 38-45% of patients with type 6 or 11 progressed. No patients with types 16 or 18 had lesions which regressed over time, while regression occurred between 23 and 45% of the time with patients having type 6 or 11 lesions (ref 8).

The association of types 16 and 18 with higher grade intraepithelial neoplasia and a high rate of lesion progression has raised the question of whether these types should be specifically identified on Pap smear or on routine evaluation of HPV infection. Routine Pap smear typing or typing of HPV lesions does not seem warranted at this time for several reasons (ref 7). Latent HPV infection, including HPV-16, is quite common among completely asymptomatic populations, and the natural history of this infection is unknown. A study of 1247 randomly selected women from Denmark and Greenland showed that 13% of cytologically normal Danish women carried latent HPV-16 infection compared with 8.8% of comparable women from Greenland (ref 7). Yet the rate of cervical cancer in Denmark is one-fifth the rate of Greenland. Thus, the utility of detecting HPV-16 on routine Pap smear has not been determined. Furthermore, although types 6 and 11 are classified as low-risk, they are not without risk. The study by Syrjanen shows that a proportion of these viral types are found in high grade intreepithelial lesions, and that these types can also show progression to higher grade lesions. Thus, there is no justification for not treating lesions caused by types 6 and 11. Finally, an optimal typing procedure for large scale use does not yet exist. Sensitive methods of DNA hybridization such as Southern blot are too unwieldy to apply on a large scale, while the more convenient methods of in-situ hybridization are relatively insensitive. At present, Pap screening with work-up by colposcopy and biopsy is still the most effective means of detecting and evaluating inapparent HPV infection.
Bibliography


